

Deep phenotyping of 89 xeroderma pigmentosum patients reveals unexpected heterogeneity dependent on the precise molecular defect

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Xeroderma pigmentosum (XP) is a rare DNA repair disorder characterized by increased susceptibility to UV radiation (UVR)-induced skin pigmentation, skin cancers, ocular surface disease, and, in some patients, sunburn and neurological degeneration. Genetically, it is assigned to eight complementation groups (XP-A to -G and variant). For the last 5 y, the UK national multidisciplinary XP service has provided follow-up for 89 XP patients, representing most of the XP patients in the United Kingdom. Causative mutations, DNA repair levels, and more than 60 clinical variables relating to dermatology, ophthalmology, and neurology have been measured, using scoring systems to categorize disease severity. This deep phenotyping has revealed unanticipated heterogeneity of clinical features, between and within complementation groups. Skin cancer is most common in XP-C, XP-E, and XP-V patients, previously considered to be the milder groups based on cellular analyses. These patients have normal sunburn reactions and are therefore diagnosed later and are less likely to adhere to UVR protection. XP-C patients are specifically hypersensitive to ocular damage, and XP-F and XP-G patients appear to be much less susceptible to skin cancer than other XP groups. Within XP groups, different mutations confer susceptibility or resistance to neurological damage. Our findings on this large cohort of XP patients under long-term follow-up reveal that XP is more heterogeneous than has previously been appreciated. Our data now enable provision of personalized prognostic information and management advice for each XP patient, as well as providing new insights into the functions of the XP proteins.

UV radiation | nucleotide excision repair | skin cancer | ocular disease | neurodegeneration

Xeroderma pigmentosum (XP) is an autosomal recessive genodermatosis, with clinical features predominantly recognized in the dermatological, ocular, and neurological systems. XP patients may present with severe sunburn on minimal sun exposure or with pigmentary changes at exposed sites and multiple early age skin cancers. The incidence has been estimated at 2.3 per million live births in Western Europe (1), but is higher in Japan (2) and North Africa (3). XP patients have a 2,000- and 10,000-fold increased incidence of melanoma and nonmelanoma skin cancers, respectively (4). About 50% of XP patients show severe sunburn reactions on minimal sun exposure (5), and approximately one third of patients have progressive neurological degeneration associated with neuronal loss (4, 5). XP has been intensively studied at the molecular and cellular levels, as affected individuals are defective in the repair of UV radiation (UVR)-induced DNA damage. The majority of patients have mutations in one of seven genes (*XPA* through *G*), whose products are involved in nucleotide

excision repair (NER) of UVR and other types of DNA damage (6) (Fig. S1).

There are two subbranches of NER distinguished by the initial recognition step (Fig. S1). Transcription-coupled NER (TC-NER) is a process whereby damage formed on the transcribed strand of actively transcribed regions of DNA is repaired rapidly. The rest of the genome is repaired by a slower global genome NER (GG-NER). Importantly the damage recognition proteins XPC and XPE are not required for TC-NER, whereas the other XP proteins are involved in both subpathways.

About 20% of XP patients, the so-called XP variants, have normal NER but are defective in translesion synthesis past UVR-induced DNA damage (Fig. S2) (7), as a consequence of mutations in the *POLH* gene encoding DNA polymerase η , an enzyme vital for replicating past unrepaired UVR photo-products (8, 9). This deficiency confers sensitivity to fibroblasts that are exposed to

Significance

Xeroderma pigmentosum (XP) is a genetic disorder caused by defective repair of DNA damage. Affected patients are mutated in one of eight genes and develop skin pigmentation changes, skin cancers, ocular surface abnormalities, and, in some cases, acute sunburn and neurodegeneration. The XP proteins are involved in different steps in the repair of DNA damage. Examination of 89 patients, the largest reported cohort under long-term follow-up, by the same multidisciplinary team of clinicians and scientists has revealed unexpected clinical heterogeneity dependent on the affected gene and the exact mutation. Our findings provide new insights into the mechanisms of carcinogenesis, ocular surface disease, and neurodegeneration, as well as providing improved clinical management and more definitive prognostic predictions.

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UVR followed by incubation with caffeine (10). This UVR+caffeine sensitivity is used as a diagnostic test (11).

XP patients in which TC-NER remains intact (groups C, E, and V) do not, in general, suffer from neurological problems (4) and have normal sunburn reactions (5), whereas those in groups in which both subbranches of NER are affected often develop neurological problems and have an abnormal sunburn reactions.

In 2010, funding was obtained from the National Commissioning Group of the UK National Health Service to establish an XP specialized clinic, where all XP patients in the United Kingdom are offered specialist dermatological, ophthalmological, neurological, psychological, and clinical genetic examination, as well as DNA repair testing and mutation analysis. Our findings on 89 XP patients, the largest reported cohort under long-term follow-up, are presented here. We estimate that this represents 90% of the XP patients in the United Kingdom. Our study differs from previous analyses in that this large cohort has been examined by the same clinicians using 60 different phenotypical end points, and in all cases, this has been correlated with the pathological mutation. These end points have enabled us to identify previously unrecognized phenotypes in some XP groups, as well as several unexpected genotype-phenotype relationships in patients with unusual phenotypes. Our findings also provide new insights into the functions of the XP proteins.

Results

A few general points have emerged from our analyses, which will be discussed in more detail below [aspects of points 1–3 have been discussed previously (5)]:

- i) Most patients in XP-A, -B, -D, -F, and -G groups have severe sunburn reactions from an early age. In contrast the TC-NER-competent groups XP-C, -E, and -V have normal sunburn reactions for skin type. We recently provided a detailed analysis of this phenomenon (5), which has also been recently noted in the US XP population (4).
- ii) XP patients with extreme sunburn reactions are likely to be diagnosed very early and photo-protected from a young age. As a consequence, in such cases pigmentary changes are relatively mild and skin cancers are relatively uncommon.
- iii) In contrast, the first symptoms in XP-C patients are photo-distributed lentigines around the age of 2 y. They have a later age of XP diagnosis and therefore accumulate more photo-damage, leading to an earlier age of first skin cancer (4, 5).
- iv) XP-E and XP-V patients tend to be diagnosed much later; they may have two decades or more without any symptoms. They therefore accumulate more UVR-induced mutations and can develop hundreds of skin tumors in later life.
- v) Paradoxically, therefore, the XP-C, -E, and -V groups, which have generally been described as milder on the basis of their cellular sensitivity to UVR and lack of neurological abnormalities (12), tend to develop more skin cancers than the groups classified as more severe using the same criteria.
- vi) XP-C patients are particularly susceptible to ocular problems relative to their skin changes compared with the other groups.
- vii) Within the XP population, XP-F and XP-G patients seem to be remarkably resistant to the development of skin cancers.

We developed a scoring system to indicate the severity of the ocular and neurological features of each patient (*SI Methods*). By necessity, this is somewhat simplistic. Note that all scores are likely to increase with age of the patients, especially those with neurological abnormalities. More detailed breakdown of dermatological, ocular disease, and neurological features by complementation group is provided in [Tables S1–S3](#), respectively.

XP-A (MIM278700). Eighteen XP-A patients from 14 families have attended the clinic (Table 1). With one exception (XP15BR), they are of South Asian or Somali origin. All are homozygous for

the causative mutations. We have divided them into two clearly distinct subgroups.

In the first subgroup (Table 1, upper group), five of eight patients, XP54PV, XP15BR, XP20BR, XP57BR, and XP111BR, have truncation or frameshift mutations in the *XP4* gene. One of these patients, XP54PV, developed her first skin cancer aged 8 y; she lived the majority of her childhood in Pakistan and Italy, without significant UVR protection. The remaining four, born and raised in the United Kingdom, have not developed any skin cancers before 30 y of age. They have moderate ocular disease and neurological abnormalities. Neurological severity is quite heterogeneous, although generally progressive with advancing age. XP111BR is homozygous for a stop codon at amino acid (aa)85 and as would be expected from the literature, she has neurological abnormalities, developmental delay, and microcephaly. XP15BR is homozygous for a frameshift mutation at aa90. At age 22, he has a neurology severity score of 5/7. Neither patient has detectable unscheduled DNA synthesis (UDS). XP57BR (p.Met214fs) with a frameshift mutation in exon 5 has severe learning difficulties, negligible speech, and pronounced hearing loss. Her affected brother died at aged 18 y. XP20BR and XP114BR, both with the p.Arg228X mutation reported in several cases in the literature (13), have somewhat slower progression of their neurological problems. There is a large cohort of XP patients with this mutation in Tunisia (14), and their clinical features were described as being less severe than the large cohort of Japanese XP-A patients with a common splicing mutation in intron 3. The latter are expected to have no XPA functional activity.

The XP-A patients in the second subgroup (Table 1, lower group) are quite different. All 10 have the mutation c.555+8A > G. This mutation has previously been reported for a 60-y-old Afghan woman of Punjabi origin, XP40BR (15). It generates a new splice donor site for intron 4, resulting in seven bases from intron 4 being incorporated between exons 4 and 5, resulting in a frameshift at this position. A very low level of normal transcript was noted, which can account for the 5–10% of normal repair in these patients (15). All of the patients in this subgroup have mild dermatological features with no neurological abnormalities and low ocular scores for their age, as described in detail elsewhere (16). This subgroup includes XP1CB, our oldest XP patient, aged 80 y. Despite working outdoors for many years, his first skin cancer was diagnosed at age 46. He has since had multiple skin cancers removed, mainly in the last decade. As previously discussed, it appears that a minimal amount of repair capability in XP-A patients is sufficient to prevent any neurodegeneration, although not to prevent the typical cutaneous features of XP (15, 17).

XP-B (MIM610651). We are aware of only two XP-B families in the United Kingdom (Table 1). XP1SA and her younger sister, XP33BR (who has not attended our clinic), have been analyzed in detail by Oh et al. (18) They are compound heterozygotes for the missense mutation p.Phe99Ser and nonsense mutation p.Arg425X. The older sister, XP1SA, aged 53 y, had moderate sunburn on minimal sun exposure and developed several skin cancers from the age of 22 y, including a malignant melanoma. She also had moderate neurological abnormalities. As a heavy smoker, she developed a lung cancer, which led to her death at age 55 y, in August 2014. Another (unrelated) individual, XP84BR, is homozygous for the same missense mutation p.Phe99Ser that is heterozygous in both XP1SA and two brothers reported in the literature (XPCS1BA and XPCS2BA) (19, 20). She suffers from severe sunburn on minimal sun exposure and sensorineural hearing loss, but otherwise her symptoms are relatively mild. Other than the hearing loss, she has minimal neurological abnormalities. She has not had any skin cancers. It is likely that XPB protein with this missense mutation retains significant activity.

Table 1. XP-A, XP-B, and XP-C patients

XP ID no	Group	Age (y)	Age diagnosis XP (y)	Ethnicity	UDS*	Allele 1	Protein change	Allele 2 [†]	Protein change	Age first cancer (y)	Type first cancer	No. MM	No. NMSC	Ocular severity score (maximum 12)	Neuro severity score (maximum 8)	
XP111BR	A	8	5	Bangladeshi	3	c.253C > T	p.Gln85X			3	—	0	0	2	3/5 [‡]	
XP54PV	A	8	7	Pakistani	ND	c.648_649delGA	p.Lys217fs			3	8	SCC	0	2	3	4/5 [‡]
XP57BR	A	15	4	Bangladeshi	1	c.640dupA	p.Met214fs			1	—	0	0	2	4	
XP80BR	A	15	8	Somalian	2	c.314G > A	p.Cys105Tyr			3	—	0	0	3	4	
XP81BR	A	19	12	Somalian	5	c.314G > A	p.Cys105Tyr			1	—	0	0	3	5	
XP15BR	A	23	<1	Caucasian	0	c.266_267dupAA	p.Val90fs			3	—	0	0	1	5	
XP114BR	A	24	22	Pakistani	8	c.682C > T	p.Arg228X			3	—	0	0	6	5	
XP20BR	A	33	13	Pakistani	8	c.682C > T	p.Arg228X			3	—	0	0	6	8	
XP9BI	A	7	6	Pakistani	ND	c.555+8A > G				0	—	0	0	1	0	
XP103BR	A	8	4	Pakistani	20	c.555+8A > G				0	—	0	0	1	0	
XP53BR	A	19	7	Pakistani	3	c.555+8A > G				1	—	0	0	2	0	
XP116BR	A	31	31	Indian Asian	12	c.555+8A > G				1	30	BCC	0	7	4	0
XP2PR	A	34	33	Pakistani	ND	c.555+8A > G				1	—	0	0	1	0	
XP89BR-S	A	35	34	Pakistani	ND	c.555+8A > G				2	—	0	0	2	0	
XP1PR	A	36	35	Pakistani	ND	c.555+8A > G				2	—	0	0	3	0	
XP88BR	A	37	31	Pakistani	15	c.555+8A > G				3	—	0	0	2	0	
XP89BR	A	44	39	Pakistani	5	c.555+8A > G				3	—	0	0	2	0	
XP1CB	A	80	67	Indian Asian	13	c.555+8A > G				0	46	MIS	10	35	3	0
XP84BR	B	35	28	Caucasian	14	c.296T > C	p.Phe99Ser			3	—	0	0	3	1	
XP1SA	B	Died55	45	Mixed	21	c.296T > C	p.Phe99Ser	c.1273C > T	p.Arg425X	2	22	MM	1	7	3	4
XP117BR	C	5	5	Caucasian	8	c.299+2delT	Splice	c.2429_2441del13	p.Gly810fs	0	4	BCC	0	0	1	0
XP93BR	C	6	1	Pakistani	1	c.1243C > T	p.Arg415X			0	—	0	2	0	0	
XP66TU	C	6	3	Caucasian	20	c.924_938del15	p.Leu309_313del	c.1021G > T	p.Ala341Ser	0	3	SCC	0	4	3	0
XP112BR-S	C	7	3	Bangladeshi	ND	c.1808G > A	p.Trp603X	c.2420+2T > C	Splice	0	—	0	0	2	0	
XP102BR	C	7	4	Caucasian	14	c.1111delA	p.Thr371fs	deletion exon 1		0	—	0	0	3	0	
XP112BR	C	9	7	Bangladeshi	9	c.1808G > A	p.Trp603X	c.2420+2T > C	Splice	0	—	0	0	2	0	
XP4LE	C	9	5	Pakistani	5	c.2176_2192del17	p.Glu726fs			0	6	BCC	0	2	5	0
XP73BR	C	10	2	Pakistani	7	c.1243C > T	p.Arg415X			0	—	0	0	6	1	
XP94BR	C	10	6	Sri Lankan	11	c.877C > T	p.Arg293X			0	—	0	0	7	0	
XP82BR	C	10	2	Caucasian	15	c.395_398delATTG	p.Asp132fs	deletion XPC exon 14–15		0	6	SCC	0	3	2	0
XP76BR	C	11	3	Pakistani	10	c.1243C > T	p.Arg415X			0	—	0	0	4	0	
XP78BR	C	12	5	Pakistani	28	c.1243C > T	p.Arg415X			0	—	0	0	5	0	
XP3LE	C	13	6	Pakistani	15	c.1243C > T	p.Arg415X			0	11	BCC	0	1	3	0
XP77BR	C	12	4	Pakistani	10	c.1243C > T	p.Arg415X			0	8	BCC	1	0	5	0
XP58BR	C	15	4	Pakistani	ND	c.1243C > T	p.Arg415X			0	—	0	0	4	0	
XP83BR	C	16	9	Pakistani	21	c.1243C > T	p.Arg415X			0	—	0	0	7	0	
XP62BR	C	17	7	Pakistani	6	c.1243C > T	p.Arg415X			0	—	0	0	4	0	
XP39BR	C	17	2	Bangladeshi	12	c.2176_2192del17	p.Glu726fs			0	—	0	0	5	0	
XP35BR	C	19	4	Bangladeshi	13	c.2176_2192del17	p.Glu726fs			0	—	0	0	6	0	
XP28BR	C	21	4	Bangladeshi	12	c.2176_2192del17	p.Glu726fs			0	8	BCC	0	8	9	0
XP51BR	C	23	11	Middle Eastern	1	c.2251-1G > C				0	12	BCC	0	1	5	0
XP6B1	C	23	5	Pakistani	13	c.1243C > T	p.Arg415X			0	22	BCC	0	5	8	0
XP15H	C	27	23	Caucasian	6	c.445_446delGA	p.Glu149fs	c.2336delT	p.Leu779fs	0	3	BCC	0	5	8	0
XP22BR	C	38	19	Middle Eastern	13	c.658C > T	p.Arg220X			0	11	BCC	0	7	7	0
XP21BR	C	Died39	6	Middle Eastern	15	c.658C > T	p.Arg220X			0	31	BCC	1	6	7	0
XP95BR	C	26	20	Caucasian	27	c.2033+5G > A				0	20	MM	9	0	0	0
XP107BR	C	37	34	Caucasian	41	c.1754A > G	p.Tyr585Cys			0	28	MIS	4	0	1	0
XP29BR	C	62	46	Asian	33	c.2033+5G > A				0	57	BCC	3	1	2	1

*UDS is measured, after a UVC dose of 10 J·m⁻², as percent of that in normal cells used as control in the same experiment. BCC, basal cell carcinoma; MIS, melanoma in situ; MM, malignant melanoma; ND, not done; NMSC, nonmelanoma skin cancer; SCC, squamous cell carcinoma; SSS, sunburn severity score (5). Note that the mutation data for the XP-A patients has been presented in ref. 16 and much of the sunburn data have been presented in ref. 5. These data are reproduced here for completeness.

[†]Where no second allele is indicated, the mutation is homozygous.

[‡]In these two cases, audiometry and neuro-ophthalmological data were not possible to obtain due to severity of cognitive impairment in each patient, so maximum score from measured parameters is only 5.

XP-C (MIM278720). Twenty-eight XP-C patients have attended the clinic (Table 1). Ten belong to an extended consanguineous kindred of Pakistani origin. The XP-C patients comprise the most homogeneous group. None shows any sign of severe sunburn reactions on minimal sun exposure. Most were diagnosed between ages 3 and 7 y because of progressive exposed-site lentiginos, especially on the malar area of the face (Fig. 1A). XP21BR and XP22BR spent the first 7 and 2 y of their lives, respectively, in Yemen and were not diagnosed definitively until their late teens. Both have had several skin cancers (Fig. 1B). With three exceptions, the XP-C patients are homozygous for truncation mutations (nonsense, frameshift, or splicing mutations) and UDS levels, as reported in the literature for other XP-C patients, are in the range of 10–20%. Skin cancers are seen relatively early in XP-C patients. However, affected younger siblings, usually diagnosed at birth, have been well protected and therefore have not developed skin cancers.

A remarkable and previously unreported observation is that progressive ocular disease in XP-C patients is much more severe than in other groups, with ocular disease scores increasing with advancing age. For example, in their first, second, and third decades, they have mean scores of 3, 4.7, and 7.5, respectively (see Table S2 for more details). If we compare the ocular scores for the non-photo-sensitive groups XP-C, XP-E, and XP-V (Tables 1 and 2) with the number of skin cancers, it is evident that patients in the latter two groups generally have many skin cancers, but relatively low ocular scores. As discussed above, the high incidence of skin cancers is a consequence of late diagnosis in these patients because of the later development of skin signs/symptoms. Despite the lack of protection from UVR, ocular scores are relatively low in these two groups. In contrast, many of the XP-C patients have very high ocular scores despite having had few or no skin cancers. For example, XP73BR and XP94BR, both aged 10, have been

well protected and have not developed any skin cancers, yet they have ocular scores of 6 and 7, respectively. In contrast, XP variant, XP63BR, aged 55, has had more than 35 skin cancers removed, yet his ocular score is just 1.

Although none of the XP-C patients has the progressive neurodegeneration found in other XP complementation groups, we observed intracranial lesions in 4 of 28 patients after routine baseline MRI scans. XP21BR developed a glioblastoma multiforme at age 38 y, which led to death less than a year later. XP28BR had a dysembryonic neuroepithelial tumor. His younger brother XP39BR had an MRI scan at age 16 y to investigate for early morning headaches. This scan showed a right temporal lobe cyst with encroachment on the orbit; he is under neurosurgical follow-up. Another patient, XP1SH, was noted on MRI to have an intraventricular mass with features most in keeping with a benign subependymoma. There have been other reports in the literature associating XP-C with the development of intracranial lesions (21).

Three of our XP-C patients (XP95BR, XP107BR, and XP29BR) fall into a distinct subgroup (bottom of Table 1) with a later presentation of cutaneous features and significantly lower ocular disease scores with respect to age. XP107BR, a Spaniard, first attended the clinic at age 33 y, with an unusual distribution of lentiginos limited to his upper back and scalp but sparing his face and forearms, despite excess UVR exposure in his youth (Fig. 1C). He developed his first melanoma at age 28 y on his scalp (despite a thick head of hair) and has subsequently developed three further melanomas at this same site (Fig. 1D). His elder brother also had several malignant melanomas on his scalp, one of which metastasized, leading to his death. UDS in his skin fibroblasts was ~40% of normal after a UVR dose of 10 Jm⁻² (Fig. 1E), and mutation analysis revealed a homozygous missense p.Tyr585Cys mutation in XPC in both brothers. The UDS level suggests that the XPC protein was partially functional. Tyr585 is conserved in all orthologs of XPC including yeast Rad4 (Fig. 1F). It is located in the inside of the protein (Fig. 1G) and is not directly involved in binding DNA (22). It is likely that this mutation destabilizes the protein, resulting in partial loss of function. The reason why this might result in these very specific clinical features remains obscure.

Another patient, XP29BR (Fig. 2A), also has unusually mild cutaneous features. He was not diagnosed until the age of 45 y, when he presented with exaggerated pigimentary changes and multiple dysplastic naevi, but no skin cancers. Fibroblasts showed about 33% UDS (Fig. 2B) and rather mild hypersensitivity to UVR-induced cell killing. Some 15 y later (Fig. 2A), he developed a few skin cancers including a malignant melanoma on his ear, which was completely excised. Molecular analysis of his genomic DNA revealed a homozygous mutation, c.2033+5G > A (intron 10). This mutation in the splice donor site of intron 10 (Fig. 2C) resulted in more or less equal amounts of two major abnormally spliced products in the mRNA, neither of which would be expected to generate functional protein. As the mutation confers only a modest change on the splice donor site, we suggest that the splicing mutation may, as with the milder XP-A patients described above, permit the read-through of a small amount of normal mRNA, which is sufficient to alleviate the clinical features, although we do not have direct evidence to support this suggestion. XP95BR has the identical mutation to XP29BR. She was diagnosed aged 20 y, but she has not adhered to strict UVR protection. In the last 6 y, she has had nine malignant melanomas excised. Her presentation may be more severe than that of XP29BR partly due to her ethnicity; she is white, whereas XP29BR is of Indian origin, or perhaps due to comparatively higher childhood UVR exposure, or there may be other factors within her genetic background that increased her susceptibility to pigimentary changes and skin cancer.

XP-D (MIM278730). Fourteen XP-D patients have attended the clinic (Table 2). Most presented initially with severe prolonged sunburn on minimal sun exposure (Fig. 3A) in their first year of life.

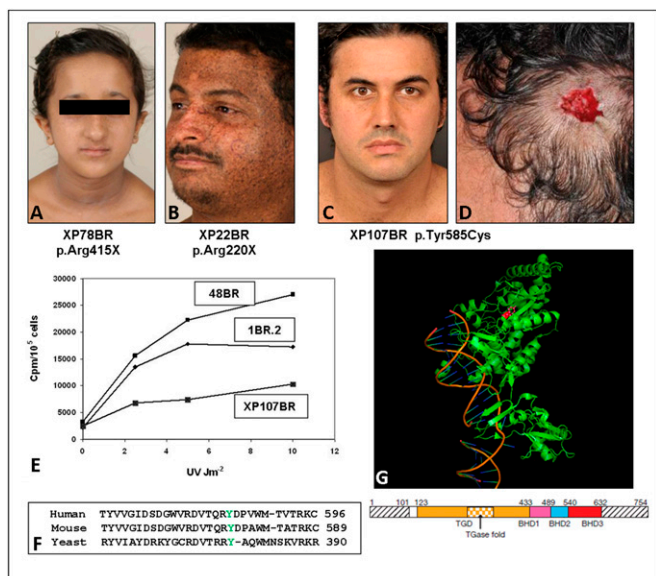


Fig. 1. XP-C patients. (A) XP78BR, aged 12 y; note lentiginos on face. (B) XP22BR, aged 38 y; note the extensive pigimentary changes. (C) XP107BR, aged 37 y; note the almost complete absence of pigimentary changes, despite not being diagnosed until 34 y of age. (D) Site of excised melanoma from XP107BR. (E) UDS, measured as incorporated ³H-thymidine cpm per 10⁵ cells following UV irradiation of XP107BR with different UVC doses compared with two normal controls: 1BR2 and 48BR. (F) Alignment of XPC protein from human and mouse and yeast Rad4 in the region around Tyr585 (indicated in green) that is mutated in XP107BR. (G) Crystal structure of Rad4 (22) with Tyr379 (corresponding to Tyr585 in human XPC) indicated in red in the structure and the schematic below.

Table 2. XP-D, XP-E, XP-F, XP-G, and XP-V patients

XP ID no	Group	Age diagnosis (y)	Age XP (y)	Ethnicity	UDS*	Allele 1	Protein change	Allele 2 [†]	Protein change	SSS	Age first cancer (y)	Type first cancer	No. MM	No. NMSC	Ocular severity score (maximum 12)	Neuro severity score (maximum 8)
XP109BR	D	4	1	Caucasian	32	c.2047C > T	p.Arg683Trp	c.1381C > G; c.2150C > G	p.Leu461Val; p.Ala717Gly	3	—	—	0	0	1	0
XP87BR	D	8	2	Caucasian	23	c.2047C > T	p.Arg683Trp	c.1867dupG	p.Val623fs	2	—	—	0	0	3	4
XP70BR	D	14	5	Caucasian	10	c.2047C > T	p.Arg683Trp	c.816-2A > G	—	3	10	SCC	0	1	4	6
XP16BR ²⁴	D	21	1	Caucasian	16	c.2047C > T	p.Arg683Trp	c.1847G > A	p.Arg616Pro	3	14	BCC	0	1	1	5
XP71BR	D	22	14	Caucasian	19	c.2047C > T	p.Arg683Trp	c.816-2A > G	—	2	10	BCC	0	4	1	5
XP17BR	D	23	2	Caucasian	16	c.2047C > T	p.Arg683Trp	c.1847G > A	p.Arg616Pro	3	21	BCC	0	2	1	5
XPJCLC	D	26	1	Caucasian	19	c.2047C > T	p.Arg683Trp	c.1933_1934delCA	p.Gln645fs	2	—	—	0	0	1	8
XP135LO-5	D	34	5	Caucasian	25	c.2047C > T	p.Arg683Trp	—	—	3	13	BCC	0	22	3	5
XP135LO	D	35	8	Caucasian	25	c.2047C > T	p.Arg683Trp	—	—	3	12	BCC	1	50	3	5
XP104LO	D	47	3	Caucasian	13	c.2047G > A	p.Arg683Trp	c.718+1C > A	—	3	23	BCC	0	4	1	7
XP59BR	D	53	9	Caucasian	24	c.2047C > T	p.Arg683Trp	c.1381C > G; c.2150C > G	p.Leu461Val; p.Ala717Gly	2	36	BCC	5	23	4	3
XP67BR	D	65	55	Caucasian	75	c.1532G > A	p.Arg511Gln	c.1381C > G; c.2150C > G	p.Leu461Val; p.Ala717Gly	2	27	BCC	0	>40	3	2
XP30BR	D	21	4	Mixed	36	c.2048G > A	p.Arg683Gln	c.1827delC	p.Phe610fs	3	—	—	0	0	1	0
XP97BR	D	34	29	Haan Chinese	53	c.2048G > A	p.Arg683Gln	c.1378-26_1383del32	—	3	—	—	0	0	3	0
XP115BR	E	30	29	Pakistani	63	c.1149delG	p.Met383fs	—	—	1	—	—	0	0	0	0
XP105BR	E	49	46	Caucasian	75	c.1070C > T	p.Pro357Leu	c.716G > T	p.Arg239Ile	1	15	MM	35	>120	4	0
XP98BR	E	62	48	Caucasian	68	c.161G > A	p.Trp54X	—	—	1	15	BCC	2	>200	4	0
XP100BR	E	62	59	Caucasian	53	c.457-2A > C	Splice	—	—	0	9	BCC	0	>100	4	0
XP72BR	F	18	9	Caucasian	36	c.1135C > T	p.Pro379Ser	—	—	3	—	—	0	0	0	0
XP32BR ³⁷	F	23	7	Caucasian	16	c.1135C > T	p.Pro379Ser	c.1765C > T	p.Arg589Trp	3	—	—	0	0	1	0
XP24BR ³⁷	F	48	30	Caucasian	4	c.1765C > T	p.Arg589Trp	c.2395C > T	p.Arg799Trp	2	—	—	0	0	3	6
XP104BR	G	6	2	Pakistani	0	c.136delC	p.His46fs	—	—	3	—	—	0	0	3	5
XP118BR	G	10	9	Caucasian	3	c.2383G > A	p.(Ala795Thr)	c.1842delT	p.(Leu615fs)	3	—	—	0	0	1	0
XP55BR	G	15	4	Somali	1	c.264+1delG	Splice	—	—	1	—	—	0	0	4	4
XP56BR	G	18	7	Somali	1	c.264+1delG	Splice	—	—	0	—	—	0	0	4	4
XP34BR	G	20	2	Caucasian	3	c.2453C > T	p.Ala818Val	c.2586_2587delTA	p.Thr863fs	3	—	—	0	0	4	4
XP120BR	G	36	36	Caucasian	ND	c.2453C > T	p.Ala818Val	c.1753G > T	p.Glu585TER	3	—	—	0	0	1	4
XP119BR	G	38	38	Caucasian	ND	c.2453C > T	p.Ala818Val	c.1753G > T	p.Glu585TER	3	20	BCC	0	1	3	4
XP101BR	G	68	64	Caucasian	14	c.869T > A	p.Ile290Asn	—	—	2	—	—	0	0	2	6
XP1NO	V	20	16	Caucasian	144	c.149dupT	p.Ser51fs	—	—	0	16	SCC	0	5	1	0
XP5B1	V	25	10	Caucasian	111	c.364A > C	p.Thr122Pro	c.1222_1225delACTT	p.Thr408fs	0	—	—	0	0	1	0
XP1CH	V	26	23	Caucasian	99	c.738delC	p.Phe247fs	c.1066C > T	p.Arg356X	0	22	MIS	1	0	2	0
XP110BR	V	27	24	Caucasian	97	c.738delC	p.Phe247fs	c.1066C > T	p.Arg356X	0	23	MM	3	0	3	0
XP136LO	V	54	53	Caucasian	81	c.25G > T > G	p.Val9Phe	c.490+3A	Splice	0	42	BCC	12	11	0	0
XP63BR	V	55	45	Caucasian	130	c.225_227delITCT	p.Leu77del	c.207delG	p.Lys70fs	0	38	BCC	15	>20	1	1
XP64BR	V	55	45	Mixed	144	c.437dupA	p.Tyr146X	—	—	0	—	—	0	0	3	0
XP115LO ²⁵	V	62	24	Middle Eastern	100	c.1117C > T	p.Gln373X	—	—	0	21	SCC	17	16	5	0
XP85BR	V	62	56	Caucasian	90	c.790G > C	p.Ala264Pro	c.490+3A > G	Splice	0	44	BCC	0	120	3	0
XP36BR	V	67	44	Caucasian	99	c.332G > A	p.Arg111His	c.1222_1225delACTT	p.Thr408fs	0	33	MM	10	>10	4	0
XP36BR-5	V	71	48	Caucasian	99	c.332G > A	p.Arg111His	c.1222_1225delACTT	p.Thr408fs	0	44	MM	13	21	1	0
XP6DU	V	75	72	Caucasian	100	c.225_227delITCT	p.Leu77del	c.207delG	p.Lys70fs	1	39	MM	>8	>30	2	0

*UDS is measured, after a UVC dose of 10 J·m⁻², as percent of that in normal cells used as control in the same experiment. Other abbreviations as in Table 1. [†]Where no second allele is indicated, the mutation is homozygous.

Several patients have been extremely well UVR protected from a very young age and have developed minimal cutaneous features even by the age of 25 y (Fig. 3 B and C). Eleven of 14 patients have severe neurodegeneration (as reflected by increasing neurological scores with advancing age).

All but one of the XP-D patients (XP67BR) is mutated at Arg683 in one allele (Fig. 3D). Strikingly, in the cohort of 11 pa-

tients in which Arg683 is replaced with tryptophan (Trp), there is a progression of neurological disease in all patients except for XP59BR, who showed no significant neurological abnormalities before the age of 50 y. XP59BR, although having minimal neurological abnormalities, has not followed UVR protection advice and has subsequently developed many skin cancers as an adult (Fig. 3E).

XP98BR is 62 y of age; cellular diagnosis was made at age 48 y, although clinical diagnosis was suspected much earlier. She developed her first skin cancer (BCC) at age 15 y and has subsequently developed hundreds of BCCs, MMs, and SCCs. These findings of multiple skin cancers are similar to those reported by Oh et al. (34) The fourth patient, XP115BR, age 30 y and of Pakistani origin, is minimally affected, with scattered lentiginos on her face and hypopigmented macules on her forearms. She has not developed any skin cancers. On initial examination, XP was not expected, but her UDS was ~50% of normal, and mutation analysis revealed a frameshift at Met383 in the 427 aa XPE/DDB2 protein. This mutation is more C-terminal than any of those previously reported (34), but nevertheless, it is expected to disrupt the protein structure (35) and therefore abolish function. Indeed, missense changes or single nucleotide deletions in the C-terminal region of XPE/DDB2 have been shown to drastically reduce the cellular protein levels (36), indicating the importance of this region for the correct structure of the XPE protein.

As expected from the literature, none of the XP-E patients has any significant neurological abnormalities. Interestingly, if we compare the ocular and skin lesions, the XP-C cohort has smaller numbers of skin cancers but quite severe ocular problems. In contrast, in the XP-E cohort, there are many more skin cancers, but the ocular lesions are significantly less severe than in the XP-C cohort.

XP-F (MIM278760). Three XP-F patients (Table 2) have attended the clinic, and all have severe sunburn on minimal sun exposure. XP32BR was diagnosed at age 7 y because of severe sunburn reactions from infancy. At age 23 y, he is surprisingly free of any cutaneous features of XP, despite his pale complexion (Fig. 4A) and low UDS level (18% of normal). He has no neurological abnormalities. He is a compound heterozygote for two missense mutations, p.Pro379Ser and p.Arg589Trp (Fig. 4D), and the properties of his cells in response to UVR have been described (37). XP72BR had several episodes of severe sunburn as a child and was diagnosed at age 9 y. At age 18 y, he has remarkably few lentiginos (Fig. 4B), and his UDS level is about 35% of normal. He is homozygous for p.Pro379Ser, the same site mutated in one

allele of XP32BR. Neither of these patients has any neurological or ocular abnormalities. Remarkably, the Pro379Ser mutation is listed in the SNP database with an allele frequency of 0.3%. If this is indeed the case, the frequency of individuals homozygous for this mutation would be ~10 per million, roughly five times the incidence of known XP cases in the United Kingdom (1). This estimate implies there might be a significant cohort of UVR-sensitive individuals not recognized as having XP, but homozygous for this mutation.

XP24BR, age 48 y, has had severe sunburn reactions from early infancy. She uses only moderate UVR protection, but despite barely detectable UDS, her skin features are fairly mild, with only a few lentiginos on her face and shoulders, within the normal range of her skin type. She has no significant ocular disease. She is compound heterozygote for two missense mutations described in several other XP-F patients (Fig. 4D), and her cellular features have been described (37). The most prominent clinical features, which have developed only in the last few years, are peripheral sensory neuropathy, progressive cerebellar ataxia, dysarthria, sensorineural hearing loss, and progressive cognitive decline, as well as sunken eyes and loss of subcutaneous fat. These features may all be characteristic of late-onset Cockayne syndrome (CS) (Fig. 4C). We recently described three early-onset CS patients with mutations in XPF or its partner protein ERCC1 (38).

It is remarkable that, despite only moderate UVR protection, none of the XP-F patients has developed any visible actinic damage or skin cancers.

XP-G (MIM278780). XP-G patients described in the literature span a wide range of clinical phenotypes from mild XP to severe combined XP-CS phenotype. Of the seven XP-G patients that we have seen (Table 2), six fit into this range. XP34BR developed severe sunburn on minimal sun exposure at age 4 wk and has since been adequately UVR protected (Fig. 5A). Because of his UVR protection, he has minimal skin changes. He has moderate learning difficulties. Another pair of siblings of Celtic origin, XP119BR and XP120BR, age 36 and 38 y, respectively, have only recently been diagnosed, although they have suffered from severe sunburn reactions on minimal sun exposure since birth, and they developed exposed-site lentiginos from 2 y of age. [Interestingly, XP119BR is the only XP-G patient who has developed a BCC (on her face at age 20 y).] They were only referred for diagnosis in their mid-30s, when both began to develop neurological problems, (more pronounced in the younger brother). The clinical features of these three patients are consistent with molecular analysis. All have a frameshift or truncation mutation in one allele and the missense mutation p.Ala818Val in the other allele (Table 2). The XPG nuclease contains two domains (N and I) required for nuclease activity, separated by a large spacer domain (Fig. 5B), whose function is unclear, although it is required for interaction with transcription factor IIIH (TFIIH) (39). Ala-818 is in the nuclease I domain and mutation at this site is likely to inactivate nuclease activity of the protein without affecting its overall structure. A similar mutation 26 aa away, p.Ala792Val, also resulted in a mild phenotype in XP124LO and 125LO (39, 40).

XP104BR is a severely affected 6-y-old child with severe sunburn on minimal sun exposure observed at age 2 wk. She has extensive freckling (Fig. 5C) and gross developmental delay. Her UDS level was undetectable, consistent with a homozygous frameshift mutation at His46.

XP55BR and XP56BR are teenage siblings of Somali origin (Fig. 5D and E). They have lentiginos at exposed sites but have been well protected from UVR exposure. Both siblings appear cachectic with sunken eyes and thin long limbs. Neurological examination revealed evidence of cognitive impairment, peripheral neuropathy, and cerebellar dysfunction. They developed sensorineural hearing loss in early childhood. MRI brain scans in both siblings revealed evidence of bilateral globus pallidus and posterior periventricular white matter calcification. These features are

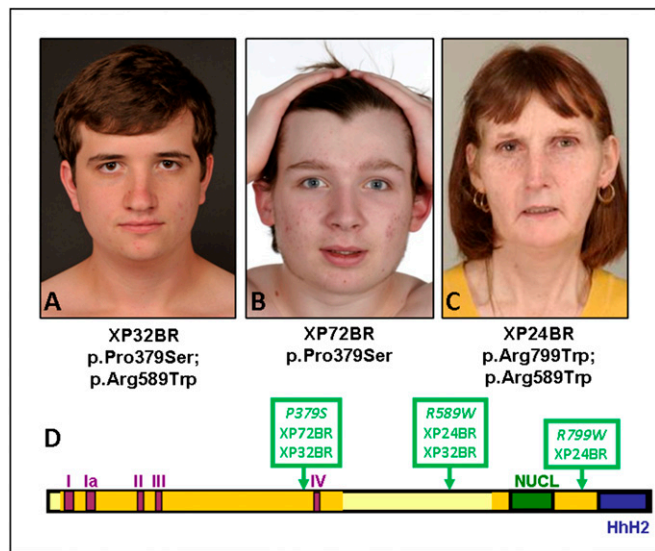


Fig. 4. XP-F patients. (A) XP32BR. (B) XP72BR. (C) XP24BR. Note the very mild skin changes despite only moderate UVR protection. (D) Scheme of XPF protein, showing the five motifs of the disrupted helicase domains (I, Ia, II, III, and IV), the nuclease domain (NUCL), and the two Helix-hairpin-helix (HhH) domains.

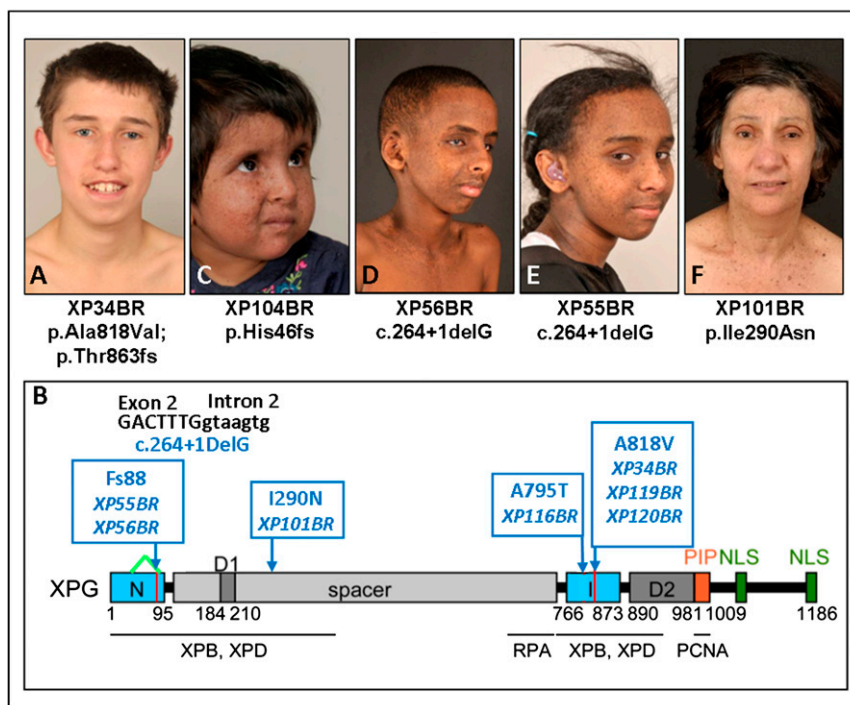


Fig. 5. XP-G patients. (A) XP34BR. (B) Structure of the XPG protein (39), showing the N and I domains required for nuclease activity, the spacer region, nuclear localization signals (NLS), and binding sites for other proteins. Sites of the mutations in the patients are indicated in blue, and the deletion of aa16–88 in XP55BR and XP56BR is indicated by a green inverted V. (C) XP104BR. (D) XP56BR. (E) XP55BR. (F) XP101BR.

typical of CS. These siblings are homozygous for a -1 -bp deletion at the boundary of exon 2 and intron 2, resulting in a frameshift mutation at aa 88 and abnormal splicing of exon 3 (Fig. 5B). Despite the similarity in the mutation to XP104BR and other severely affected XP-CS patients described in the literature (41) (homozygous frameshift or nonsense mutation close to the N terminus, presumably resulting in no functional protein, no detectable UDS), these siblings have much less severe neurological abnormalities than would be expected from patients with apparently similar truncation mutations (41–43). Sequencing of the cDNA reveals an in-frame 216-nucleotide deletion from G48 in the middle of exon 1 to G263 at the end of exon 2 (Fig. 5B) as a minor product. The encoded protein would be completely missing aa16–88, containing most of the N domain, consistent with the undetectable level of UDS. However, the rest of the 1,186 aa protein would be intact and this might account for the clinical features being less severe than anticipated from a frameshift so close to the N terminus.

The most unusual XP-G patient is XP101BR (Fig. 5F), a 68-y-old woman of Greek Cypriot parentage. She has severe sunburn on minimal sun exposure and rarely ventures outdoors. Her skin changes are relatively mild with minimal exposed-site lentiginos and she has not developed any skin cancers. Neurologically, she had normal developmental milestones, living a fully active and independent life for more than 50 y. However, over the last 10 y, she has shown cognitive decline and behavioral changes similar to those seen in Alzheimer's patients, as well as impaired balance and sensorineural hearing loss. Three of her six siblings in Cyprus are reported to have similar cognitive changes in association with hypersensitivity to sunlight, whereas the other three are completely normal, suggesting linkage with XP. The parents were not known to be related, but originated from the same village in Cyprus, consistent with the patient's homozygosity for a missense mutation in XPG. p.Ile290Asn is located in the spacer region of the protein, between the two nuclease domains (Fig. 5B). One affected sister of XP101BR is confirmed to be homozygous, whereas an unaffected brother is heterozygous for the same mu-

tation. Although little is understood about the detailed function of the spacer region, Ile290 is conserved in mammals, fish, frog, and chicken and is in the region thought to be involved in interaction with TFIIF (44).

XP-V (MIM278750). These individuals have normal sunburn reactions for skin type and have no neurological abnormalities. Typically they are not diagnosed until their second or third decade at the earliest. At this point, they have accumulated years of UVR-induced mutations, leading to development of multiple skin malignancies at a later age (Table 2). As with the XP-E patients, XP-V patients develop hundreds of skin cancers, but ocular disease is relatively mild compared with that found in XP-C patients. XP-V patients display a range of mutations in the *POLH* gene (Table 2), both missense and truncations, but the cellular and clinical phenotypes are quite similar in most cases.

Discussion

The literature on XP suggests that there are gradations in severity of the clinical features, dependent on the complementation group. Patients in groups XP-A, -D, and -G are considered to be the most severe (12), with early-onset neurological degeneration and abnormally severe sunburn reactions, whereas XP-C is considered to be intermediate, and XP-E and variant are the least severely affected. Although in some respects this is correct, especially in conditions in which UVR protection and early diagnosis is difficult, we found that early diagnosis and rigorous UVR protection can have profound effects on clinical features. For example, most of our cohort of XP-D patients with very early sunburn episodes were diagnosed in the first year of their lives and have therefore exercised rigorous UVR protection. As a consequence, subsequent skin damage in these individuals is barely detectable. In marked contrast, the groups conventionally described as milder (XP-E and variant) generally are not diagnosed until they are adults, due to subtlety and later onset of skin alterations. During this undiagnosed period, they accumulate large

quantities of precarcinogenic lesions, and as a consequence, they may develop hundreds of skin cancers, even if they are well protected after they have been diagnosed. However, there is an additional management problem; whereas it is relatively easy to persuade parents of a child who has an extreme sunburn reaction to protect their child from all UVR exposure, it is much more difficult to persuade an adult, who has had a hitherto normal lifestyle, to instigate rigorous UVR protection.

A striking aspect of our analysis of the UK cohort of more than 80 XP patients has been the unanticipated heterogeneity of clinical features, not only between, but also within, complementation groups. We highlighted here and elsewhere a cohort of mild XP-A patients originating from the Indian subcontinent, in which the mutation causes aberrant splicing, but we can attribute the mild phenotype to a low level of read-through from the normal splice site (16). Likewise the absence of neurological abnormalities in XP-D patient XP59BR with a splicing mutation most likely results from a small amount of read-through from the normal splice site. We also discussed a late-onset XP-C patient, XP29BR, and XP-G siblings (XP55BR and XP56BR) with milder than expected phenotypes. In all these cases, the mutation generates aberrant splice products, and we speculated that small amounts of either normal read-through, or of splicing that results in in-frame products, might alleviate the anticipated phenotype. These findings highlight the importance of sequencing both genomic DNA and cDNA and of carrying out functional DNA repair assays to obtain accurate genotype-phenotype relationships.

A remarkable and unexpected finding is the specific propensity to ocular surface problems in the XP-C group compared with XP-E and XP-V patients, who have many more skin cancers. Very little is known about the etiology of these ocular surface problems, and there are no studies on the control of cell cycle, DNA replication, or response to DNA damage in ocular surface cells. One possible explanation for the lack of ocular damage in XP-V patients may lie in the different nature of the defect in this group, which is only manifest when cells replicate their DNA. It may be that dividing cells in the ocular surface proliferate more slowly than those in the skin. If this is the case, there would be more time before DNA replication for NER (which is unaffected in XP-V and only reduced to 50% of normal in XP-E) to repair DNA damage in the eye than in the skin. This hypothesis is entirely speculative and much more work needs to be done to understand the nature and origin of UVR-induced ocular surface problems.

XP-F is one of the rarer XP groups and only about 30 cases have been described in the literature (45, 46). All three of our patients have severe sunburn on minimal sun exposure, but they have minimal exposed-site lentiginos and no skin cancers. In fact, very few XP-F patients have developed skin cancers [see table 3 in Gregg et al. (45), but note that contrary to what is indicated in this table, XP24BR and XP32BR have not had any skin cancers]. The few XP-F patients that have developed skin cancers did so as adults (47, 48), mostly above the age of 40 y (46, 48–50). Nevertheless it appears that, whereas XP-C patients have pigmentary changes and skin cancers without abnormally severe sunburn

reactions, XP-F patients, in direct contrast, have severe sunburn reactions with only modest pigmentary changes and relatively few skin cancers. These observations provide further insight into the function of the XPF protein, and it will be of interest to determine in future studies whether *XPF* mutations do in some ways protect against the anticipated skin cancers.

Among our XP-G patients, as with XP-F, we observed neurological abnormalities of varying severity associated with acute sunburn reactions, but just one skin cancer and little evidence of premalignant skin lesions. A survey of the literature does indeed suggest that skin cancers are rare in this group (51). Many of the severely affected cases with XP-CS died at a very young age, before any cancers had time to develop. Three Japanese XP-G patients developed skin cancers, but only at ages 40, 54, and 32 y, respectively (52–54).

In summary, detailed clinical and molecular analysis, together with functional assay of a large cohort of XP patients by the same multidisciplinary team, has enabled us to provide improved clinical management of patients with this DNA repair disorder. Furthermore, insights gained from previously unreported genotype-phenotype relationships have enabled us in many cases to make prognostic predictions about the likelihood of the development of neurological abnormalities, which is a major concern to patients who control their skin problems by rigorous photo-protection.

The importance of early diagnosis of XP cannot be over-emphasized, particularly for patients with normal sunburn reactions for skin type (XP-C, -E, and variant groups), in whom overt skin signs may not become apparent for several years. XP should be considered as a differential diagnosis in any patient who has unusually early or exposed-site patterns of freckling, so that UVR protection can be instigated as early as possible, to improve morbidity and mortality from skin cancer.

Methods

Systematic analysis of all patients' notes was undertaken to assess more than 60 different genotypic and phenotypic variables. The clinical studies have been carried out in great detail; however, to simplify the data for the reader and to make objective comparisons between the severity of different phenotypic data, we used three severity scoring systems (described in *SI Methods*). For cellular analyses, UDS and UV+caffeine sensitivity for XP-V cells were carried out with cultured skin fibroblasts as described previously (11, 55). Mutations in cDNA and genomic DNA were analyzed as described in *SI Methods*. Informed consent was obtained from all patients, and this study was performed in accordance with protocols approved by the Research Ethics Committee of Guy's and St Thomas' Foundation Trust (reference 12/LO/0325).

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