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# A Distinct Genotype of XP Complementation Group A: Surprisingly Mild Phenotype Highly Prevalent in Northern India/Pakistan/Afghanistan

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Xeroderma pigmentosum (XP) is a rare inherited disorder of DNA repair. Affected individuals cannot repair ultraviolet radiation (UVR)–induced DNA damage, resulting in an increased skin cancer risk (Bradford et al., 2011), severe sunburn in approximately 50% of patients (Sethi et al., 2013), and progressive neurodegeneration in approximately 30% (Kraemer et al., 1987; Totonchy et al., 2013). XP can result from defects in any of eight genes (*XPA–XPG* and *POLH*). *XPA–XPG* are involved in nucleotide excision repair (NER) of DNA damage (Cleaver et al., 2009).

Xeroderma pigmentosum complementation group A (XP-A) patients usually have a severe phenotype, with exaggerated sunburn and early onset of progressive neurodegeneration, which results in death, usually in the second or third decade (Anttinen et al., 2008). XPA protein is required for damage verification in the NER pathway. More than 20 different mutations have been identified in the *XPA* gene (States et al., 1998; Takahashi et al., 2010). Many of the reported cases come from Japan because of a founder mutation (c.390-1G>C) carried by 1% of the Japanese population (Hirai et al., 2006; Satokata et al., 1990). This mutation results in abnormal splicing of mRNA and subsequent production of truncated, nonfunctioning XPA protein and the typically severe clinical phenotype.

Although a diagnosis of XP-A has usually been associated with a poor prognosis, a number of XP-A patients undergoing long-term follow-up at the UK National XP Clinic have a surprisingly mild phenotype. To examine this finding further, a detailed genotype-phenotype study in this cohort was conducted. Neurological analysis included audiometry, nerve conduction studies, brain magnetic resonance imaging and neuropsychometric evaluations. Informed written consent was obtained from all patients. The study was performed in accordance with protocols approved by the Research Ethics Committee of Guy's and St. Thomas' Hospitals NHS Foundation Trust, London (reference 12/LO/0325).

Nineteen of 90 patients being studied at the UK National XP clinic were assigned to complementation group A (Table 1). Twelve of these patients, from eight consanguineous families, displayed a mild XP-A phenotype with no ocular surface disease, delayed onset or lack of skin cancer, and normal neurological and neuropsychometric evaluations (Figure 1a–h). Mean age at assessment was 32 years (range 6–79 years) and mean age at clinical diagnosis was 26 years (range 4–46 years), significantly higher than in the more severely affected XP-A group of patients, who showed progressive neurodegeneration presenting as developmental delay and cognitive impairment, sensorineural hearing loss, microcephaly, neuropathy, and cerebellar signs (Table 1). Remarkably, one of the patients, XP1CB, is aged 79 years without any XP-related neurological problems. He spent the first 30 years of his life in India working mostly outdoors and was only diagnosed clinically at age 46 years. These 12 patients all were homozygous for the mutation c.555+8A>G, which previously was reported by Sidwell et al. (2006) in a 61-year-old Punjabi woman with no neurological problems. All 12 patients included in this study, as well as the case described by Sidwell et al., originate from a 950-km stretch of land

*Abbreviations: NER, nucleotide excision repair; UVR, ultraviolet radiation; XP, xeroderma pigmentosum; XP-A, xeroderma pigmentosum complementation group A*

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**Table 1. Summary of clinical features in the XP-A patient cohort**

| XP number | Age (sex) | Country of origin | Age at clinical diagnosis | Cutaneous features | SSS | Developmental/ neuropsychometric, and neurological evaluation | Age at first mucocutaneous cancer (type) | Mutation in *XPA* gene |
|---|---|---|---|---|---|---|---|---|
| XP9BI | 6 (F) | Pakistan | 6 | Lentigines | 0 | Normal | | c.555+8A>G |
| XP103BR | 7 (F) | Pakistan | 4 | Lentigines | 0 | Normal | | c.555+8A>G |
| XP53BR | 18 (M) | Pakistan | 7 | Lentigines/photosensitivity | 1 | Normal | | c.555+8A>G |
| XP121BR | 25 (F) | Pakistan | 25 | Lentigines | 1 | Normal | | c.555+8A>G |
| XP116BR | 31 (M) | India | 31 | Lentigines | 1 | Normal | 31 (BCC) | c.555+8A>G |
| XP2PR | 32 (F) | Pakistan | 32 | Lentigines | 1 | Normal | | c.555+8A>G |
| XP89BR-S | 34 (M) | Pakistan | 33 | Lentigines | 2 | Normal | | c.555+8A>G |
| XP1PR | 35 (M) | Pakistan | 35 | Lentigines | 2 | Normal | | c.555+8A>G |
| XP88BR | 36 (M) | Pakistan | 31 | Lentigines | 3 | Normal | | c.555+8A>G |
| XP49BR | 38 (M) | Afghanistan | 24 | Lentigines/photosensitivity | 1 | Normal | | c.555+8A>G |
| XP89BR | 43 (F) | Pakistan | 39 | Photosensitivity | 3 | Normal | | c.555+8A>G |
| XP1CB | 79 (M) | India | 46 | Lentigines | 0 | Normal | 46 (MM) | c.555+8A>G |
| XP111BR | 7 (F) | Bangladesh | 5 | Lentigines | 3 | Abnormal | | c.253C>T p.(Gln85TER) |
| XP57BR | 14 (F) | Bangladesh | 1 | Photosensitivity | 1 | Abnormal | | c.640dupA p.(Met214fs) |
| XP80BR | 14 (F) | Somalia | 8 | Photosensitivity | 3 | Abnormal | | c.314G>A p.(Cys105Tyr) |
| XP81BR | 18 (M) | Somalia | 12 | Photosensitivity | 1 | Abnormal | | c.314G>A p.(Cys105Tyr) |
| XP15BR | 22 (M) | UK | 0.5 | Photosensitivity | 3 | Abnormal | | c.266_267dupAA p.(Val90fs) |
| XP114BR | 24 (M) | Pakistan | 22 | Photosensitivity | 3 | Abnormal | 22 (SCC) | c.682C>T p.(Arg228TER) |
| XP20BR | 32 (M) | Pakistan | 13 | Photosensitivity | 3 | Abnormal | 22 (ocular CIN3) | c.682C>T p.(Arg228TER) |

Abbreviations: BCC, basal cell carcinoma; CIN, conjunctival intraepithelial neoplasia; F, female; M, male; MM, malignant melanoma; SCC, squamous cell carcinoma; SSS, sunburn severity score (Sethi et al.,2013); XP, xeroderma pigmentosum; XP-A, xeroderma pigmentosum complementation group A.

around the Northern India/Pakistan/Afghanistan borders (Figure 1i), suggesting a founder effect present in this population.

The c.555+8A>G mutation at the eighth nucleotide of intron 4 generates a new splice donor site and results in aberrant splicing of intron 4 and nonfunctional, truncated XPA protein. However, there is a small amount of normally spliced mRNA (Sidwell et al., 2006), which results in production of residual normal XPA protein detectable in immunoblots (Figure 1j). Comparison of the upper XPA band in lanes 9–12 with the calibration in lanes 1–6 suggests that 50-μg extract from the mild XP-A cells has the same amount of (or less) XPA protein as 2.5-μg normal extract (lane 2), indicating the presence of <5% of the normal level of XPA protein in the mild XP-A cells. In contrast, no protein is detectable in the XP-A null cell line XP15BR (lanes 1 and 13). This residual protein carries out NER, consistent with the 5–15% of normal unscheduled DNA synthesis found in these patients (Figure 1k). This most likely explains their normal neurological phenotype. It has been shown that low levels of XPA protein, transfected into XPA-deficient cells, are able to significantly protect against DNA damage (Muotri et al., 2002).

Interestingly, the sunburn reactions in this group are variable, even though all patients are of similar ethnicity. This may be explained by the fact that the very small amount of functioning XPA protein may not be sufficient to repair the high level of photoproduct accumulation after sun exposure, resulting in moderate sunburn severity. However, endogenous neurological damage is likely to be generated continually at a low rate so that the low level of functioning XPA protein may be sufficient to repair the damage as it occurs, resulting in a normal neurological phenotype.

A handful of other XP-A patients with mild phenotype have been reported in the literature, although none as mild as our cohort. Four middle-aged Japanese XP-A patients presented with late-onset neurological impairment and moderate sunburn reactions, without development of skin cancer (Takahashi et al.,

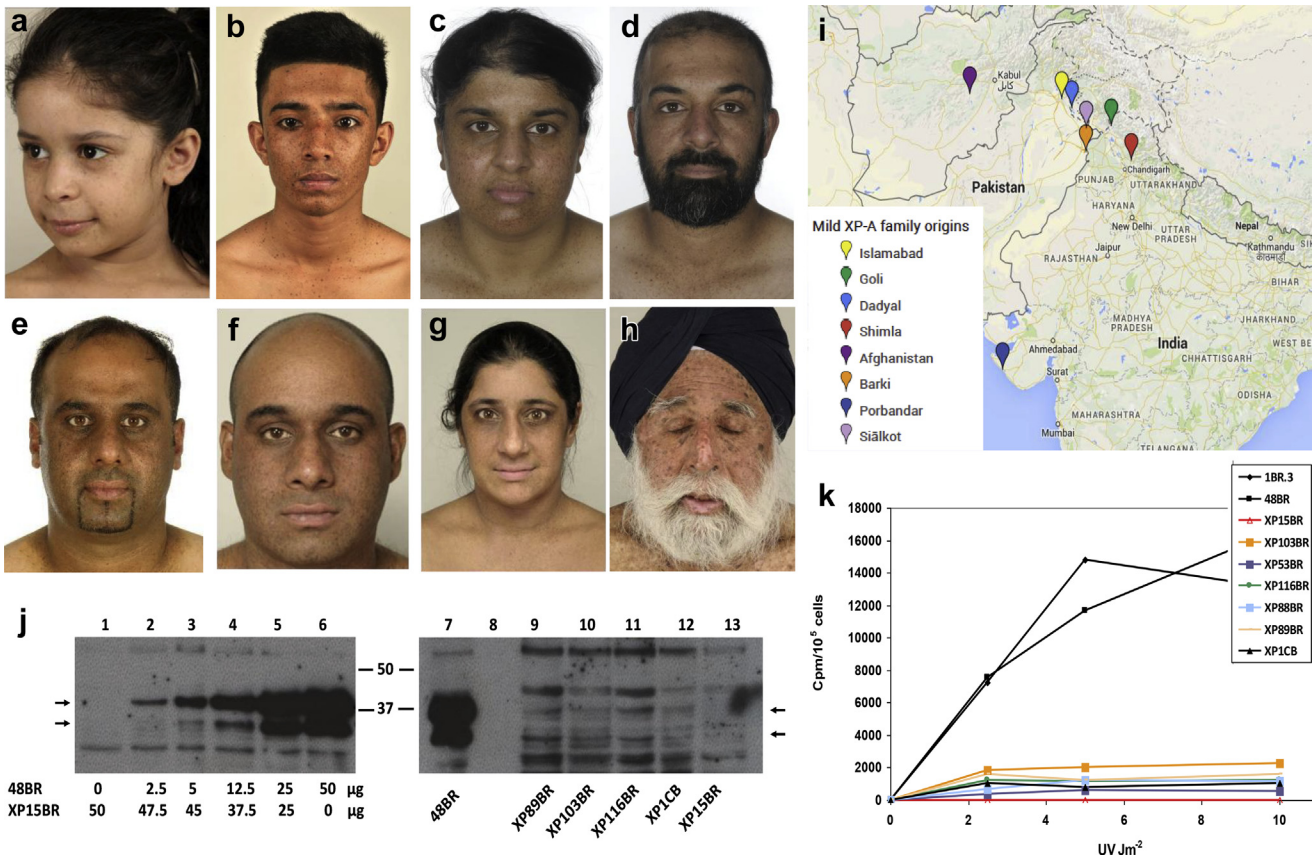


**Figure 1. Face views of mild xeroderma pigmentosum complementation group A (XP-A) patients and map of origins, immunoblots of protein extracts probed with monoclonal antibody to XPA protein, and measurement of UDS in mild XP-A and control cell lines.** (**a**) XP103BR: Seven-year-old girl with a few facial lentigines (distantly related to patients shown in e–g). (**b**) XP53BR: Eighteen-year-old man who presented with sunburn lasting 1 week and increased lentigines at exposed sites. (**c, d**) XP2PR, XP1PR: Two siblings aged 32 and 35 years, respectively, who developed facial lentigines at age 2 years (distantly related to patients shown in e-g). (**e**–**g**) XP89BR-S, XP88BR, XP89BR: Three siblings, currently aged 34, 36, and 43 years, respectively, who presented with increased facial lentigines and easy sunburn. (**h**) XP1CB: Seventy-nine–year-old man who developed lentigines at exposed sites at age 6 years. Of the seven other siblings in his family, four have XP. Until 30 years of age he had worked outdoors as a veterinarian in India, with high cumulative ultraviolet radiation exposure. He then moved to the United Kingdom and worked indoors as a pathologist until his retirement. He developed melanoma in situ on his left cheek at age 46 years and since then has developed 8 melanomas and >20 nonmelanoma skin cancers. He underwent a left hemicolectomy for a sigmoid colon adenocarcinoma Dukes B at age 55 years, followed by further surgery for mucinous adenocarcinoma Dukes B2 the following year. He subsequently developed keratoacanthomas and a sebaceous adenoma, leading to a diagnosis of Muir-Torre syndrome [mutation c.306G>T in *MLH-1* (Thompson et al., 2014)] unrelated to his mutation in the *XPA* gene. (**i**) Origins of the eight families with mild XP-A are indicated on the map. (**j**) Immunoblots of protein extracts probed with monoclonal antibody to XPA protein (BD Bioscience, Oxford, UK, #556453). Left: Calibration: The 50-µg protein extract is made up of the indicated quantities from normal 48BR cells and XPA-null XP15BR cells. Right: The 50 µg of extract from the indicated cells. The positions of the 50-kDa and 37-kDa markers are indicated between the panels. XPA protein, indicated with arrows, runs as two bands on either side of the 37-kDa marker. Normal XPA protein level of ≤5% is detected in the mild XP-A cases but not in XP15BR. (**k**) UDS measured by incorporation of $^{3}$H-thymidine into nondividing cells after ultraviolet-C irradiation with the indicated doses. From 5% to 15% of normal UDS is detected in cells from the mild XP-A patients but is undetectable in XP15BR. UDS, unscheduled DNA synthesis. All patients pictured here have provided written and oral consent for publication of these photographs.

2010). Their milder phenotype was attributed to frameshift mutations in exon 6 [c.689dupT p.(Arg231fs) in one patient and c.779delCinsTTCTT p.(Thr260fs) in the other three] resulting in truncated XPA proteins.

This study highlights the importance of genotype-phenotype correlations in XP, not only for diagnosis but also for prognosis and genetic counseling. Here we present 12 XP-A patients with variable sunburn reactions and normal cognitive and neurological phenotype, the largest number of mild XP-A patients reported to date. Although the milder skin phenotype may be related to some extent to the more pigmented Fitzpatrick skin types, in our cohort of patients, homozygous for c.555+8A>G, we are now able to give cautiously optimistic prognostic information with regard to lack of neurodegeneration and later onset of skin cancer. A diagnosis of mild XP-A should be considered in individuals with facial lentigines, originating from the borders of Northern India, Pakistan, and Afghanistan, as early photoprotection can reduce further development of lentigines and potential skin cancers. Our findings from a relatively small immigrant population in the United Kingdom imply that there may be many such individuals in the area of origin, who are likely to be undiagnosed because of the mildness of symptoms and who may suffer from excessive skin damage later in life.

## CONFLICT OF INTEREST
The authors state no conflict of interest.

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***Mieran Sethi*[1,2], *Shaheen Haque*[3], *Heather Fawcett*[4], *Jonathan F. Wing*[4], *Natalie Chandler*[5], *Shehla Mohammed*[5], *Ian M. Frayling*[6], *Paul G. Norris*[3], *David McGibbon*[2], *Antony R. Young*[1], *Robert P.E. Sarkany*[2], *Alan R. Lehmann*[4] and *Hiva Fassihi*[2,*]**

*[1]King's College London, Kings Health Partners, Division of Genetics and Molecular Medicine, St. John's Institute of Dermatology, Guy's Hospital, London, United Kingdom; [2]National Xeroderma Pigmentosum Service, Department of Photodermatology, St. John's Institute of Dermatology, Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom; [3]Department of Dermatology, Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, United Kingdom; [4]Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton, United Kingdom; [5]Genetics Department, Guy's and St. Thomas' NHS Foundation Trust, London, United Kingdom; and [6]Institute of Medical Genetics, University Hospital of Wales, Cardiff, United Kingdom*
*[*]Corresponding author e-mail: hiva.fassihi@gstt.nhs.uk*

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*See related commentary on pg 742*

# Meganuclease-Mediated *COL7A1* Gene Correction for Recessive Dystrophic Epidermolysis Bullosa

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## TO THE EDITOR

Dystrophic epidermolysis bullosa (DEB) is a rare and severe genetic skin disease inherited in a dominant (DDEB) or recessive (RDEB) manner, responsible for blistering of the skin and mucosa after mild trauma (Bruckner-Tuderman, 2010; Uitto et al., 1992). DEB is caused by a wide variety of mutations in *COL7A1* encoding type VII collagen, the major component of anchoring fibrils which form key attachment structures for dermal-epidermal adhesion (Hovnanian et al., 1997; Varki et al., 2007).

Gene correction approaches based on sequence-specific DNA double strand breaks (DSB)-mediated homology-directed repair (HDR) allow precise and accurate correction of mutations (Yanez-Munoz et al., 2006). They have the potential to restore stable expression and function of the defective gene and to reverse the disease phenotype

*Abbreviations: DDEB, dominant dystrophic epidermolysis bullosa; DSB, double strand breaks; HDR, homology-directed repair; IDLVs, integration-deficient lentiviral vectors; MNs, meganucleases; RDEB, recessive dystrophic epidermolysis bullosa*

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