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

M. Sethi¹, S. Haque², H. Fawcett³, J. F. Wing³, N. Chandler⁴, S. Mohammed⁴, I. M. Frayling⁵, P. G. Norris², D. McGibbon⁶, A. R. Young¹, R. P. E. Sarkany⁶, A. R. Lehmann³, H. Fassihi⁶

¹King’s College London, Kings Health Partners, Division of Genetics and Molecular Medicine, St John’s Institute of Dermatology, Guy’s hospital, London, UK.
²Department of Dermatology, Addenbrooke’s Hospital, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK.
³Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton, UK.
⁴Genetics Department, Guy’s & St Thomas’ NHS Foundation Trust, London, UK.
⁵Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK.
⁶National Xeroderma Pigmentosum Service, Department of Photodermatology, St John’s Institute of Dermatology, Guy’s and St Thomas’ NHS Foundation Trust, London, UK

Corresponding author: Dr Hiva Fassihi, Consultant Dermatologist and Clinical Lead for the UK National XP Service, St John’s Institute of Dermatology, 1st Floor Counting House, Guy’s Hospital, London SE1 9RT, UK

Tel: +44 (0)207 188 0847 Fax: +44 (0)207 188 8907 Email: hiva.fassihi@gstt.nhs.uk

Short title: XP complementation group A with a mild phenotype

Abbreviations:

XP xeroderma pigmentosum
UVR ultraviolet radiation
NER nucleotide excision repair
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 (XP-A to XP-G and variant), XPA-XPG being involved in nucleotide excision repair (NER) of DNA damage (Cleaver et al., 2009).

XP-A patients, deficient in XPA protein, 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. Over 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 results in abnormal splicing of mRNA and subsequent production of truncated, non-functioning XPA protein and the typically severe clinical phenotype.
Although a diagnosis of XP-A has usually been associated with poor prognosis, a number of XP-A patients, under 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. Specifically, patients were examined by specialists, and underwent audiometry, nerve conduction studies, brain MRI scans, and neuropsychometric evaluations. 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’ Hospitals NHS Foundation Trust, London (reference 12/LO/0325).
Nineteen out of 90 patients under study at the UK National XP clinic were assigned to complementation group A (Table 1). Twelve of these, 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). Their mean age at assessment was 32 years (age range 6 to 79 years) and mean age at clinical diagnosis was 28 years (range 4 to 46 years), significantly higher than the more severely affected XP-A group with progressive neurogeneration 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 thirty years of his life in India working largely outdoors and was only diagnosed clinically aged 46 years. These 12 patients were all homozygous for the mutation c.555+8A>G, which has been previously reported by Sidwell et al., 2006 in a 61 year-old Punjabi woman with no neurological problems. All 12 patients included here, as well as the case described by Sidwell et al. originate from a 950km stretch of land around the Northern India/ Pakistan/ Afghanistan borders, suggesting a founder effect present in this population (Figure 1I).
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 non-functional truncated XPA protein. However, there is a small amount of normally spliced mRNA (Sidwell et al., 2006), which results in production of detectable residual normal XPA protein 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 as or less XPA protein than 2.5 µg normal extract (lane 2), indicating the presence of less than 5% of XPA protein in the mild XP-A cells. In contrast no protein is detectable in XP-A null cell line XP15BR (lanes 1 and 13). This residual protein carries out NER, consistent with the 5-15% of normal UDS found in these patients (Figure 1K). 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). This most likely explains their normal neurological phenotype.

Interestingly, the sunburn reactions in this group were variable, despite all patients being 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 and therefore the low level of functioning XPA protein may be sufficient to repair the damage as it forms, resulting in a normal neurological phenotype.
There has been a handful of other mild XP-A patients 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., 2010). Their milder phenotype was attributed to frameshift mutations in exon 6 (c.689dupT p.(Arg231fs) in one patient, c.779delCinsTTCTT p.(Thr260fs) in the other three) producing truncated XPA proteins.

This study highlights the importance of genotype-phenotype correlations in XP, not only for diagnosis but also for prognosis and genetic counselling. In this paper 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 to some extent related to their 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 regards 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 UK, 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 may suffer from excessive skin damage later in life.

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References


Figure legend

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 hemicolecetomy 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 3H-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.
Table 1. Summary of clinical features in XP-A patient cohort

<table>
<thead>
<tr>
<th>XP number</th>
<th>Age (Sex)</th>
<th>Country of Origin</th>
<th>(^2) Age at XP diagnosis</th>
<th>SSS</th>
<th>Cutaneous features</th>
<th>Developmental/neuropsychometric and neurological evaluation</th>
<th>Age 1st mucocutaneous cancer (type)</th>
<th>Mutation in XPA gene</th>
</tr>
</thead>
<tbody>
<tr>
<td>XP9BI</td>
<td>6 (F)</td>
<td>Pakistan</td>
<td>6</td>
<td>0</td>
<td>Lentigines</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP103BR</td>
<td>7(F)</td>
<td>Pakistan</td>
<td>4</td>
<td>0</td>
<td>Lentigines</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP53BR</td>
<td>18(M)</td>
<td>Pakistan</td>
<td>7</td>
<td>1</td>
<td>Lentigines / Photosensitivity</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP121BR</td>
<td>25 (F)</td>
<td>Pakistan</td>
<td>25</td>
<td>1</td>
<td>Lentigines</td>
<td>Normal</td>
<td>31 (BCC)</td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP116BR</td>
<td>31(M)</td>
<td>India</td>
<td>31</td>
<td>1</td>
<td>Lentigines</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP12PR</td>
<td>32(F)</td>
<td>Pakistan</td>
<td>32</td>
<td>1</td>
<td>Lentigines</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP89BR-S</td>
<td>34(M)</td>
<td>Pakistan</td>
<td>33</td>
<td>2</td>
<td>Lentigines</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP1PR</td>
<td>35(M)</td>
<td>Pakistan</td>
<td>35</td>
<td>2</td>
<td>Lentigines</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP88BR</td>
<td>36(M)</td>
<td>Pakistan</td>
<td>31</td>
<td>3</td>
<td>Lentigines</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP49BR</td>
<td>38(M)</td>
<td>Afghanistan</td>
<td>24</td>
<td>1</td>
<td>Lentigines / Photosensitivity</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP89BR</td>
<td>43(F)</td>
<td>Pakistan</td>
<td>39</td>
<td>3</td>
<td>Photosensitivity</td>
<td>Normal</td>
<td></td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP1CA</td>
<td>79(M)</td>
<td>India</td>
<td>67</td>
<td>0</td>
<td>Lentigines</td>
<td>Normal</td>
<td>46 (MM)</td>
<td>c.555+8A&gt;G</td>
</tr>
<tr>
<td>XP111BR</td>
<td>7(F)</td>
<td>Bangladesh</td>
<td>5</td>
<td>3</td>
<td>Lentigines</td>
<td>Abnormal</td>
<td></td>
<td>c.253C&gt;T  p.(Gln85STER)</td>
</tr>
<tr>
<td>XP57BR</td>
<td>14(F)</td>
<td>Bangladesh</td>
<td>1</td>
<td>1</td>
<td>Photosensitivity</td>
<td>Abnormal</td>
<td></td>
<td>c.640dupA  p.(Met214fs)</td>
</tr>
<tr>
<td>XP80BR</td>
<td>14(F)</td>
<td>Somalia</td>
<td>8</td>
<td>3</td>
<td>Photosensitivity</td>
<td>Abnormal</td>
<td></td>
<td>c.314G&gt;A  p.(Cys105Tyr)</td>
</tr>
<tr>
<td>XP81BR</td>
<td>18(M)</td>
<td>Somalia</td>
<td>12</td>
<td>1</td>
<td>Photosensitivity</td>
<td>Abnormal</td>
<td></td>
<td>c.314G&gt;A  p.(Cys105Tyr)</td>
</tr>
<tr>
<td>XP15BR</td>
<td>22(M)</td>
<td>UK</td>
<td>0.5</td>
<td>3</td>
<td>Photosensitivity</td>
<td>Abnormal</td>
<td></td>
<td>c.266_267dupAA  p.(Val90fs)</td>
</tr>
<tr>
<td>XP114BR</td>
<td>24(M)</td>
<td>Pakistan</td>
<td>22</td>
<td>3</td>
<td>Photosensitivity</td>
<td>Abnormal</td>
<td>22 (SCC)</td>
<td>c.682C&gt;T  p.(Arg228TER)</td>
</tr>
<tr>
<td>XP20BR</td>
<td>32(M)</td>
<td>Pakistan</td>
<td>13</td>
<td>3</td>
<td>Photosensitivity</td>
<td>Abnormal</td>
<td>22 (ocular CIN3)</td>
<td>c.682C&gt;T  p.(Arg228TER)</td>
</tr>
</tbody>
</table>

SSS: Sunburn severity score (Sethi et al., 2013), BCC: basal cell carcinoma, MM: malignant melanoma, SCC: squamous cell carcinoma, CIN: conjunctival intraepithelial neoplasia.
Age of diagnosis by cellular measurement of defective NER or identification of pathogenic mutation(s). Clinical diagnosis may have been earlier.