Xeroderma Pigmentosum Groups C and F: Additional Assignments and A Review of the Subjects in Japan

YOSHISADA FUJIWARA1, MASAMITSU ICHIHASHI2, YOSHIHIKO UEHARA1, AKIRA MATSUMOTO1, YOKO YAMAMOTO1, YOSHIO KANO1 and YOSHIMASA TANAKURA1

1Department of Radiation Biophysics and 2Department of Dermatology, Kobe University School of Medicine, Kobe 650, Japan

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The differential bead-labelling method for heterodikaryon complementation enabled us to allocate xeroderma pigmentosum patients XP40KO and XP38KO to complementation groups C and F, respectively. Group C XP40KO cells exhibited 15% UDS and the 5 and 1.5-fold hypersensitivities to UV and 4-nitroquinoline-1-oxide (4NQO) killings, respectively, while group F XP38KO cells showed a higher residual level of 20–25% UDS and a less UV/4NQO hypersensitivity than did the previously assigned group F strains. We reviewed the thus far assigned Japanese groups C and F subjects for the repair and clinical characteristics. The present group C XP40KO was typical, while XP38KO was heterogeneous within group F with respect to repair.

INTRODUCTION

Autosomal recessive XP is a high skin cancer-susceptible disorder, now consisted of 9 complementation groups (A through I) and a variant form (for the latest review, ref. 1; for group I, ref. 2). Takebe’s survey3,4 of XP patients in Japan until 1982 disclosed their genetic characteristics, differing from those in the USA, Europe and Egypt, that many patients belonged to groups A and variant, while only 4, 1 and 4 patients to groups C (most common in the US and Europe), D, and F (only in Japan) respectively without allocations to rare groups B, E and G. In 1983 Moshell et al.5 found a single group H patient (GM3248) with Cockayne syndrome, and group I was found in 19852. Our subsequent complementation tests for new entries supplemented 2 and 4 more subjects to rare groups D6 and F7 respectively, with new assignments of 2 group E8 and 1 group G9 patients in Japan.

This short report further describes (i) additional allocations of a group C, XP40KO and a
Table 1. Repair, survival and clinical phenotypes of Japanese XP groups C and F subjects so far reported.

<table>
<thead>
<tr>
<th>Patient (cell strain)</th>
<th>Age&lt;sup&gt;b&lt;/sup&gt;, sex</th>
<th>UDS (%)</th>
<th>UV survival&lt;sup&gt;c&lt;/sup&gt;</th>
<th>4NQO survival&lt;sup&gt;g&lt;/sup&gt;</th>
<th>Clinical phenotype</th>
<th>Parental relation</th>
<th>Affected sibs</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n Do (J/m²)</td>
<td>n Do (µM)</td>
<td>Acute sun sensitiv.</td>
<td>Dermal symptom</td>
<td>Skin cancers&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Neuro-mental defects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Complementation group C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XP2KA</td>
<td>5 F 10–16</td>
<td>1.0</td>
<td>1.0 0.080</td>
<td>+ Severe + (SCC)</td>
<td>–</td>
<td>Sibs</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>XP3KA</td>
<td>10 M 10–16</td>
<td>1.0</td>
<td>1.0 0.080</td>
<td>+ Severe + (SCC)</td>
<td>–</td>
<td>–</td>
<td>M. Inoue&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>XP4KA</td>
<td>21 F 20</td>
<td>f</td>
<td>nt –</td>
<td>Mild +</td>
<td>–</td>
<td>None</td>
<td>None</td>
<td>17</td>
</tr>
<tr>
<td>XP40OS</td>
<td>30 M 14&lt;sup&gt;e&lt;/sup&gt;</td>
<td>nt</td>
<td>nt +</td>
<td>Moderate + (BCE, KA)</td>
<td>–</td>
<td>None</td>
<td>None</td>
<td></td>
</tr>
<tr>
<td>XP40KO</td>
<td>11 F 10–15</td>
<td>1.0</td>
<td>1.0 0.080</td>
<td>+ Severe + (BCE)</td>
<td>–</td>
<td>1st cousin</td>
<td>None</td>
<td>This paper</td>
</tr>
<tr>
<td>Complementation group F</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XP23OS</td>
<td>45 F 10&lt;sup&gt;h&lt;/sup&gt;</td>
<td>1.0</td>
<td>1.7&lt;sup&gt;i&lt;/sup&gt;</td>
<td>Very mild –</td>
<td>–</td>
<td>–</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>XP2YO&lt;sup&gt;f&lt;/sup&gt;</td>
<td>65 F 10–15</td>
<td>1.2</td>
<td>1.7 1.0 0.043</td>
<td>Mild + (SCC)</td>
<td>–</td>
<td>–</td>
<td>14</td>
<td></td>
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<tr>
<td>XP3YO</td>
<td>29 M 10–15</td>
<td>1.2</td>
<td>1.7 1.0 0.043</td>
<td>Moderate –</td>
<td>–</td>
<td>3, 13, 17</td>
<td></td>
<td></td>
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<tr>
<td>XP101OS</td>
<td>49 F k</td>
<td>k</td>
<td></td>
<td>Mild + (BCE, KA)</td>
<td>–</td>
<td>–</td>
<td>21</td>
<td></td>
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<tr>
<td>XP25KO</td>
<td>8 F 10–15</td>
<td>1.2</td>
<td>1.7 1.0 0.043</td>
<td>+ Mild –</td>
<td>–</td>
<td>None</td>
<td>None</td>
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<tr>
<td>XP27KO</td>
<td>11 F 10–15</td>
<td>1.2</td>
<td>1.7 1.0 0.043</td>
<td>+ Mild –</td>
<td>–</td>
<td>–</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>XP28KO</td>
<td>8 F 10–15</td>
<td>1.2</td>
<td>1.7 1.0 0.043</td>
<td>+ Mild –</td>
<td>–</td>
<td>2nd cousin</td>
<td>Sibs</td>
<td>7</td>
</tr>
<tr>
<td>XP41KO</td>
<td>5 M Biopsy not available</td>
<td></td>
<td></td>
<td>Mild –</td>
<td>–</td>
<td>–</td>
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<td></td>
</tr>
<tr>
<td>XP38KO</td>
<td>44 F 20–25</td>
<td>1.7</td>
<td>2.2 1.0 0.080</td>
<td>+ Very mild –</td>
<td>–</td>
<td>None</td>
<td>1</td>
<td>This paper</td>
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<tr>
<td>Normals</td>
<td>100</td>
<td>1.5</td>
<td>5.0 1.3 0.130</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>-Open frames mean "not sure" or "not presented in the literature"; <sup>b</sup>-Age at skin biopsy; <sup>c</sup>-From the present Figures 2 and 3 and from our unpublished survival curves; <sup>d</sup>-SCC, squamous cell carcinoma, BCE–basal cell epithelioma, KA–keratoacanthoma; <sup>e</sup>-Personal communication; <sup>f</sup>-Post-UV colony-forming ability was said to be the same as that of XP2KA (M. Inoue, person. commun.); g-From ref. 20, h-From ref. 12, i-Our estimation from the UV survival curve in ref. 12, j-XP2YO is a sister of the grandfather of XP3YO (ref. 17), k-UDS and UV hypersensitivity was said to be similar to those of XP2YO, l-XP38KO appears to have 2 more affected among 7 sibs.
group F, XP38KO, and (ii) the present status and characteristics of Japanese patients belonging to groups C and F.

**MATERIALS AND METHODS**

The 2 strains, XP38KO and XP40KO (see Table 1) for test, of the Kobe University XP Registry and the reference strains of normal NHSF6 and XP groups A through H except unavailable group B (see Fig. 1 legend) were used. The complementation analysis following cell fusion and specific heterodikaryon identification from the test and reference homodikaryons by differential Latex-bead labelling has been described in detail previously\(^6\) and is briefly

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**Fig. 1.** Complementation tests. The XP38KO and XP40KO cells prelabelled with large Latex beads (1.09 µm) and the reference strains (normal (N), NHSF6; group A, XP6KO; C, XP3KA; D, XP59TO; E, XP2RO; F, XP2YO; G, XP2BI; H, GM3248; group B, no longer available)\(^6\) with small beads (0.48 µm) were fused with 45% polyethylene glycol. Cells were sparsely spread 2 h later, incubated for 24 h, UV-irradiated with 10 J/m², and reincubated in 10 µCi/ml of \(^{3}H\) thymidine (53 Ci/m mole, Amersham) for an additional 3 h. Autoradiography was made with a Sakura NR-M2 liquid emulsion. Mean grains per nucleus of dikaryons (background < 1.21 grains/cell) for a measure of UDS were estimated from 50 cells each of heterodikaryons with both large and small beads, test-cell homodikaryons with only large beads and reference-cell homodikaryons with only small beads. (A) XP40KO, (B) XP38KO. Closed bar, heterodikaryons; shaded bar, test-cell homodikaryons; open bar, reference-cell homodikaryons. In Figure only, the cell-strain prefixes (NH, XP, GM) were omitted for short designations. The bar of histogram indicates standard deviation. The various XP homodikaryons show their own low levels of UDS, relative to the NHSF6/NHSF6 homodikaryons.
presented in Figure 1 legend. Absolute and relative amounts of UV-induced UDS in heterodikaryons and homodikaryons were also determined (see Fig. 1). Clonogenic UV and 4NQO (4-nitroquinoline-1-oxide) survival curves were assayed for the XP strains and analyzed by extrapolation number ($n$) and mean lethal dose ($D_0$).

RESULTS AND DISCUSSION

Figure 1A shows the complementation results for XP40KO. Only the XP40KO/XP3KA(C) heterodikaryons failed to give normal level of UDS, despite sufficient complementation (90–108% UDS) in all other heterodikaryons between XP40KO and the reference XP groups A, D, E, F, G and H. Thus, we can allocate XP40KO to group C. Both XP40KO (test) and XP3KA (reference) cells showed 15% UDS, falling into the ordinary group C range of 10–25% UDS$^{1, 10}$. Figure 2A indicates that, based on the $D_0$ comparison, XP40KO and XP3KA cells were 5 times as sensitive to UV ($n=1$, $D_0=1.0 \text{ J/m}^2$) as normal cells ($n=1.5$, $D_0=5.0 \text{ J/m}^2$) and twice less sensitive than group A XP6KO cells ($n=1$, $D_0=0.4 \text{ J/m}^2$). However, both group C strains showed only 1.5 times higher sensitivity to 4NQO killing ($n=1$, $D_0=0.08 \mu\text{M}$) than did normal cells ($n=1.3$, $D_0=0.13 \mu\text{M}$), although group A XP6KO was extremely hypersensitive (Fig. 2B). Such

![Graph](image-url)

**Fig. 2.** Survival curves of XP40KO cells. The assay method for UV and 4NQO survivals has been described elsewhere$^4$ (4NQO treatment was for 1 h at 37°C in phosphate-buffered saline). (A) 254 nm UV, (B) 4NQO. ○, normal (mean of 3 strains); · · · · · , group A XP6KO: $n=1$, $D_0=0.4 \text{ J/m}^2$ for UV; $n=1$, $D_0=0.01 \mu\text{M}$ for 4NQO; ●, reference group C XP3KA; •, XP40KO. Each point was a mean of 3 determinations (SD ≤ 4.3% of mean). The $n$ and $D_0$ values were described in the text except for XP6KO.
killing results with XP3KA and XP6KO are consistent with our previous data.\textsuperscript{11}

The similar complementation results (Fig. 1B) allowed us to assign XP38KO to group F, since it failed to complement only the reference group F, XP2YO. The XP38KO homokaryons from the 8 combination sets had 20–25% UDS, being higher than the usual group F level of 10–15%. Correspondingly, Figure 3A shows reproducibly a slightly less UV hypersensitivity of XP38KO (n=1.7, Do=2.2 J/m\textsuperscript{2}) than that of the previously assigned group F strains (XP2YO, XP25KO, XP27KO: n=1.2, Do=1.7 J/m\textsuperscript{2}). In Figure 3B, XP38KO cells were twice as resistant to 4NQO (n=1.0, Do=0.08 µM) as the 3 group F strains (n=1, Do=0.043 µM). Such an XP38KO response mimics that of group C (Fig. 2B).

Table 1 has compiled the repair, survival and clinical characteristics of the reported Japanese groups C and F patients (5 and 9 respectively). First, 4 of the 5 group C patients had commonly acute sunburn in childhood, severe skin lesions and skin cancers (100% at age between 5 and 20 years), but not neurologic defects. Clinically mild XP4KA is an exception. XP2KA, XP3KA and XP40KO group C cells were 5 and only 1.5 times more sensitive to UV and 4NQO respectively than normal (Fig. 2; Table 1), as described previously.\textsuperscript{11} Such differential response to UV and 4NQO of group C cells deviates from the identical supersensitivity to both agents of typical group A cells, which may lack the repair of both pyrimidine dimers and 4NQO-purine adducts.\textsuperscript{15} In XP cells, the extent of dimers remaining in DNA correlates

Fig. 3. Survival curves of XP38KO. (A) UV, (B) 4NQO. o, normal (see Fig. 2); --, group A XP6KO (see Fig. 2); A, mean of group F XP2YO, XP25KO and XP27KO; ■, XP38KO. Each was a mean of 3 determinations (SD ≤ 4.2% of mean). The Do value for UV of XP38KO is significantly different from those of the other group F strains (p < 0.05).
with UV hypersensitivity\textsuperscript{10, 11, 14}. Thus, the less lethal response to 4NQO than to UV of the group C cells may suggest the presence of substantial repair of 4NQO lesions, compared with much defective repair of pyrimidine dimers.

Regarding group F, the number of subjects has increased, and its occurrence has been still specific in Japan, except for 1 case (XP126LO) from England\textsuperscript{16}. All the group F patients manifest mild skin lesions without neuro-mental complications (Table 1), as originally described\textsuperscript{12, 14}. Skin cancers occurred in 2 out of 9 patients at relatively higher age (Table 1) due presumably to their repair ability at a half rate of normal\textsuperscript{7, 12-14}. Usually, the group F cells exhibit 10–15\% UDS after [\textsuperscript{3}H] thymidine labelling for the initial 3 h following UV. Such initial low UDS arises by normal slow repair due to the specific lack of early rapid repair\textsuperscript{7, 13, 14}. Group F cells are twice more 4NQO-sensitive than group C cells (Table 1). However, the present very mild XP38KO revealed a higher residual level of 20–25\% UDS and the group C-equivalent 4NQO resistance (Table 1), suggesting a heterogeneity within group F, as previously noted in groups A\textsuperscript{10, 16}, C\textsuperscript{18} and G\textsuperscript{19}.

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REFERENCES


