Clinical Characteristics, DNA Repair, and Complementation Groups in Xeroderma Pigmentosum Patients from Egypt

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ABSTRACT

Xeroderma pigmentosum (XP) has been reported to be unusually frequent among Middle Eastern populations. This report describes the first survey of DNA repair characteristics among Egyptians. Sixteen XP patients were contacted, and biopsies from eight were analyzed for unscheduled DNA synthesis, strand breakage during pyrimidine dimer excision, and complementation groups. The patients were equally distributed between Complementation Groups A and C. Unscheduled synthesis and strand breaks were significantly higher in Group C than in Group A cells. Central nervous system disorders were found in all of the Group A patients and in none of the Group C patients. No clinical symptoms were observed in the heterozygotes. A 2-month-old sib of an XP patient was free of symptoms, but unscheduled synthesis and strand breakage in cultures from this sib were the same as in the related XP homozygote. From the relative frequencies of each complementation group found in various parts of the world, we offer a hypothesis concerning the relative sizes and roles for gene products specified by the alleles or genes corresponding to each complementation group.

INTRODUCTION

In surveys of DNA repair and clinical characteristics of XP patients reported for the United States (41) and Japan (45), several common features of this human, hereditary, high-cancer disease have emerged. Distinct groups with reduced levels of excision repair of UV damage in DNA can be identified by somatic cell hybridization experiments and have been designated A, B, C, D, E, F, G, and variant (2, 7, 15, 21, 32, 44). One of the oldest and most comprehensive surveys of XP was made in Egypt (26, 27). In view of the large number of Egyptian cases, high frequency of consanguinity, the relatively uniform high levels of sunlight exposure, and the proposed linkage of XP with the ABO blood group system (27), we considered it worthwhile to conduct a biochemical investigation of a number of these patients. We report here observations of their clinical and familial features, DNA repair levels, complementation groups, and incision rates. In addition to characterizing the patients, we used a number of new approaches that may prove useful in subsequent investigations of DNA repair.

MATERIALS AND METHODS

Cell Culture. Skin biopsies of selected patients and one female heterozygote were taken in the Human Genetics Clinic, Ain-Shams University, Cairo, placed in tubes containing Eagle’s minimal essential medium with 10% calf serum, and delivered to the Institute for Medical Research, Camden, N. J., where they were established in culture. Homozygotes were designated XP1CA, XP2CA, etc., and the heterozygote was designated XPX4CA according to the convention proposed by Cleaver et al. (16) (CA represented Cairo, the city of origin). Once cultures were established, they were shipped to other laboratories for biochemical investigations. Normal human fibroblasts in current use in the respective laboratories were used for comparison.

Repair DNA Synthesis. Repair DNA synthesis was measured in confluent cell populations treated with Sendai virus, which contained a large number of homokaryons. After fusion, the cells were grown for 4 days in Ham’s F-10 medium with 7.5% fetal calf serum. Cultures were incubated for 30 min in this medium supplemented with 10 μM hydroxyurea and irradiated with 0 or 10 J UV per sq m as described previously (20). After irradiation, the cells were grown for 2 hr in F-10 medium containing 7.5% fetal calf serum, 10 μM hydroxyurea, and 10 μCi [3H]dThd per ml (specific activity, 18.5 Ci/mmol). The cells were lysed in 0.2 M NaOH, and the lysates were kept overnight at 4°C. The labeled DNA was precipitated with HCl, collected on Whatman GF/C filters, and counted by liquid scintillation. The protein content of the lysates were measured autoradiographically without hydroxyurea as described earlier (20).

In several experiments, unscheduled DNA synthesis was measured autoradiographically without hydroxyurea as described earlier (20).

Complementation Analysis. Cell cultures from different patients were fused with previously characterized XP cells of known complementation groups using inactivated Sendai virus (20). These cultures were grown and treated as described above. In a few experiments, autoradiography was used (20).

Inhibition of Sealing of Excision Gaps with ara-C. To measure the accumulation of incision events, cultures labeled by growth for 24 to 48 hr in 0.05 μCi [3H]dThd per ml were rinsed, irradiated with 0 to 13 J UV per sq m, and grown for up to 5 hr in ara-C (10 μM) and hydroxyurea (2 mM). Cells were then...
harvested, and approximately 10^6 cells in 0.5 ml were added to a 0.5-ml layer of 0.01 M EDTA:0.1 N NaOH on the top of 5 to 20% alkaline sucrose gradients in 36-ml tubes as described previously (18). DNA molecular weights (weight and number averages) were determined after centrifugation for 3 to 5 hr at 25,000 rpm in an SW27 rotor, fractionation, and scintillation counting as described elsewhere (17, 18). The number of breaks in DNA from cells grown in ara-C and hydroxyurea after irradiation were determined (18) for each cell type and expressed as a ratio relative to the normal cell type.

RESULTS

Clinical Information on Biopsied Patients and Relatives. We obtained biopsies from 8 patients and one mother, and within these 6 families, there were 7 other genetically related patients we did not sample. In these families, there was a total of 54 recorded children, of whom 15 were clinically diagnosed as having XP. Another XP case was recorded in a family distantly related to Family 6. When these numbers are corrected for ascertainment bias by the simple sib method (42), the frequency of homozygotes among sibs of the probands is 9 of 48, or 19%, in comparison with an expected 25% for a simple recessive disease. Because some of the children in the families we studied died within the first few years of life, we cannot determine whether any of these would have developed XP symptoms. The frequency of 19% is therefore a minimal estimate for our small population group. All of the patients investigated were young (2 months to 13 years) and exhibited sun-induced pigmentation and ocular and neoplastic symptoms characteristic of XP (Chart 1; Table 1; Refs. 14 and 41). In 2 families, the patients also had neurological disorders involving low IQ, dwarfed stature, and microcephaly characteristic of the De Sanctis-Cacchione syndrome (19). Marriage between relatives, a common cultural pattern in Egypt, is evident in our survey (Chart 1; Table 1). Only one of 6 families we investigated had no record of intermarriages (XP3CA), so that in 5 families we are certain of consanguineous origin for the patients' homozygosity. A sib (XP8CA) of an XP patient was 2 months old and exhibited erythema and a single large (2 to 5 mm in diameter) pigment patch on the bridge of the nose; this child proved to be an XP homozygote case. One-half of our families originated from the delta zone of lower Egypt and one-half originated from upper Egypt, regions that tend to be separate and where people marry within their groups.

Heterozygotes were without clinical symptoms, and one was biopsied for further investigation. Studies of United States families have shown no excessive incidence of cancers in heterozygotes in most families except for those with high levels of sun exposure in the South (43) and then only in about 10% of the heterozygotes studied. Therefore, despite the severe sun exposure in Egypt, our relatively small heterozygote sample size would not be expected to reveal significant increases in skin cancers (43).

Levels of DNA Repair and Complementation Analysis. DNA repair was measured by liquid scintillation counting in populations treated with Sendai virus 4 days before UV irradiation. At the time of irradiation, the cultures were confluent and con-
would have been missed, but these have not been observed in
of XP2CA, to the same group. Similarly, the assignment of
studied by hybridization; so cells with double or linked defects
XP6CA to Group C was confirmed in autoradiographic experi-
ments with XP7CA, which has a rather high residual repair activity,
 Egyptian patients to Groups A and C. Liquid scintillation count-
large increase in [3H]dThd incorporation occurred as a result
repair DNA synthesis observed in a culture in hybridizations
ing from semiconservative DNA synthesis and had no quanti-
tative effect on unscheduled synthesis.

The liquid scintillation counting data obtained with XP25RO,
XP20RO, and XP3NE cells (Table 2) fit very well with the
results of unscheduled DNA synthesis measured by autoradi-
ography (5). All our cell strains showed decreased levels of
repair, the lowest being XP2CA (and the related XP1CA) and
XP5CA cells.

To develop a simple, rapid technique for analyzing hybridized
cells in complementation analysis that would be easier than
using autoradiography (20), we used liquid scintillation count-
ing. This technique is limited by 2 factors: (a) the presence of a
small number of cells still in semiconservative synthesis
cannot be prevented, and these cells may cause variability in
the background level of ³H activity; (b) variations in the fre-
cquency of hybrid cells will produce variations in the amount of
repair DNA synthesis observed in a culture in hybridizations
between complementing cell types. Therefore, we have ana-
lyzed this set of data simply on the basis of whether or not a
large increase in [³H]dThd incorporation occurred as a result of
hybridization (Table 3). On this basis, we assigned the
Egyptian patients to Groups A and C. Liquid scintillation count-
ing with XP7CA, which has a rather high residual repair activity,
was inconclusive. The experiment was repeated with autoradi-
ography, which definitely assigned XP7CA to Group C. The
assignment of XP2CA to Group A was confirmed in autoradi-
ographic experiments by the assignment of XP1CA, the brother
of XP2CA, to the same group. Similarly, the assignment of
XP6CA to Group C was confirmed in autoradiographic experi-
ments with XP8CA cells. Only selected pairs of cells were
studied by hybridization; so cells with double or linked defects
would have been missed, but these have not been observed in

TABLE 1
Clinical and epidemiological data for 16 XP homozygous patients

<table>
<thead>
<tr>
<th>Family</th>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Disease onset (mos.)</th>
<th>Birthplace</th>
<th>Parental relation</th>
<th>Affected sibs</th>
<th>Dyspigmentation</th>
<th>Occular</th>
<th>IQ and development</th>
<th>Neoplasia</th>
<th>Heterozygote skin symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>XP1CA</td>
<td>F</td>
<td>9 yr</td>
<td>4</td>
<td>UE&lt;sup&gt;b&lt;/sup&gt;</td>
<td>First cousin</td>
<td>1 male, 2 females</td>
<td>Very severe</td>
<td>+</td>
<td>20% dwarfed microphthalmic</td>
<td>Squamous carcinoma</td>
<td>Facial hyperpigmented nodules (mother)</td>
</tr>
<tr>
<td>2</td>
<td>XP3CA</td>
<td>F</td>
<td>13 yr</td>
<td>9</td>
<td>UE</td>
<td>None</td>
<td>1 male</td>
<td>Severe</td>
<td>+</td>
<td>Normal</td>
<td>Keratocon-thoma</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>XP4CA</td>
<td>F</td>
<td>7 yr</td>
<td>6</td>
<td>LE</td>
<td>First cousin</td>
<td>2 females</td>
<td>Severe</td>
<td>+</td>
<td>30%</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>XP6CA</td>
<td>M</td>
<td>10 yr</td>
<td>4</td>
<td>UE</td>
<td>First cousin</td>
<td>1 female (half-sib)</td>
<td>Very severe</td>
<td>+</td>
<td>Normal</td>
<td>Papilloma</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>XP7CA</td>
<td>M</td>
<td>5 yr</td>
<td>10</td>
<td>LE</td>
<td>Second cousin</td>
<td>1 male cousin</td>
<td>Very severe</td>
<td>+</td>
<td>Normal</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

<sup>a</sup> Age at first symptoms of erythema or dyspigmentation.
<sup>b</sup> All show photophobia; all except XP8CA have conjunctivitis.
<sup>c</sup> At least one and in most cases both parents were observed.
<sup>d</sup> XP1CA and XP2CA are cousins (see Chart 1).
<sup>e</sup> UE, upper Egypt; LE, lower Egypt.
<sup>f</sup> XP6CA and XP8CA are sibs.

XP studies and are considered unlikely.

Accumulation of Strand Breaks in the Presence of ara-C and HU. When UV-irradiated cells are analyzed directly by
alkaline sucrose gradients, technical reasons limit the maxi-
mum-control, single-strand molecular weight to about 2 to 5 × 10<sup>6</sup> (12, 36). Excision repair breaks are barely detectable in
these gradients because of rapid coordinated incisions, exci-
sions, and ligations (18, 24). However, when irradiated cells
were grown in ara-C (10 µM) and hydroxyurea (2 mM) the
molecular weights of single-stranded DNA decreased because
excision repair was blocked before completion (Chart 2) (25, 31).
Hypothesis: ara-C and hydroxyurea alone did not cause strand breaks in unirradiated cells. Breaks accumulated most rapidly in the

TABLE 2
Repair DNA synthesis in XP cell populations<sup>a</sup> irradiated with 10 J UV per sq m

<table>
<thead>
<tr>
<th>Cell strain&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Incorporated [³H]dThd&lt;sup&gt;c&lt;/sup&gt;</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>C5RO (control)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>28.3</td>
<td>100</td>
</tr>
<tr>
<td>C5RO (control)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>29.3</td>
<td>100</td>
</tr>
<tr>
<td>XP25RO (Group A)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>XP20RO (Group C)</td>
<td>4.6</td>
<td>17</td>
</tr>
<tr>
<td>XP3NE (Group D)</td>
<td>11.3</td>
<td>43</td>
</tr>
<tr>
<td>XP1CA (GM 2990)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>XP2CA (GM 2991)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>XP3CA (GM 2992)</td>
<td>1.9</td>
<td>7</td>
</tr>
<tr>
<td>XP4CA (GM 2993)</td>
<td>5.8</td>
<td>20</td>
</tr>
<tr>
<td>XP5CA (GM 2994)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>XP6CA (GM 2995)</td>
<td>3.3</td>
<td>12</td>
</tr>
<tr>
<td>XP7CA (GM 2997)</td>
<td>7.1</td>
<td>24</td>
</tr>
<tr>
<td>XP8CA (GM 2998)</td>
<td>3.3</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> The populations contained a high frequency of homokaryons since these cells were fused 4 days before UV irradiation.
<sup>b</sup> XP1CA (cousin of XP2CA) and XP8CA (brother of XP6CA) were tested autoradiographically. The results confirmed the liquid scintillation counting data obtained with cells of the related patient.
<sup>c</sup> The figure is obtained by subtraction of the 0 J/sq m value (0.4 to 1.5 cpm/µg) from the 10 J/sq m value.
<sup>d</sup> Two independent experiments with the same control strains.
Repair DNA synthesis in cell populations obtained after fusion of various Egyptian XP cells with XP cells belonging to complementation groups A, C, or D

<table>
<thead>
<tr>
<th>Egyptian XP strain</th>
<th>A (XP2sRO)</th>
<th>C (XP2gRO)</th>
<th>D (XP3NE)</th>
<th>Conclusion (group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>XP1CA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+&lt;sup&gt;c&lt;/sup&gt;</td>
<td>A</td>
</tr>
<tr>
<td>XP2CA</td>
<td>0.5</td>
<td>21.1</td>
<td>ND&lt;sup&gt;c&lt;/sup&gt;</td>
<td>A</td>
</tr>
<tr>
<td>XP3CA</td>
<td>18.5</td>
<td>4.9</td>
<td>21.3</td>
<td>C</td>
</tr>
<tr>
<td>XP4CA</td>
<td>12.2</td>
<td>4.1</td>
<td>36.3</td>
<td>C</td>
</tr>
<tr>
<td>XP5CA</td>
<td>0.6</td>
<td>14.3</td>
<td>ND</td>
<td>A</td>
</tr>
<tr>
<td>XP6CA</td>
<td>11.1</td>
<td>5.0</td>
<td>ND</td>
<td>A</td>
</tr>
<tr>
<td>XP7CA</td>
<td>11.0</td>
<td>11.5</td>
<td>31.5</td>
<td>Indeterminate</td>
</tr>
<tr>
<td>XP7CA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>—</td>
<td>+</td>
<td>C</td>
</tr>
<tr>
<td>XP8CA&lt;sup&gt;a&lt;/sup&gt;</td>
<td>+</td>
<td>—</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

<sup>a</sup> The figure is obtained by subtraction of the 0 J/sq m value (0.4 to 1.5 cpm/µg) from the 10 J/sq m value.
<sup>b</sup> Repair DNA synthesis was measured by autoradiography.
<sup>c</sup> +, complementation; —, no complementation; ND, not done.

first hr after irradiation but then more slowly and saturated at doses between 10 and 20 J/sq m (Chart 3). These kinetics are similar to those observed for other events during excision repair (13).

XP cells were surveyed at the single dose (13 J/sq m) and time (5 hr) at which the largest number of breaks was observed in normal cells. All XP cells showed the accumulation of a small number of breaks which were readily observable in these assays because of the sensitivity of alkaline sucrose gradient profiles to small amounts of breakage (Charts 2 and 3; Table 4). Therefore, these results clearly demonstrate that a low amount of incision occurs in all of these XP cells.

Each cell type appears to accumulate a slightly different number of breaks (Table 4), and when cells are viewed according to complementation group, the cells in Group A fall significantly below those of Group C (Table 4). Lower repair in the Group A cells compared to the group C cells was also observed in the measurements of unscheduled synthesis (Table 2). The heterozygote cell line showed the same frequency of strand breaks as normal cells (Table 4), in keeping with most other measurements that show apparently normal repair in XP heterozygotes (6, 10).

DISCUSSION

The patients we investigated fall in the 2 XP groups previously defined as Groups A and C (34). If we assume that sibs and close relatives of our patients who also show clinical symptoms of XP will fall in the same complementation groups as the probands, then these cases are equally divided among Groups A and C. This assumption has proved true in the past and is confirmed by the assignments of the related patients (XP1CA and XP2CA; XP6CA and XP8CA) in the present study. However, in view of the complexity of this disease and the heterogeneity of some complementation groups, such an assumption must be used cautiously and explicitly.

Our results also exemplify some of the characteristics and heterogeneity previously reported for Groups A and C. Our observations indicate that Group A patients who have both cutaneous and neurological disorders have the lowest levels of repair. Previous observations indicated that Group A patients with these symptoms have levels of unscheduled DNA synthesis (9, 15, 41, 44) or repair replication (9, 45) that are undetectable or at the level of resolution. However, our experiments with ara-C show that there is a measurable level of strand breakage even in Group A cells (Table 3). An exceptional Group A patient (XP8LO) with no neurological disorders has a higher level of unscheduled synthesis (23).

Our Group C patients have higher levels of repair than do Group A patients (Tables 2 and 4), and both repair synthesis
and strand breakage seem to vary considerably among different strains. All these patients are without overt neurological disorders, in keeping with the general pattern of Group C patients (1, 15, 41), but again, there are exceptions because one Group C patient has been reported with mild neurological symptoms of deafness and mental retardation (30). The higher level of repair in Group C than Group A cells (Tables 3 and 4) correlates with the higher survival seen in Group C after UV irradiation (1). The precise molecular basis for differences between Groups A and C remains unexplained.

The most common forms of XP are Groups A and C. The next most frequent are Group D and variant with the remaining Groups B, E, F, and G thus far represented by only 1 or 2 families (Table 5). No variants were found in our limited survey, but they have been found among other Middle Eastern populations (e.g., XP3ORO from Lebanon and XP7TA from Israel), so their absence from our study may simply be a consequence of the small sample size. The low frequency of Group C cases among Japanese (45) was prefigured in a study of gene frequencies by Neel et al. (39) who suggested that the number of alleles involved in XP was fewer in Japanese than in Caucasian populations.

A consideration from these various population studies is whether the complementation groups thus far discovered represent different alleles or genes and whether complementation involves intercistronic or intracistronic events. Most of the complementation groups contain one or more patients who are known to come from consanguineous marriages, and therefore we will assume that cells within one complementation group are homozygous at a particular locus. Complementation Groups A through G all appear to involve an early step in excision repair associated with recognition of damaged sites and incision of DNA. The variant (11) appears to involve a later stage in excision repair associated with polymerization and/or ligation (17, 25, 28) that may increase the error rate of excision repair (37) and produce secondary alterations in DNA replication (13, 17, 35, 40). The high frequency of Groups A and C suggests that these groups may represent major, large proteins involved in an early step of incision for which there are many potentially mutable sites resulting in loss of function (3, 33). The infrequent occurrence of other groups suggests that these may represent small proteins or polypeptides with few potential mutational sites at risk or proteins whose function comes under stringent selection criteria (3, 33). Studies of DNA repair in heterokaryons in the presence of cycloheximide (29) and the ability of cell extracts to perform repair in cell-free conditions (38) indicate that some of the XP complementation groups may represent regulatory factors that control the synthesis of repair enzymes and/or their access to DNA damage within chromatin structure.

The consequences of these various enzymatic defects in...
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repair involves high levels of sister chromatid exchange (4, 22, 46), mutation (37), and cancer (41). It is hoped that by field studies like this and by making cell lines from these patients generally available we may continue unravelling important features of this disease as a model for environmentally induced human cancer.

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REFERENCES


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