Am. J. Hum. Genet. 50:677–689, 1992

Xeroderma Pigmentosum and Cockayne Syndrome: Overlapping Clinical and Biochemical Phenotypes

G. A. Greenhaw,* A. Hebert,† M. E. Duke-Woodside,‡ I. J. Butler,‡ J. T. Hecht,* J. E. Cleaver,§ G. H. Thomas,§ and W. A. Horton*

Departments of *Pediatrics, †Dermatology, and ‡Neurology, University of Texas Medical School, Houston; and §Laboratory of Radiobiology and Environmental Health, University of California, San Francisco

Summary

Two siblings are described whose clinical presentation of cutaneous photosensitivity and central nervous system dysfunction is strongly reminiscent of the DeSanctis-Cacchione syndrome (DCS) variant of xeroderma pigmentosum. An extensive clinical evaluation supported a diagnosis of DCS and documented previously unreported findings. In vitro fibroblast studies showed UV sensitivity that was two to three times that of normal controls. However, neither a post-UV-irradiation DNA excision-repair defect indicative of XP nor a semiconservative DNA replication defect indicative of XP variant was found. Rather, a failure of RNA synthesis to recover to normal levels after UV exposure was observed, a biochemical abnormality seen in Cockayne syndrome (CS), one of the premature-aging syndromes with clinical UV sensitivity. These patients, therefore, clinically have XP, but their biochemical characteristics suggest CS. The reason(s) for the severe neurologic disease, in light of the relatively mild cutaneous abnormalities, is unclear. Other cases with unusual fibroblast responses to irradiation have been noted in the literature and, along with the data from our patients, reinforce the notion of the complexity of DNA maintenance and repair.

Introduction

Xeroderma pigmentosum (XP) is a rare autosomal recessive condition which presents clinically as photosensitivity of the skin and eyes, with premature cutaneous aging and neoplasias. The exact gene defect(s) is unknown, but it has been recognized for a number of years that a defect in excision repair of UV-induced DNA damage is found in the cells of most affected individuals (Cleaver and Kraemer 1989). The incidence of XP in the United States is approximately 1/250,000 but is greater in Egypt and Japan. This disorder has been observed worldwide in all ethnic groups. Males and females are equally affected (Cleaver and Kraemer 1989). The XP variant group has a clinical presentation indistinguishable from that of classic XP and is UV sensitive but has no excision-repair defect. Rather, the defect is an abnormality in replication past repaired sites of UV-damaged DNA (Kraemer et al. 1987; Cleaver and Kraemer 1989).

In addition to the cutaneous manifestations, 20%–35% of those affected with XP also have progressive neurologic degeneration (Reed et al. 1977). This may either begin in early childhood or not present until the second decade. The DeSanctis-Cacchione syndrome (DCS) constitutes a subgroup of XP with severe neurologic involvement. These patients have short stature, microcephaly, mental retardation, hypogonadism, ataxia, spasticity, peripheral neuropathy, and sometimes sensorineural deafness (Reed et al. 1977). Significantly, patients with DCS are among those with the greatest UV sensitivity and poorest DNA repair (Cleaver et al. 1984; Cleaver and Thomas 1988). Presumably, the neurologic problems result from neuronal death or dysfunction because of an inability to repair DNA (Andrews et al. 1978).

Cockayne syndrome (CS) is a very rare autosomal recessive, premature-aging syndrome that has some

Received June 21, 1991; revision received November 25, 1991.
Address for correspondence and reprints: William A. Horton, M.D., Department of Pediatrics, University of Texas Medical School, P.O. Box 20708, Houston, TX 77225.
© 1992 by The American Society of Human Genetics. All rights reserved.
0002-9297/92/5004-0003$02.00

clinical and biochemical similarities to XP, including UV photosensitivity, mental retardation, complex nervous system abnormalities, and cutaneous pigmentary changes. Patients with CS are distinguished clinically from those with XP by, among other things, the presence of cachectic dwarfism, pigmentary retinal degeneration, and absence of actinically induced cancer (Timme and Moses 1988). Cells from patients with CS demonstrate in vitro UV sensitivity, but, unlike the case for cells from XP patients, excision repair of UV-induced damage is normal. Rather, in vitro studies demonstrate a characteristic failure of fibroblasts to show recovery of RNA and DNA synthesis after UV exposure (Mayne and Lehmann 1982). In addition, cells from patients with CS are known to be unable to repair transcriptionally active DNA as rapidly and as efficiently as do normal cells (Venema et al. 1990). Several patients have been documented with presentations typical of both XP and CS (Robbins et al. 1974; Dupuy et al. 1982). The patients demonstrated neurologic abnormalities characteristic of CS, as well as cutaneous findings indicative of XP. Excision repair is significantly reduced in these patients.

We present a Hispanic family in which three siblings demonstrated clinical manifestations consistent with DCS. Two of the three siblings underwent extensive investigation, including detailed neurologic and dermatologic evaluations which supported this diagnosis. It is surprising that in vitro analysis of UV sensitivity and DNA repair capacity of cultured fibroblasts was not consistent with DCS but instead was consistent with CS.

Clinical Case Presentations

Patient 1

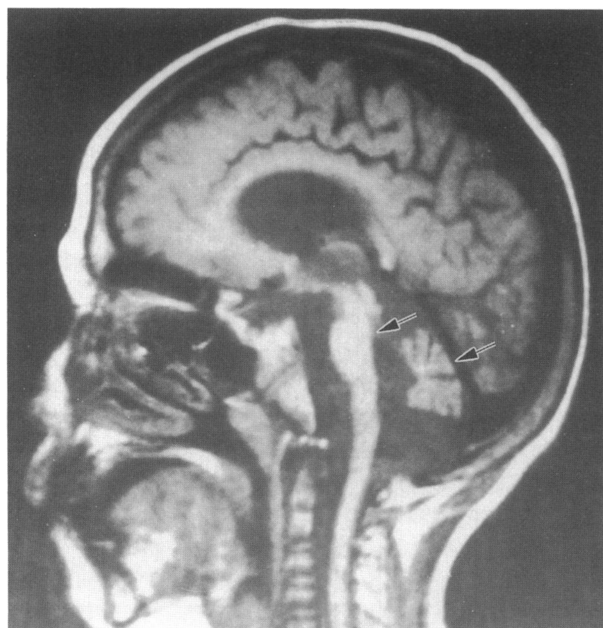
GU was an 8½-year-old male referred for evaluation of developmental delay and seizures. He was the seventh of 11 children; pregnancy, labor, and delivery were uncomplicated. Although consanguinity was not definitely determined, there was a high probability that the parents were related, since pedigree analysis showed ancestors with the same family name in both maternal and paternal lines. Both families had lived in the same small Mexican village for many years. Although considered a normal infant, he always sunburned quite easily. At 1 year of age his development ceased; he never walked or developed speech and was never toilet trained. Facial freckling was noted at age 2–3 years, and at age 5 years he developed grand mal seizures. Bilateral equinovarus deformities developed in early childhood. Otherwise, his health had been excellent.

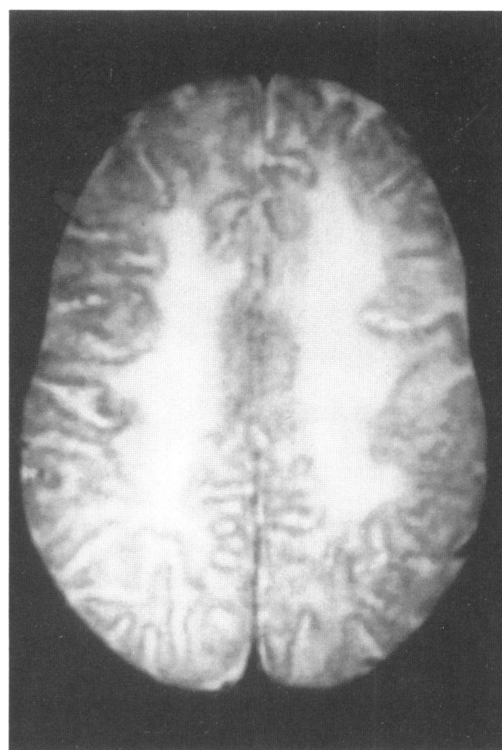
Pertinent physical findings were as follows: height 116 cm (<3% for age, 50% for age 6 years), weight 19 kg (<3% for age, 50% for age 5½ years), and head circumference 49 cm (<3% for age, 50% for age 2 years). He appeared alert and smiled responsively but had limited purposeful activity. No external ocular abnormalities were noted, except for mild conjunctival erythema. Funduscopic examination revealed bilateral optic atrophy without pigmentary abnormality. Thoracic and abdominal examinations were unremarkable, and testes were undescended bilaterally. Marked bilateral, fixed equinovarus deformities were present. Skin pigmentation was moderately dark and similar to that of his mother. Generalized facial freckling was noted, with multiple hyperpigmented macules and scattered telangiectasias. Nails, scalp, and hair were unremarkable. Deep-tendon reflexes were diminished, and the lower extremities were hypertonic with extensor plantar responses.

Laboratory studies were extensive. Those with unremarkable or normal results included blood chemistries, urinalysis, routine chromosome analysis, quantitative immunoglobulins and T-cell panel, urine and fecal porphyrin analyses, cerebrospinal fluid (CSF) studies for syphilis, bacterial, fungal, acid-fast bacillus, or herpes infections. A mild microcytic anemia was present; serum iron and TIBC were normal, and hemoglobin electrophoresis revealed 100% type A hemoglobin. CSF biogenic amine neurotransmitter metabolite analysis revealed expected levels of homovanillic acid (HVA) and 3-methoxy,4-hydroxy-phenylethylene glycol (MHPG); however, the level of 5-hydroxyindole-acetic acid (5-HIAA) was half the expected value. CSF IgG and albumin were also decreased.

X-ray studies included a complete skeletal survey. A chest X-ray was unremarkable. Hip and lower-extremity films revealed bilateral coxa valga with dislocation of the right hip and bilateral equinovarus deformities. No evidence of a skeletal dysplasia was seen. Magnetic resonance imaging (MRI) of the brain demonstrated ventricular dilatation and diffuse leukodystrophy with generalized cerebral atrophy and olivo-ponto-cerebellar atrophy (OPCA) (fig. 1, top two panels). Computed tomography (CT) of the brain did not show any intracranial calcifications (fig. 1, bottom panel).

The results of other studies were as follows: Brain-





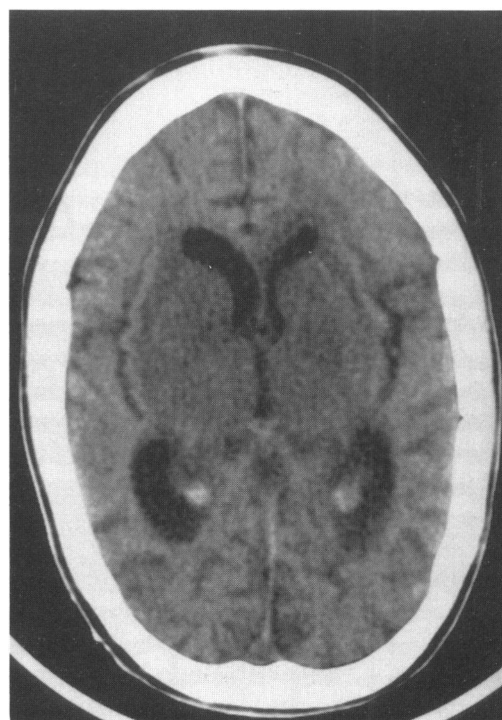


Figure 1 Brain MRI of AU. *Above,* Imaging revealed generalized cerebral atrophy, ventriculomegaly, and severe OPCA (arrows). Sagittal T_1–weighted spin echo TR = 800 and TE = 20. *Top right,* Extensive abnormal signal throughout white matter of cerebral hemispheres, consistent with leukodystrophy. Axial T_2–weighted spin echo TR = 2,000 and TE = 80. *Bottom right,* Brain CT of GU. Generalized cerebral atrophy, ventriculomegaly, and severe OPCA were again seen. No intracranial calcifications characteristic of CS were observed.

stem auditory-evoked responses (BAER) demonstrated a sensorineural hearing deficit. Electroencephalography was abnormal, with left temporal and parietal slowing. Electromyelography and nerve-conduction studies were normal. Light and electron microscopy (EM) of conjunctiva obtained by biopsy showed axonal dropout within nerve bundles, with collagen-filled Schwann cell troughs. EM studies of sural nerve biopsies showed severe axonal dropout of both myelinated and unmyelinated axons, with evidence of Wallerian degeneration as well as numerous collagen-filled Schwann cell troughs and bands of Buenger. Many unmyelinated axons were swollen, while myelinated axons had accumulations of tubules, filaments, and prominent cisternae in their cytoplasm (fig. 2). Histochemistry and EM of skeletal muscle revealed severely atrophic fibers with striking subsarcolemmal folds, redundant basal lamina, and myofilament dissolution, with normal mitochondria. These findings were most consistent with a peripheral neuropathy.

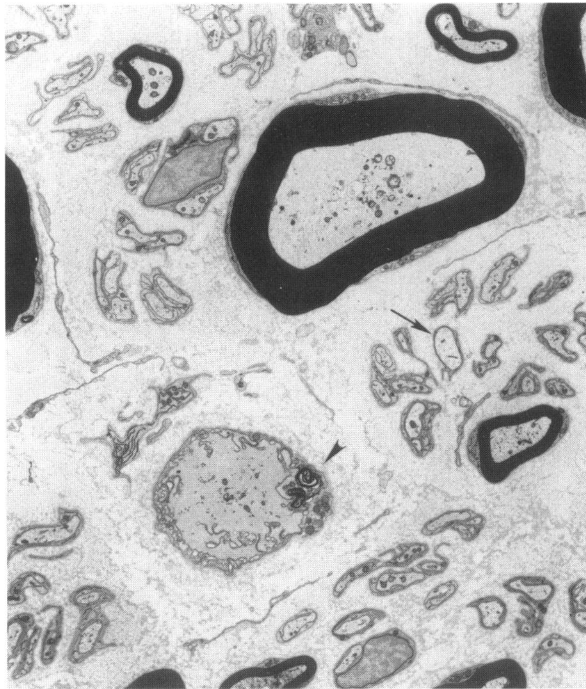


Figure 2 Sural nerve biopsy. Severe axonal dropout of both myelinated and unmyelinated axons was observed. Ongoing axon degeneration was demonstrated by Wallerian degeneration (arrowhead) and swollen unmyelinated axons (arrow). (Electron micrograph, × 3,000)

Urodynamic evaluation revealed detrusor hyperreflexia, small bladder capacity, and small postvoid residual. There was no evidence of dyssynergia. A renal sonogram was unremarkable.

Biopsy of sun-damaged and undamaged facial skin exhibited spongiosis, focal parakeratosis, and perivascular lymphocytes. Melanin incontinence and telangiectasia without solar elastosis were also present. The patient died at the age of 10 years, of complications of uncontrolled seizures and aspiration pneumonia after surgical repair of his clubbed feet.

Patient 2

AU was the younger sister of GU and was 7½ years old at evaluation. Her past history was similar to her brother's, including normal early development and lifelong solar sensitivity. She achieved more advanced milestones than did GU, including walking, talking, and toilet training. At 3 years of age, her development ceased; she lost both her ability to walk and most of her speech. Facial freckling appeared at the same time. She never developed seizures, and her health had otherwise been excellent (fig. 3).

Pertinent physical findings were as follows: height 113 cm (<3% for age, 50% for age 5½ years) and weight 17.2 kg (<3% for age, 50% for age 15 mo). She followed single-step instructions given in Spanish and ambulated very short distances with an ataxic, scissoring gait while supported. She had a vocabulary of a few Spanish words. She had mild strabismus and bilateral conjunctival erythema. Thoracic, abdominal, and external genitalia examinations were unremarkable. A mild equinovarus deformity of her right foot was present. Deep-tendon reflexes were diminished, and bilateral extensor plantar responses were present. Muscle tone was generally increased. Basic skin pigmentation was similar to her brother's, and she also had similar facial freckling, multiple telangiectasias, and hyperpigmented macules. Nails, scalp, and hair were unremarkable. Results of laboratory studies on blood and CSF; X-rays of chest, hips, and lower extremities; MRI of the brain; skin biopsy; microscopic (light and EM) and histochemical analyses of conjunctiva, sural nerve and muscle; EMG and nerve conduction studies; urologic evaluation; and porphyrin screening were very similar to those for GU. In particular, MRI of her brain showed the same diffuse leukodystrophy with OPCA. CSF levels of 5-HIAA were also half that expected; levels of HVA and MHPG were normal. An EEG was normal.

Patient 3

This older sibling of GU and AU had a past medical history and clinical course very similar to those of GU. She died at 10 years of age, in Mexico, of unknown causes, possibly pneumonia.

Cellular Studies

Fibroblasts were obtained from GU and AU by sterile punch biopsy. Cultures were prepared from both sun-exposed and unexposed skin and were grown under routine conditions in Eagle's minimal essential medium supplemented with 10% (v/v) FBS, 2 mM L-glutamine, 50 units penicillin/ml, and 50 μg streptomycin/ml. Cell stocks were subcultured every 1 or 2 wk, and cell strains of less than passage 15 were used for all experiments. Cultures were designated with UCSF (University of California, San Francisco) numbers according to one author's (J.E.C.) consecutive series of diagnostic studies for XP (table 1). A culture from one patient (GU, XP63SF) has been deposited

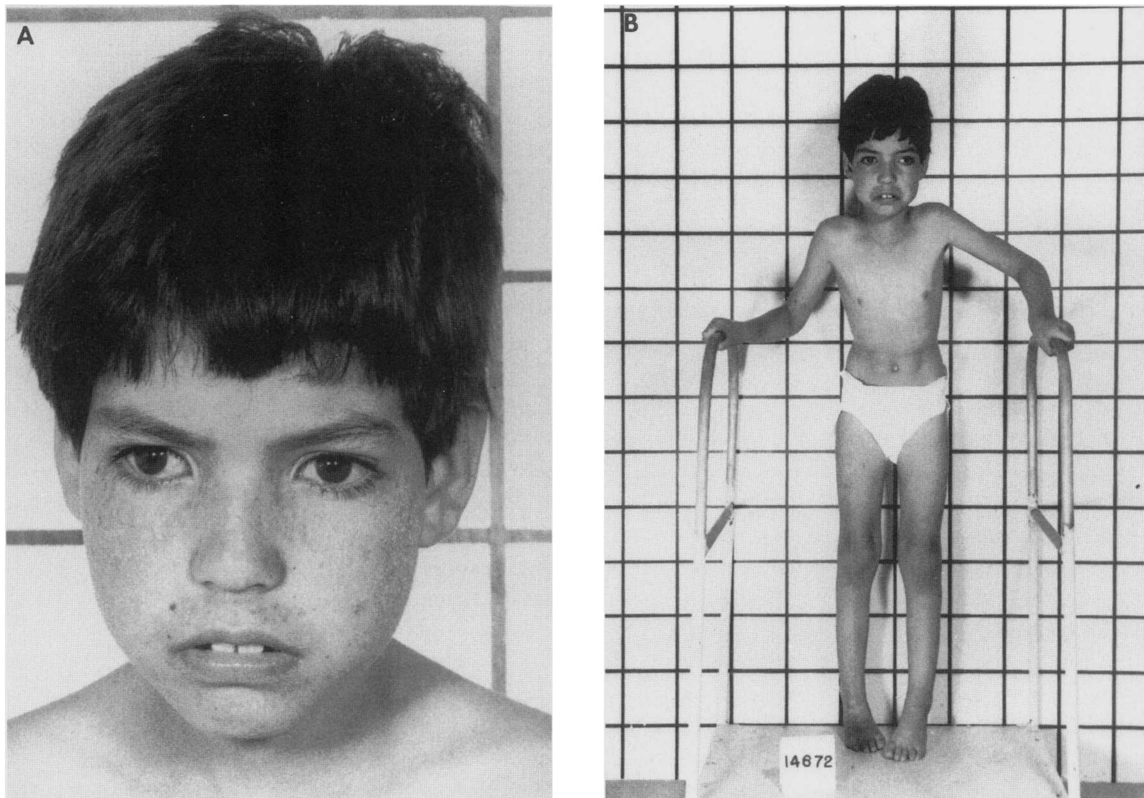


Figure 3 AU at 7½ years of age. The patient had marked growth retardation without signs of the cachectic dwarfism seen in CS. She also had microcephaly, facial hyperpigmented macules and telangiectasias, spasticity of the limbs (especially the lower extremities), and ocular and cutaneous solar sensitivity. Her brother's appearance was very similar.

with the NIGMS Human Genetic Mutant Cell Repository (Camden, NJ) and designated GM10905. Fibroblasts derived from unaffected controls, known CS patients, and one patient suspected to have CS were grown in the same manner.

Sensitivity of the fibroblasts to UV light was determined by relative cell survival after UV irradiation. The rapid well assay method (Cleaver and Thomas 1988) was used. One milliliter of cultured cells (10^4 cells/ml) was seeded into well culture trays. The cells were irradiated with various doses of UV light and allowed to grow for 4–6 d. At this time, the culture medium was changed to one containing 2.0 μCi [^{3}H]hypoxanthine (9.1 Ci/mmol)/ml, and cells were incubated for 4 h. After the staining, cultures were fixed and air-dried, and the radioactivity was extracted. Previous studies have shown that, under these conditions, label is incorporated primarily into RNA rather than into DNA. Also, RNA synthesis continues regardless of the degree of confluence on the culture.

During the first 24 h after UV irradiation, cells with phenotypes such as XP and CS show degrees of inhibition and recovery of RNA synthesis that are different than those of normal cells. By the time of labeling for

Table 1

Patient and Cell-Line Designation

Patient	Source of Cells[a]	Designation
GU (proband) ...	Uninvolved skin	XP63SF
	Involved skin	XP64SF and XP66SF[b]
AU (proband) ...	Uninvolved skin	XP61SF
	Involved skin	XP62SF and XP67SF[b]
Normal	Foreskin	FS
	Adult skin	AG2602
CS	Uninvolved skin	CS73SF and CS41SF
XP	Uninvolved skin	XP99SF
Sun sensitivity ...	Uninvolved skin	80SF

[a] Biopsies were taken from skin with obvious actinic changes and from normal appearing skin on the same patient.

[b] Repeated independent biopsies of a single patient.

survival assays, however, these transient changes are over, and rates of synthesis should have returned to control rates. This was confirmed by measuring the rates of RNA synthesis per cell, in both control and irradiated cultures, for several cell types (table 2). For each cell type the incorporation per cell was found to be the same in control and irradiated cultures at the time of measurement, and therefore the radioactivity per well can be used as an indicator of the number of cells in each well. The relative survival was calculated from the incorporation of ^{3}H by the cells in each well, relative to that of unirradiated cells, and was used as a measure of relative UV sensitivity.

The relative amount of DNA excision repair of UV-induced pyrimidine dimers that was performed by fibroblasts during the first 6 h after irradiation was determined by a method which converts repair patches into DNA breaks that can be quantified on calibrated alkaline sucrose gradients (Cleaver et al. 1984). In brief, cells were grown until confluent in [^{14}C]thymidine-containing medium. Confluent cultures were used to reduce the contribution of semiconservative DNA replication to changes in relative DNA sizes. Cultures were irradiated with UV light (254 nm, 13 J/m^2 at 1.3 $J/m^2/s$) and then incubated for 6 h in medium containing [^{3}H]thymidine and 100-fold excess cytosine arabinoside. These conditions allowed the incorporation of cytosine arabinoside into excision-repair patches, resulting in chain termination and the accumulation of single-strand DNA breaks at the sites of DNA repair. The cells were then layered onto a lysis solution on top of alkaline sucrose gradients and centrifuged at 25,000 *g* for 4 h to separate DNA fragments according to size. Fractions were collected and processed for determination of [^{3}H] and [^{14}C] radioactivity according to a method described elsewhere (Cleaver 1975). Molecular weight and the number of breaks accumulated per control DNA molecule (approximately 2×10^8 daltons) were calculated (Ehmann and Lett 1973). This permits detection of the excision-repair defect present in XP.

Assessment of the size of DNA fragments resulting from semiconservative DNA replication after low doses of UV light was determined in exponentially growing cells labeled with [^{14}C]thymidine, as above. Cells were drained of medium and were irradiated with a dose of either 1.3 J/m^2 or 1.65 J/m^2 to produce pyrimidine dimers. Cells were returned to fresh unlabeled medium and incubated for 60 min to allow excision repair and resumption of DNA synthesis. Unlabeled medium was then replaced with [^{3}H]thymidine-labeled medium, and the cells were incubated for 15 min. Cells were harvested, lysed, and analyzed by alkaline sucrose gradient sedimentation. [^{3}H] counts were normalized in each gradient to the same absolute amount of [^{14}C] in the gradient. This assesses DNA fragment size and the amount of each size present, permitting detection of the XP variant group (Cleaver et al. 1981*a;* Kaufmann and Cleaver 1981).

Rates of RNA synthesis after UV irradiation were determined by labeling newly transcribed RNA for 30 min with [^{3}H]hypoxanthine (0.5 μCi/ml, 9.1 Ci/mmol), at various times up to 8 h after irradiation with UV light. Cultures were fixed and extracted with 5% perchloric acid for scintillation counting. Recovery of RNA synthesis after UV irradiation is very depressed in patients with CS (Mayne and Lehmann 1982).

Table 2

Rates of [^{3}H]hypoxanthine Incorporation per Cell in Cultures Grown for 4 d after Irradiation with 5.2 J/m^2

Cell Line and Dose	Mean ± SE ^{3}H Radioactivity/10^4 Cells[a]
FS:	
Control	398.5 ± 90.8 (4)
5.2 J/m^2	578.5 ± 143.0 (4)
XP63SF:	
Control	331.6 ± 25.3 (8)
5.2 J/m^2	304.1 ± 56.4 (8)
XP99SF:	
Control	217.9 ± 14.3 (8)
5.2 J/m^2	193.2 ± 13.9 (8)

[a] The incorporation per cell for each cell type was the same in control and irradiated cultures. Each pair of control and UV-irradiated cultures were compared by Student's *t*-test, and the differences were not significant at $P < .5$. Numbers in parentheses are number of samples.

Results

UV Sensitivity

The cell lines from both patients were more sensitive to UV irradiation than were normal cells (fig. 4). Although this degree of sensitivity was not as extreme as can be observed for XP, it was still substantial and similar to that observed with a CS cell line (table 3).

Proficiency of Dimer Excision Repair

The determination of DNA repair produced values which were very close to normal (table 4). Results

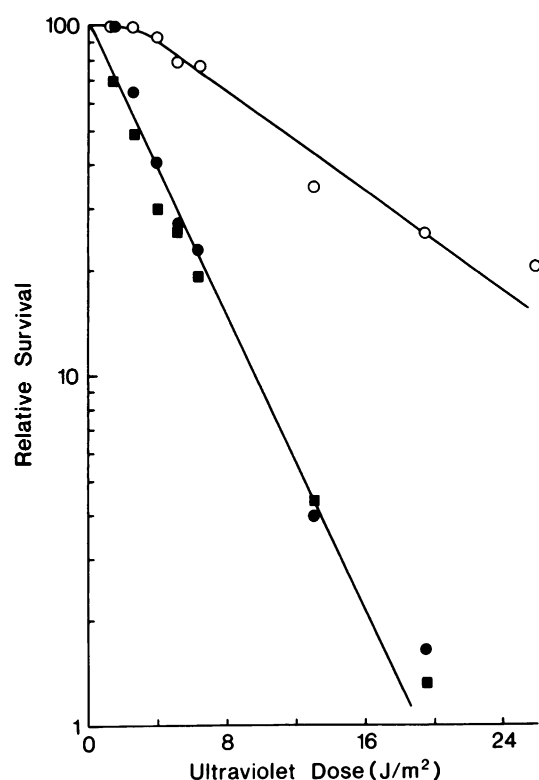


Figure 4 Relative cell survival in normal and patient cell lines. Survival was assayed by growth in 4 × 6-well trays for 7 d after exposure to various doses of UV radiation. Survival was significantly decreased in cells taken from AU and GU. ○ = Normal fibroblasts; ● = XP66SF; and ■ = XP67SF.

Table 3

Sensitivity of Various Cell Lines to UV Light

	Cell Line	D_{37}[a] (J/m^2)
FS (normal)		15.7 ± 1.9[b]
Patient GU	XP63SF	4.4
	XP64SF	4.2
	XP66SF	5.0
Patient AU	XP61SF	3.5
	XP62SF	3.5
	XP67SF	6.0
XP	Group A	2.0–3.3[c]
	Group C	5[c,d]
	Group D	1.5[c]
CS	CS41SF	3.0

[a] In vitro dose of UV exposure at which 37% of exposed fibroblasts survive.
[b] Mean ± SE of nine determinations.
[c] Previously published (Cleaver and Thomas 1988) value, included here for comparison with values for cell lines from patients GU and AU.
[d] Higher values are obtained when cells are seeded at higher than usual densities in this assay (Cleaver and Thomas 1988).

obtained overlapped the normal range; thus, it was not possible to do XP complementation analyses. Results were much higher than values observed for cell lines from patients with DCS or more limited neurological defects. By this assay, XP complementation group A typically has 2%–5% of normal levels of repair, and XP group D has about 10%–50% of normal repair (Cleaver and Kraemer 1989). Patients with CS have normal excision repair (Ahmed and Setlow 1978).

Determination of Post-UV DNA Daughter-Strand Fragments Size

For fibroblasts cultured from normal controls (FS) and patient AU (XP62SF), the effect of 1.6 J/m^2 UV was a similar suppression of [^{3}H] incorporation, with no prominent peaks at low molecular weights. XP variant is distinguished from classic XP by a defect associated with replication of UV-damaged DNA (Lehmann èt al. 1975; Cleaver et al. 1981*a;* Kaufmann and Cleaver 1981), which can be clearly delineated by analyzing the size distributions of DNA fragments synthesized after irradiation. At a UV dose of 1.6 J/m^2, an XP variant cell line displays a marked block to DNA replication past sites of dimer repair, with accumulation of [^{3}H]-labeled DNA at the shorter size ranges, which approximate the dimer-dimer spacing along template DNA. These are observed as a major [^{3}H] peak in the region of fractions 15–20 of the alkaline sucrose gradients (fig. 5) (Cleaver et al. 1981*a;* Kaufmann and Cleaver 1981). By this analysis, no detectable differences were seen between normal controls and cells obtained from AU, thus excluding the XP variant group.

Rates of RNA Synthesis

After exposure to UV radiation, the rate of DNA or RNA synthesis in normal or XP cells shows an initial decline followed by recovery to preirradiation levels. Cells from CS patients show the same initial decline but are very slow in returning nucleic acid synthesis to preirradiation levels (Mayne and Lehmann 1982). Quite delayed recovery of RNA synthesis was observed in cell lines from our patients and from a patient (CS73SF) with clinically apparent CS tested concurrently (fig. 6). A patient (80SF) with idiopathic sun

Table 4

Relative Amount of DNA Repair in Normal and Affected Fibroblasts

Cell Line(s) 77[c]	No. of Single-Strand Breaks/2 × 10^8 Daltons			% of Normal Repair
	No UV	UV	UV Control	
FS (normal)[a]	1.07 ± .1	8.3 ± .40	6.9 ± .41	100
XP61SF[b]	.78	5.51 ± .49	4.73 ± .49	68.3 ± 7.8
XP62SF and XP67SF[b]	.70	6.56 ± .70	5.86 ± .70	84.6 ± 11.3
XP63SF[b]	.63	7.03 ± .51	6.40 ± .51	92.4 ± 9.2
XP64SF and XP66SF[b]	...	6.57 ± .77	5.78 ± 77[c]	83.4 ± 12.1

Note.—Repair ability was sufficiently close to normal to prevent complementation-group analysis against XP complementation groups A–G.

[a] Data are mean ± SE of five determinations.

[b] Data are mean and range of duplicates, for cultures from repeat biopsies combined.

[c] Pooled control value of 0.80 was used.

sensitivity showed normal recovery, indicating the ability of this method to discriminate specific metabolic defects in recovery from UV irradiation.

Discussion

The ability to repair damaged DNA is critical for all organisms, and several different biochemical repair systems are assumed to be present in humans, including excision repair and postreplication repair (Cleaver and Kraemer 1989). There exists a group of inheritable disorders, including XP and CS, in which the ability to repair DNA damaged by physical or chemical mutagens is compromised, resulting in characteristic clinical syndromes. The processes and gene products involved are poorly understood, and, until recently, no specific mutations which could cause the various clinical syndromes were known (Tanaka et al. 1990; Weeda et al. 1990). A large body of clinical and experimental data has accumulated from the study of affected subjects which has been helpful in developing a systematic approach to understanding these disorders. It is the combination of specific clinical presentations and in vitro characterization of DNA repair defects which has evolved into the present method used to make specific diagnoses. This, of course, is done without a clear understanding of the underlying pathogenesis. The siblings presented here cannot be easily diagnosed and prompt a review of the criteria used to delineate DCS and CS.

Attempts to understand the DNA repair defect in XP and CS resulted in discovery of complementation groups (Cleaver and Kraemer 1989). For XP, complementation-group designation is made on the basis of the ability of fused fibroblasts from two different patients to restore excision repair of DNA to normal levels in vitro. Seven different XP complementation groups (A–G) have been described in addition to the XP variant group. Neurologic involvement has been found in most but not all XP patients in groups A, B, D, and G but rarely either in groups C, E, and F or in the variant group. Sensitivity in vitro to UV radiation—and the ability to repair DNA after exposure to UV light—varies among the groups. Groups A and D usually demonstrate both the greatest sensitivity to UV and the least ability to repair damaged DNA. Patients with DCS usually belong to complementation group A or D. Three complementation groups are known for CS, on the basis of restoration of a normal rate of recovery of RNA synthesis after exposure of heterokaryons to UV radiation (Timme and Moses 1988). Several patients with clinical characteristics of both XP and CS are known. One is the only member of XP group B and CS group C (Robbins et al. 1974). Several others, formerly constituting a separate XP group (H), have been placed in already established XP groups (Dupuy et al. 1982; D. Bootsma, personal communication).

Various clinical studies and case reports, in addition to complementation group analyses, have helped establish both the DCS and CS phenotypes. When autopsies and clinical findings of several patients with DCS were reviewed (Reed et al. 1977), all patients were found to have microcephaly, mental retardation, short stature, facial freckling, spasticity, and hypogonadism. All showed similar OPCA. Sural nerve biopsies from three patients with clinical findings consistent with DCS (Tachi et al. 1988) demonstrated

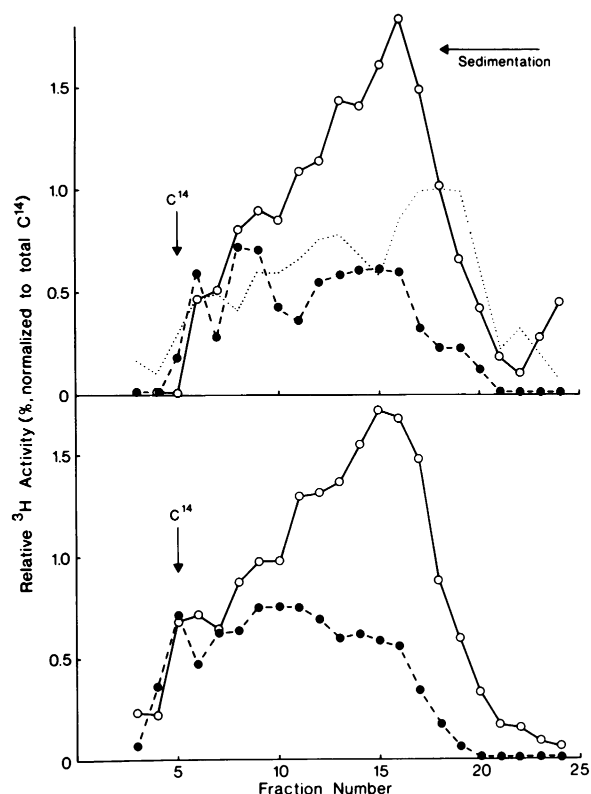


Figure 5 Alkaline sucrose gradients of normal fibroblasts and XP30RO (known XP variant) (*top graph*) and XP62F fibroblasts (*bottom graph*) labeled with ^{3}HdThd (10 μCi/ml, 80 Ci/mmol) for 15 min starting 60 min after irradiation with 1.6 J/m^2. Relative ^{3}HdThd incorporation was 42% for the normal control fibroblasts and was 52% for XP62SF fibroblasts. ○ = Control incorporation; ● = 1.6 J/m^2 UV; and ---- = XP variant XP30RO, redrawn from Kaufmann and Cleaver (1981), at a UV dose of 1.3 J/m^2. Data have been normalized so as to represent both the effect of UV on total quantitative uptake and size classes of DNA. Sedimentation was from right to left, at 25 krev/min for 4 h. No lower-molecular-weight peak (typical of XP variant) was seen.

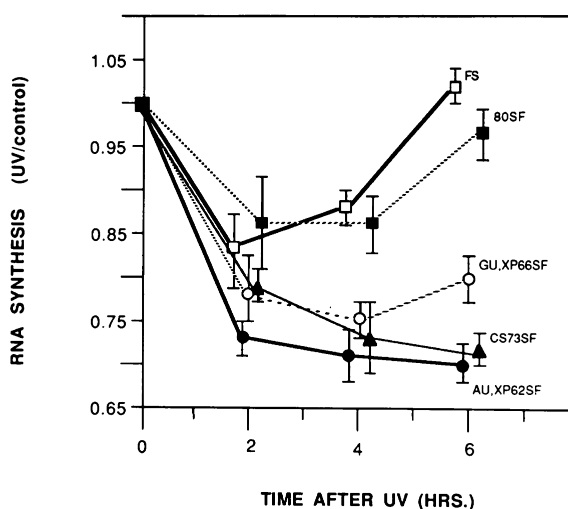


Figure 6 Failure of recovery of nucleic acid synthesis after UV exposure. The rate of RNA synthesis failed to return to preirradiation levels within 6 h. Normal fibroblasts and those from a patient suspected clinically to have CS had depressed RNA synthesis initially, but it rapidly returned to preirradiation rates. Suppression of this recovery is characteristic of CS. □ = Normal fibroblasts (FS); ■ = patient exhibiting idiopathic sun sensitivity (80SF); ▲ = patient (CS73SF); ● = proband fibroblasts (AU,XP62SF); and ○ = proband fibroblasts (GU,XP66SF). Mean and standard errors are displayed.

reduced numbers of myelinated fibers with signs of Schwann cell degeneration, indicative of a peripheral neuropathy secondary to neuronal atrophy. The summarized findings of a large number of XP cases with neurologic abnormalities (Robbins 1989) established diagnostic criteria for "XP neurologic disease." Patients appeared normal at birth and initially had normal developmental milestones. Solar sensitivity was present from birth, and the other classic cutaneous manifestations of XP appeared early in life and were lifelong. Various neurologic abnormalities also appeared early, usually within the first few months to years of life, and included microcephaly, mental retardation, central nervous system (CNS) ventricular dilatation with cortical atrophy, extrapyramidal and cerebellar disturbances, corticospinal tract abnormalities, sensorineural deafness, and a characteristic peripheral neuropathy which was clinically evident as hyporeflexia progressing to areflexia. Studies of CSF immunoglobulins and biogenic amine neurotransmitters in XP or CS have not been previously reported.

Extensive study of CS patients similarly produced a characteristic CS phenotype, although, as with XP, not all findings were found in all patients (Otsuka and Robbins 1985). Patients appeared normal at birth, but severe cachectic dwarfism developed at age 6–12 mo. This included loss of subcutaneous fat, disproportionately long extremities, sunken eyes, large ears, and a thin prominent nose (Timme and Moses 1988). Major neurologic complications included sensorineural hearing loss, ataxia, choreoathetosis, spasticity, and gait disturbance with many similarities to patients with DCS. However, the central and peripheral nervous system abnormalities seen in CS were quite distinct from those seen in DCS. Neuroradiological scanning, biopsy, or autopsy examinations of CS patients showed a marked generalized demyelination in addi-

tion to cerebral and cerebellar atrophy, and most patients had intracranial calcifications within the basal ganglia (Ohnishi et al. 1987; Nishio et al. 1988; Bolsthauser et al. 1989). A distinctive skeletal dysplasia has also been reported (Silengo et al. 1986). A photosensitive dermatitis was usually present, but development of cutaneous malignancies did not occur (Otsuka and Robbins 1985).

The results of neurotransmitter analyses of CFS taken from GU and AU are interesting and have not, to our knowledge, been documented previously in either XP or CS. Expected levels of HVA and MHPG were found, but levels of 5-HIAA were significantly decreased. CSF contents of HVA and MHPG are good indexes of CNS dopamine and norepinephrine metabolism, respectively (Seifert et al. 1980). The content of 5-HIAA indirectly reflects serotonin metabolism, and CSF deficiency of this metabolite indicates abnormal serotonergic neuronal function. Serotonin-containing neurons cluster in or near the midline of the pons and upper brain stem, a region that MRI shows as being affected in these two subjects. Furthermore, these neurons project to other brain regions, including the cerebral and cerebellar hemispheres (Cooper et al. 1986), and thus present a possible explanation for the central neurologic deficits such as growth deficiency, microcephaly, and loss of developmental milestones. Similar findings have been observed in patients with OPCA from other causes (Duvoisin and Plaitakis 1984). It seems likely that the changes seen are the result of neuronal atrophy in the affected areas rather than of hypotrophy, since early development was normal. The reason that serotonergic neurons either are more sensitive indicators of internal mutagens or have poorer DNA repair mechanisms than do other neurons is unclear. Also unclear is how the relatively mild skin changes and intact in vitro excision repair correlate with the severe neurologic problems observed clinically.

A review of the presentations of our patients strongly suggests they have DCS, a subgroup of XP, rather than CS. The clinical skin and neurologic manifestations are typical. Although no cutaneous malignancies were found, skin biopsy showed evidence of premature solar damage with changes typical of XP. Sural nerve, conjunctival, and muscle-biopsy studies all showed abnormalities consistent with axonal degeneration rather than demyelination. MRI scanning showed OPCA, a finding well documented in DCS but not reported in CS. Furthermore, within the basal ganglia intracranial calcifications suggestive of CS were not seen.

Therefore, our patients pose a special difficulty in classification with respect to XP and CS. Even though solar sensitivity, neurologic dysfunction, microcephaly, premature solar damage, in vitro UV sensitivity, and a special central and peripheral neuropathy are consistent with a diagnosis of DCS, and even though major clinical criteria for the diagnosis of CS are absent, significant deficits in DNA excision repair or replication in fibroblasts from these patients could not be identified. Rather, a prolonged suppression of RNA synthesis after UV exposure in vitro, a well documented finding in CS, was observed (table 5).

This family is not the only example of a clinical presentation which resembles XP but without in vitro findings typical of the excision-defective XP or XP variant groups. In a survey of XP cases in Egypt, two unrelated patients were examined who unequivocally had XP clinically but who, at the cellular level, after UV irradiation, exhibited normal UV sensitivity, excision repair, and DNA replication (J. German, N. Hashem, D. Karentz, and J. E. Cleaver, unpublished observations). Another patient (cell line XP3BR) with clinical immunodeficiency and cellular sensitivity to DNA-damaging agents has yet to be given a specific clinical diagnosis or to show demonstrable repair deficiency (Teo et al. 1982, 1983). Another case (GM2881) has been reported with apparent dominant inheritance of XP-like symptoms, but no other cellular abnormalities in DNA repair or replication were found (Cleaver et al. 1981*b*). In the patient surveys carried out by one of our laboratories (J.E.C. and G.H.T.) over the past 6 years, cells from 57 patients have been analyzed on the basis of a clinical presentation sufficiently close to XP to merit evaluation. Of these, 24 did not exhibit either in vitro UV sensitivity or DNA excision-repair deficits, a number too large for all to be XP variants.

In addition to these patients with clinical presentations suggestive of XP, two groups of individuals with trichothiodystrophy (TTD) and deficient ability to repair UV-induced DNA damage have been described (Broughton et al. 1990). Cell strains from one group (type 2) were very sensitive to the lethal effects of UV radiation and were dimer excision-repair deficient, with in vitro properties indistinguishable from those of XP group D. Cells from a second group (type 3) had normal in vitro survival after UV exposure but showed reduced rates of both incision and repair synthesis.

Therefore, as in the present sibship, a significant number of patients have been identified whose clinical features or in vitro cellular behavior overlap with those of XP. This reflects the complexities of the repair

Table 5

Clinical and Laboratory Features

Type of Feature	DCS	CS	AU/GU
Solar sensitivity	+	+	+
Actinic skin changes	+	−	+
Skin cancers	+	−	−
Eye findings:			
Optic atrophy	−	+	+
Conjunctivitis	+	−	+
Cataracts	−	+	−
Lid atrophy and neoplasia	+	−	−
Corneal ulcers and scarring	+	−	−
Pigmentary retinopathy/degeneration	−	+	−
Other:			
Mental retardation	+	+	+
Seizures	+	−	+(AU)
Sensorineural hearing loss	+	+	+
Microcephaly	+	+	+
Hyporeflexia	+[a]	−	+
Poor linear growth	+	+	+
Cachetic dwarfism	−	+	−
Skeletal dysplasia	−	+	−
Large ears, beak nose, and long extremities	−	+	−
Brain imaging:			
Cortical atrophy	+	+	+
Cerebellar atrophy	−	+	−
OPCA	+	−	+
Demyelination	−	+	−
Basal ganglia calcifications	−	+	−
Peripheral nerve biopsy:			
Patchy or generalized demyelination	−	+	−
Neuronal degeneration	+	−	+
Axon dropout	+	−	+
Laboratory:			
UV sensitivity	+	+	+
Dimer excision-repair defect	+	−	−
Delay in RNA synthesis recovery after UV exposure	−	+	+

[a] Progressive.

systems involved, the poorly understood nature of these systems, and the difficulties encountered in attempting to define these systems in the laboratory. For instance, previously reported typical cases of DCS generally demonstrated good correlation between in vitro fibroblast survival and excision-repair ability and the severity of neurologic involvement (Andrews et al. 1978). However, the neurologic involvement seen in XP and DCS cannot be wholly explained by the degree of fibroblast UV sensitivity or excision-repair defect, since neurons are not exposed to UV radiation in vivo. Patients with DCS often belong to XP group D, a group with high UV sensitivity and very poor ability to repair UV-damaged fibroblast DNA. However, not all patients in group D have neurologic problems (Cleaver and Kraemer 1989). So, even within complementation groups the specific repair defect may vary. Furthermore, patients in group C, whose fibroblasts typically also have excision-repair ability only 10%–20% that of normal cells, do not, with one possible exception, have any neurologic sequelae (Robbins 1989). Thus, the degree of fibroblast UV sensitivity does not necessarily reflect either the pathogenesis of the repair defect or the extent of its effects. It is possible that, although our patients and those with DCS, CS, and TTD each have distinct presentations, they may share similar defective DNA repair systems.

It is doubtful that these atypical cases can be classified using currently established criteria. Both susceptibility to DNA damage and the ability to repair such damage appear to be complex processes and may vary from tissue to tissue. Additional explanations of the

actinic sensitivity and neurological complications of XP may be necessary. Recently, differences in the rates of repair of UV-induced damage in human cells, differences that are based on the specific type of lesion produced (cyclobutane pyrimidine dimers vs. 6-4 photoproducts; Broughton et al. 1990) and on the transcriptional state of the damaged gene (active versus inactive; Venema et al. 1990), have been demonstrated. Thus, when a cell is not repair competent, the type of accumulated damage, the specific genes involved, and the importance of the damaged genes to the function of the specific cell or tissue type seem critical in producing the phenotype observed. It could be expected, then, that in conditions which result from abnormalities of DNA repair, clinical heterogeneity would be the rule rather than the exception. The most important next step in the analysis of our patients will be to determine their relationship to known CS complementation groups. This will involve cell fusion studies with CS groups A and B cell lines, as well as determination of rates of RNA synthesis after UV irradiation in cell hybrids (Lehmann 1982). These experiments are underway. Complementation against the XP groups or CS group C would not be feasible, because these groups are characterized by deficient excision repair, whereas our patients are competent at such repair. It would also be interesting to see whether a specific defect in the repair of transcriptionally active genes could be demonstrated.

When sufficient numbers of bona fide XP and CS genes have been cloned, atypical patients such as AU and GU may provide important insights into the molecular basis of DNA repair mechanisms. At this moment, only two genes have been cloned for XP and CS (Tanaka et al. 1989, 1990; Rinaldy et al. 1990; Weeda et al. 1990). These correspond to XP complementation group A (Rinaldy et al. 1988, 1990; Tanaka et al. 1989; 1990) and to XP group B, which is the same as CS group C and the hamster complementation group ERCC3 (Weeda et al. 1990). Of special interest is the XP-B/CS-C/ERCC3 gene product. The gene coding this protein, probably a helicase, contains a mutation that causes abnormal splicing of mRNA. This defect was identified in the heterozygous state, in both the patient and her clinically normal mother. The mutation in the other defective allele, presumably received from the father, has not yet been elucidated. Such variety of mutation is common among heritable conditions, and patients with the same clinical disorder may have diverse DNA mutations within the same gene. This presumed allelic heterogeneity may explain the clinical variability seen in these disorders. It seems obvious that many different gene products are involved in DNA repair. Mutations either in the genes coding for these proteins or in genes involved in their regulation could have diverse effects on the competency and efficiency of these repair systems, resulting in a variety of clinical phenotypes difficult to categorize and making the characterization and biochemical classification of conditions with abnormal DNA repair quite challenging.

Acknowledgments

This work was supported in part by the U.S. Department of Energy, Office of Health and Environmental Research, contract DE-AC03-76-SF01012. We are grateful to W. C. Charles for technical assistance in performing the alkaline sucrose gradient analyses. We also are thankful to J. Lynn Grace for assistance in the preparation of the manuscript.

References

Ahmed FE, Setlow RB (1978) Excision repair in ataxia telangiectasia, Fanconi's anemia, Cockayne syndrome, and Bloom's syndrome after treatment with ultraviolet radiation and N-acetoxy-2-acetylaminofluorene. Biochim Biophys Acta 521:805–809

Andrews AD, Barrett SF, Robbins JH (1978) Xeroderma pigmentosum neurologic abnormalities correlate with colony-forming ability after ultraviolet radiation. Proc Natl Acad Sci USA 75:1984–1988

Bolsthauser E, Yalcinkaya C, Wichmann W, Reutter F, Prader A, Valavanis A (1989) MRI in Cockayne syndrome type 1. Neuroradiology 31:276–277

Broughton BC, Lehmann AR, Harcourt SA, Arlett CF, Sarasin A, Kleijer WJ, Beemer FA, et al (1990) Relationship between pyrimidine dimers, 6-4 photoproducts, repair synthesis and cell survival: studies using cells from patients with trichothiodystrophy. Mutat Res 235:33–40

Cleaver JE (1975) Methods for studying repair of DNA damaged by physical and chemical carcinogens. In: Busch H (ed) Methods in cancer research, vol 11. Academic Press, New York, pp 123–165

Cleaver JE, Charles WC, Kong SH (1984) Efficiency of repair of pyrimidine dimers and psoralen monoadducts in normal and xeroderma pigmentosum human cells. Photochem Photobiol 40:621–629

Cleaver JE, Greene AE, Coriell LL, Mulivor RA (1981*a*) Xeroderma pigmentosum variants. Cytogenet Cell Genet 31:188–192

Cleaver JE, Greene EA, Coriell LL, Riccardi VM (1981*b*) An autosomal dominant inheritance for multiple sunlight-induced malignancy in a patient without abnormalities in DNA repair or replication: repository identification no. GM2881. Cytogenet Cell Genet 29:122–124

Cleaver JE, Kraemer KH (1989) Xeroderma pigmentosum.

In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds) Metabolic basis of inherited disease, McGraw-Hill, New York, pp 2949–2971

Cleaver JE, Thomas GH (1988) Rapid diagnosis of sensitivity to ultraviolet light in fibroblasts from dermatologic disorders, with particular reference to xeroderma pigmentosum. J Invest Dermatol 90:467–471

Cooper JR, Bloom FE, Roth RH (1986) The biochemical basis of neuropharmacology. Oxford University Press, New York

Dupuy JM, Moshell AN, Lutzner MA, Robbins JH (1982) A new patient with both xeroderma pigmentosum and Cockayne syndrome is not complementation group B. J Invest Dermatol 78:356

Duvoisin RC, Plaitakis A (eds) (1984) Advances in neurology: the olivopontocerebellar atrophies. Raven, New York

Ehmann UK, Lett JT (1973) Review and evaluation of molecular weight calculations from the sedimentation profiles of irradiated DNA. Radiat Res 54:152–162

Kaufmann WK, Cleaver JE (1981) Mechanisms of inhibition of DNA replication by ultraviolet light in normal human and xeroderma pigmentosum fibroblasts. J Mol Biol 149:171–187

Kraemer KH, Lee MM, Scotto J (1987) Xeroderma pigmentosum. Arch Dermatol 123:241–250

Lehmann AR (1982) Three complementation groups in Cockayne syndrome. Mutat Res 106:347–356

Lehmann AR, Kirk-Bell S, Arlett CF, Paterson MC, Lohman PH, de Weerd-Kastelein EA, Bootsma D (1975) Xeroderma pigmentosum cells with normal levels of excision repair have a defect in DNA synthesis after UV-irradiation. Proc Natl Acad Sci USA 72:219–223

Mayne LV, Lehmann AR (1982) Failure of RNA synthesis to recover after UV-irradiation: an early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. Cancer Res 42:1473–1478

Nishio H, Kodama S, Matsuo T, Ichihashi M, Ito H, Fujiwara Y (1988) Cockayne syndrome: magnetic resonance images of the brain in a severe form with early onset. J Inherited Metab Dis 11:88–102

Ohnishi A, Mitsudome A, Murai Y (1987) Primary segmental demyelination in the sural nerve in Cockayne's syndrome. Muscle Nerve 10:163–167

Otsuka F, Robbins JH (1985) The Cockayne syndrome—an inherited multisystem disorder with cutaneous photosensitivity and defective repair of DNA. Am J Dermatopathol 7:387–392

Reed WB, Sugarman GI, Mathis RA (1977) DeSanctis-Cacchione syndrome. Arch Dermatol 113:1561–1563

Rinaldy A, Bellew T, Egli E, Lloyd RS (1990) Increased UV resistance in xeroderma pigmentosum group A cells after transformation with a human genomic DNA clone. Proc Natl Acad Sci USA 87:6818–6822

Rinaldy A, Dodson ML, Darling TL, Lloyd RS (1988) Gene cloning using cDNA libraries in a differential competition hybridization strategy: application to cloning XP-A related genes. DNA 7:563–570

Robbins JH (1989) A childhood neurodegeneration due to defective DNA repair: a novel concept of disease based on studies of xeroderma pigmentosum. J Child Neurol 4:143–146

Robbins JH, Kraemer KH, Lutzner MA, Festoff BW, Coon HG (1974) Xeroderma pigmentosum: an inherited disease with sun sensitivity, multiple cutaneous neoplasms and abnormal DNA repair. Ann Intern Med 80:221–248

Seifert WE, Foxx JL, Butler IA (1980) Age effect on dopamine and serotonin metabolite levels in cerebrospinal fluid. Ann Neurol 8:38–42

Silengo MC, Franceschini P, Bianco R, Biagioli M, Pastorin L, Vista N, Baldassar A, et al (1986) Distinctive skeletal dysplasia in Cockayne syndrome. Pediatr Radiol 16:264–266

Tachi N, Sasaki K, Kusano T, Wakai S, Nagaoka M, Annaka S, Minami R, et al (1988) Peripheral neuropathy in four cases of group A xeroderma pigmentosum. J Child Neurol 3:114–119

Tanaka K, Miura N, Satakata I, Miyamoto I, Yoshida MC, Satoh Y, Kondo S, et al (1990) Analysis of a human DNA excision repair gene involved in group A xeroderma pigmentosum and containing a Zinc-finger domain. Nature 348:73–76

Tanaka K, Satokata I, Ogita Z, Uchida T, Okada Y (1989) Molecular cloning of a mouse DNA repair gene that complements the defect of group A xeroderma pigmentosum. Proc Natl Acad Sci USA 86:5512–5516

Teo IA, Arlett CF, Harcourt SA, Priestly A, Broughton BC (1982) Multiple hypersensitivity to mutagens in a cell strain (46BR) derived from a patient with immunodeficiencies. Mutat Res 107:371–376

Teo IA, Broughton BC, Day RS, James MR, Karran P, Mayne LV, Lehmann AR (1983) A biochemical defect in the repair of alkylated DNA in cells from an immunodeficient patient (46BR). Carcinogenesis 4:559–564

Timme TL, Moses RE (1988) Review: diseases with DNA damage-processing defects. Am J Med Sci 295:40–48

Venema J, Mullenders LHF, Natarajan AT, van Zeeland AA, Mayne LV (1990) The genetic defect in Cockayne syndrome is associated with a defect in repair of UV-induced DNA damage in transcriptionally active DNA. Proc Natl Acad Sci USA 87:4707–4711

Weeda G, van Ham RCA, Vermeulen W, Bootsma D, van der Eb AJ, Hoeijmakers JHJ (1990) A presumed DNA helicase encoded by ERCC-3 is involved in the human repair disorders xeroderma pigmentosum and Cockayne's syndrome. Cell 62:777–791