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Defects in the DNA Repair and Transcription Gene *ERCC2(XPD)* in Trichothiodystrophy

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Summary

Trichothiodystrophy (TTD) is a rare autosomal recessive disorder characterized by brittle hair with reduced sulfur content, ichthyosis, peculiar face, and mental and growth retardation. Clinical photosensitivity is present in ~50% of TTD patients but is not associated with an elevated frequency of cancers. Previous complementation studies show that the photosensitivity in nearly all of the studied patients is due to a defect in the same genetic locus that underlies the cancer-prone genetic disorder xeroderma pigmentosum group D (XP-D). Nucleotide-sequence analysis of the *ERCC2* cDNA from three TTD cell strains (TTD1VI, TTD3VI, and TTD1RO) revealed mutations within the region from amino acid 713–730 and within previously identified helicase functional domains. The various clinical presentations and DNA repair characteristics of the cell strains can be correlated with the particular mutations found in the *ERCC2* locus. Mutations of Arg658 to either His or Cys correlate with TTD cell strains with intermediate UV-sensitivity, mutation of Arg722 to Trp correlates with highly UV-sensitive TTD cell strains, and mutation of Arg683 to Trp correlates with XP-D. Alleles with mutation of Arg616 to Pro or with the combined mutation of Leu461 to Val and deletion of 716–730 are found in both XP-D and TTD cell strains.

Introduction

Trichothiodystrophy (TTD) is a rare autosomal recessive disorder characterized by brittle hair with reduced sulfur content, ichthyosis, peculiar face, and mental and growth retardation (Itin and Pittelkow 1990). Clinical photosensitivity is present in ~50% of TTD patients but is not associated with an elevated frequency of cancers. Analysis of the DNA repair characteristics of cells from TTD patients has revealed a remarkable heterogeneity. Initial studies reported that some cells show normal DNA repair, some show a severe deficiency in nucleotide-excision repair, and some show a specific deficiency in the repair of UV-induced (6-4)photoproducts by nucleotide-excision repair (Lehmann et al. 1988; Broughton et al. 1990). Strains reported as having normal repair of cyclobutane dimers and reduced repair of (6-4)photoproducts were also found to demonstrate UV sensitivity only at doses $>\sim 10$ J/m^2. However, a recent study, which includes the same cell strains, reports that all photosensitive TTD strains studied display wild-type or near-wild-type (50%–70% of normal) repair of (6-4)photoproducts in the overall genome and reduced repair (10%–50% of normal) of cyclobutane dimers (Eveno et al. 1995).

Complementation studies show that the photosensitivity in nearly all of the studied TTD patients is due to a defect in the same genetic locus that underlies the cancer-prone genetic disorder xeroderma pigmentosum (XP) group D (Stefanini et al. 1993*a*). Some XP group D patients present with clinical symptoms characteristic of both XP and Cockayne syndrome (CS; Johnson and Squires 1992). The DNA repair and transcription gene *ERCC2* has been shown to correct the photosensitivity of XP-D, XP-D/CS, and the major group of TTD cells (Flejter et al. 1992; Lehmann et al. 1992; Mezzina et al. 1994; Takayama et al., 1995). Mutations in the *ERCC2* gene in five XP-D, two XP-D/CS, and four TTD cell strains have been reported (Broughton et al. 1994, 1995; Frederick et al. 1994; Takayama et al. 1995), demonstrating that *ERCC2* is the gene underlying the repair defect in this complementation group comprising these three clinically distinct disorders.

The ERCC2 protein is highly conserved with 52%, 55%, 83%, and 98% identity to Rad3 from *Saccharomyces cerevisiae* (Weber et al. 1990), Rad15 from *Schizosaccharomyces pombe* (Murray et al. 1992), ERCC2 from *Xiphophorus maculatus* (Coletta et al.

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1995), and ERCC2 from Chinese hamster (Kirchner et al. 1994), respectively. The human ERCC2 and yeast Rad3 proteins have single-stranded DNA-dependent ATPase and ATP-dependent 5′→3′ DNA helicase activities (reviewed by Prakash et al. 1993; Sung et al. 1993). DNA•RNA helicase activity has also been demonstrated for Rad3 (Bailly et al. 1991).

Defects in two other genes, *TTDA* and *ERCC3/XPB,* can be responsible for the photosensitivity of TTD patients. There are one known patient (TTD1BR) in TTD group A (Stefanini et al. 1993*b*) and two known TTD patients (siblings TTD4VI and TTD6VI) with a defect in *ERCC3/XPB* (Vermeulen et al. 1994). The genes for all three photosensitive TTD complementation groups encode subunits of transcription initiation factor TFIIH (Schaeffer et al. 1993, 1994; Drapkin et al. 1994; Vermeulen et al. 1994). TFIIH is required for transcription initiation by RNA polymerase II and for nucleotide-excision repair (Drapkin et al. 1994). We report here the causative mutations in the *ERCC2/XPD* gene in three TTD cell strains and correlate the mutations with the clinical presentations and DNA repair characteristics.

Material and Methods

Cell Strains and Culture Conditions

The origins of TTD1VI and TTD3VI are described by Sarasin et al. (1992) and of TTD1RO are described by Kleijer et al. (1994). Cells were cultured in α-modified Eagle's medium (MEM) or Eagle's MEM with 15% fetal bovine serum, 1.5 μg/ml glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin.

Cell Survival after UV Irradiation

Cell survival was measured by the colony-forming ability of cells following UV-irradiation, as described by Lehmann et al. (1977).

DNA Repair Measurements by Unscheduled DNA Synthesis (UDS)

Low serum, coverslip cultures of skin fibroblasts were prelabeled with 370 KBq ^{3}H-thymidine (284 GBq/mmol; New England Nuclear) per milliliter 1 h before UV-irradiation. The coverslip cultures were then washed twice with PBS and irradiated under a germicidal lamp (primarily 254 nm) with a dose rate of 0.12 J/m^2/s, at the doses indicated. The cells were further labeled in growth medium containing ^{3}H thymidine for 2 h, followed by a 1 h chase incubation with cold thymidine (10^{-5} M). The coverslip cultures were then washed, fixed in methanol, and dried. The mounted coverslip cultures were treated with emulsion (Ilford Nuclear Research) and exposed for 1 wk at 4°C. After development, the cells were stained with Giemsa, and the average number of grains over the nucleus scored, cells undergoing replication synthesis being excluded.

Reverse Transcription and PCR Amplification

For TTD1VI and TTD3VI, RNA isolation, reverse transcription, and PCR amplification, using primers determined from the cDNA sequence (table 1), were carried out as described by Takayama et al. (1995). For TTD1RO, RNA isolation, reverse transcription, and PCR amplification were performed as described by Broughton et al. (1994).

Genomic DNA Preparation and PCR Amplification

Genomic DNA was prepared and PCR amplification was carried out as described by Takayama et al. (in press) for TTD1VI and TTD3VI and by Broughton et al. (1994) for TTD1RO.

Cloning and Nucleotide-Sequence Analysis

For TTD1VI and TTD3VI, PCR products were cloned (pGEM-T vector; Promega) and plasmid DNA was prepared and analyzed as described by Takayama et al. (1995) with 8–15 clones per pool for cDNA analysis and 5–12 clones per pool for genomic DNA analysis. For TTD1RO, procedures were as described by Broughton et al. (1994).

Results

Nucleotide-Sequence Analysis of ERCC2 in TTD Cells

To determine the defects in *ERCC2* responsible for the UV-sensitivity of TTD1VI, TTD3VI, and TTD1RO cells, the nucleotide sequence of the coding region of the *ERCC2* gene in these cells was analyzed by using overlapping fragments obtained by reverse transcription and PCR amplification (see Material and Methods).

TTD1VI

Patient TTD1VI, a boy, was born in 1986 at 33 wk gestation with markedly reduced weight (1,480 g), cranial circumference, and body length. At birth, he had scaling feet, and his hair was short and brittle. At 9 mo, his weight and height were below the third percentile, and he was unable to sit up. He had bilateral cataracts, moderately ichthyotic skin, nail abnormalities, spastic neurological abnormalities, and severe photosensitivity. There was a deficiency of sulfur-containing amino acids in the hair (cystine content reduced by ~30%). He died at the age of 12 mo from cachexia and septicaemia (Sarasin et al. 1992). The cell strain is very sensitive to UV light and exhibits a low level of UDS, and the initial study reported that it is deficient in the removal of both cyclobutane dimers and (6-4)photoproducts (table 2; Broughton et al. 1990). The recent study reports that it is deficient in the removal of cyclobutane dimers and

Table 1

PCR Primers for Amplification of *ERCC2/XPD* from TTD1VI and TTD3VI

Amplified Region[a]	5′ Primer	3′ Primer	Size (bp)
51–790	GACCCCGCTG CACAGTCCGG	TGTGGGCCTC GTCGAAGACC	740
483–2090	CAGCCTCACA GCCTCCTATG	TTGCCCCTGA TGGCCCGACC	1,608
1001–2397	CCGTGCTGCC CGACGAAGTG	GTCACCAGGA ACCGTTTATG GCCCCACCCG	1,397
2001–2397[b]	GGACCAGTTC CAGATTCGTG	GTCACCAGGA ACCGTTTATG GCCCCACCCG	1,037

[a] Base numbers are cDNA position, where coding is from 79 to 2361 (Weber et al. 1990).
[b] The final fragment is amplified from genomic DNA. Base numbers are the corresponding cDNA base position, because complete genomic sequence data are not available.

near wild type in the removal of (6-4)photoproducts (table 2; Eveno et al. 1995).

In one allele of TTD1VI, three alterations in *ERCC2* were identified: (1) a silent A-to-C transversion at position 546; (2) a C-to-G transversion at position 1459 resulting in a conservative Leu461-to-Val substitution in helicase domain III; and (3) a 45-base deletion (bases 2224–2268) resulting in a 15–amino acid deletion near the C-terminus (amino acids 716–730) (fig. 1*B*). Position 1459 is within both the 483–2090 and the 1001–2397 cloned PCR-amplified fragments. Thus, we were able to determine that all three alterations are from one allele (fig. 1*B*). The equivalent of amino acid position 461 is Leu in the *S. pombe,* fish, and hamster homologues, but is Ile in the *S. cerevisiae* homologue (fig. 2*A*). The 15–amino acid deletion includes a region with 8 of 10 positions identical in all five homologues (fig. 2*B*).

Genomic DNA analysis revealed a C-to-G transversion at base 2228 of the corresponding cDNA that creates a new splice-donor site for the final intron resulting in the 45-base deletion (fig. 1*E*). When a matrix was used to score potential splice-site sequences (Stormo 1987), the new splice donor site created at 2223 scored +52, whereas prior to the mutation this site scored +5. Although not altered, the donor at 2268, which scored +49, is apparently not used when the mutation creating the site at 2223 is present.

The second *ERCC2* allele from TTD1VI also has three alterations: (1) a silent A-to-C transversion at position 546; (2) a silent C-to-T transition at position 2211; and

Table 2

Characteristics of the Patients and Cell Strains

Cell Strain	Clinical Phenotype	UDS (% of normal)	Cell Survival (UV) (D_{37} % of normal)	Cyclobutane Dimer Repair[a] (% of normal)	(6-4)photoproduct Repair[b] (% of normal)
TTD1VI	TTD; male	20[c]–35[d] (28[e])	18[c]–21[e]	40[c,f]	18[c]; 90[f]
TTD3VI	TTD; male	30[e]–40[d]	50[e]–57[d]	...	...
TTD1RO	TTD; female	35–50[d,g]	50[d]–67[c]	100[c]; 40[f]	50[c]; 95[f]
TTD1BEL	TTD; male	15[h]	20[h]	...	...
XP102LO	XP-D; female	10[i]–20[j]	23[i]	0[j]	15[j]
XP17PV	XP-D; male	5[k]	12[l]	...	...

[a] Cyclobutane dimers removed 24 h after 8 Jm^{-2} UV irradiation (10 Jm^2 for XP102LO).
[b] (6-4) photoproducts removed 6 h after 10 Jm^{-2} UV irradiation.
[c] Broughton et al. 1990.
[d] Stefanini et al. 1993*a*.
[e] This study. UDS computed at 5, 10, and 15 Jm^{-2}.
[f] Eveno et al. 1995.
[g] Kleijer et al. 1994.
[h] Broughton et al. 1994.
[i] Johnson et al. 1985.
[j] Johnson and Squires 1992.
[k] Mariani et al. 1992.
[l] Stefanini et al. 1992.

(3) a C-to-T transition at position 2242, resulting in a nonconservative Arg722-to-Trp substitution (fig. 1*B*). The equivalent amino acid position is Arg in all five homologues (fig. 2*B*).

TTD3VI

Patient TTD3VI, a boy, was born in 1979. He was hospitalized for extreme light sensitivity and growth retardation (−1 SD). He had typical PIBI(D)S symptoms (Photosensitivity, Ichthyosis, Brittle hair, Intellectual impairment, Decreased fertility, and Short stature) at the age of 6–7 years. There was a congenital cataract, low serum concentration of vitamin B6, and hyper-IgE (Van Neste et al. 1989). The cell strain derived from an unexposed part of the body is sensitive to UV at doses >10 J/m^2 (a survival pattern characteristic of strains initially reported to display normal repair of cyclobutane dimers and reduced repair of [6-4] photoproducts) and exhibits a low level of UDS (table 2; Stefanini et al. 1993*a*).

In one allele of TTD3VI, the same three alterations in *ERCC2* as in one allele of TTD1VI were identified, namely, (1) a silent A-to-C transversion at position 546; (2) a C-to-G transversion at position 1459, resulting in a conservative Leu461-to-Val substitution in helicase domain III; and (3) a 45-base deletion (bases 2224–2268) resulting in a 15–amino acid deletion near the C-terminus (amino acids 716–730) (fig. 1*C*). Again, genomic DNA analysis revealed a C-to-G transversion at base 2228 of the corresponding cDNA.

The second allele of TTD3VI has a G-to-A transition

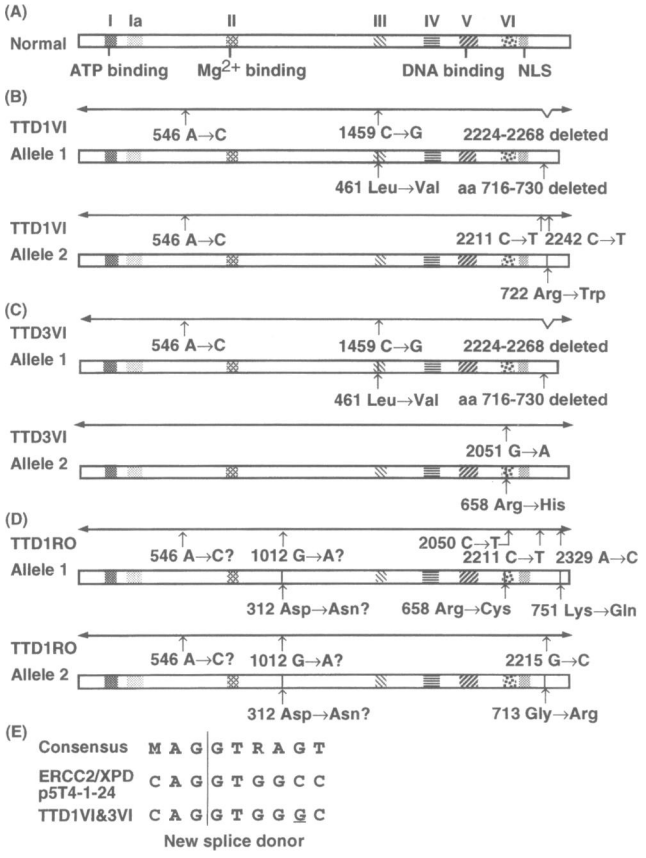

Figure 1 *ERCC2* cDNA mutations and resulting ERCC2 protein alterations, diagrammed for TTD1VI (*B*), TTD3VI (*C*), and TTD1RO (*D*). Mutations with a question mark (?) are heterozygous in that cell strain, but the linkage relationship with the other mutations has not been determined. Numbers below cDNAs indicate the affected nucleotide positions where coding is from 79 to 2361, including the stop codon. Numbers below proteins indicate the affected amino acid positions. Normal ERCC2 protein, with the seven conserved domains identified for a superfamily of known and putative helicases (I, Ia, and II-VI) (Gorbalenya et al. 1989) and the putative NLS (Weber et al. 1990) regions marked, is shown in *A*. The region of the base substitution in the genomic DNA causing the splicing alteration seen in allele 1 of TTD1VI and TTD3VI is shown in *E*. p5T4-1-24 is a functional genomic cosmid clone of *ERCC2* (Weber et al. 1988).

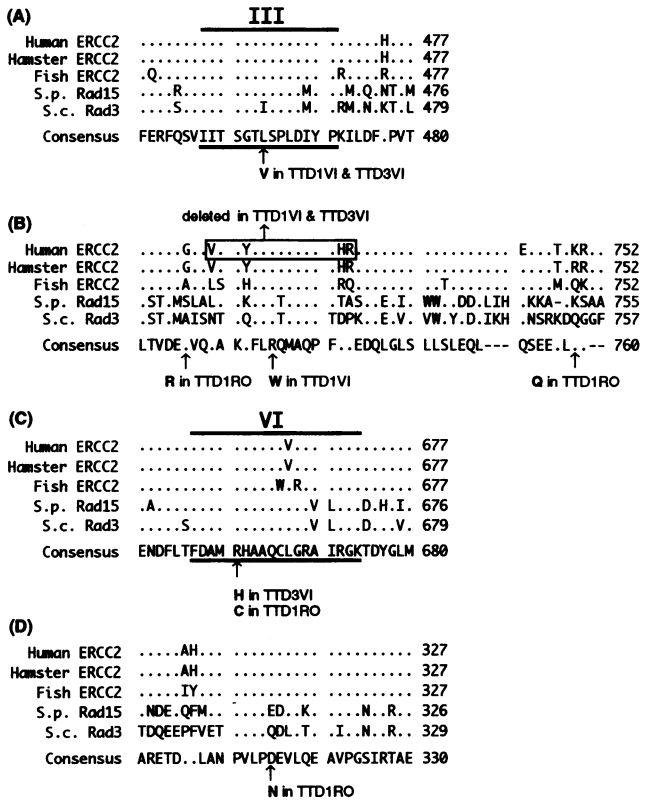

Figure 2 Alignment of the ERCC2/XPD homologues from five species in the regions of the TTD1VI (*A* and *B*), TTD3VI (*A*, *B*, and *C*), and TTD1RO (*B*, *C*, and *D*) mutations. Rad15 is from *S. pombe*, hamster ERCC2 is from Chinese hamster, fish ERCC2 is from *Xiphophorus maculatus*, and Rad3 is from *S. cerevisiae* (SwissProt accession nos.: Rad15, P26659; human ERCC2, P18074; Rad3, P06839; and genomic DNA sequence Genbank accession nos.: hamster ERCC2, U04967 and U04968; fish ERCC2, 417896). The under- and overlined regions are the conserved helicase domains (see legend, fig. 1). Arrows indicate the position of the identified amino acid substitution, and boxed amino acids represent deletions in the corresponding cell strain.

at position 2051, resulting in a conservative Arg-to-His substitution at position 658 in helicase domain VI (fig. 1C). The equivalent amino acid position is Arg in all five homologues (fig. 2C).

TTD1RO

Patient TTD1RO, a girl, was born at 37 wk gestation. Birth weight was 2,140 g (P30 [percentile]) and length was 44 cm (P<3). She had growth retardation (−5 SD for height and −4 SD for both weight and head circumference at the age of 2 years), mental retardation, mild psychomotor retardation, bilateral cataracts, and photosensitivity. Periodically she almost completely lost her scalp hair, which subsequently regrew. Ichthyosis was not present from birth but appeared intermittently, together with respiratory tract infections and nearly complete loss of scalp hair (which would subsequently regrow with a simultaneous improvement in skin symptoms). She died unexpectedly in her sleep at 2 years and 8 mo of age (Kleijer et al. 1994). The fibroblast cell strain is sensitive to UV at doses >10 J/m^2 and exhibits a moderate level of UDS (table 2; Broughton et al. 1990; Stefanini et al. 1993*a*). The initial study of this strain found that excision of cyclobutane dimers is normal, but removal of (6-4)photoproducts is deficient (table 2; Broughton et al. 1990). The recent study of this strain found that repair of cyclobutane dimers is deficient and repair of (6-4)photoproducts is near wild type (table 2; Eveno et al. 1995).

One *ERCC2* allele of TTD1RO has at least three changes: (1) a C-to-T transition at position 2050, resulting in an Arg658-to-Cys substitution in helicase domain VI; (2) a silent C-to-T transition at position 2211; and (3) an A-to-C transversion at position 2329, resulting in a Lys751-to-Gln substitution (fig. 1*D*). Arg658 is the same conserved Arg that is mutated to His in TTD3VI (fig. 2C). For Lys751, the equivalent amino acid position is also Lys in *S. pombe* but is Arg in the hamster homologue and Gln in the fish and *S. cerevisiae* homologues (fig. 2*B*).

The second *ERCC2* allele of TTD1RO has at least one change, a G-to-C transversion at position 2215, resulting in a Gly713-to-Arg substitution (fig. 1*D*). The equivalent amino acid position is also Gly in the hamster homologue but is Ala in the fish and *S. cerevisiae* homologues and Ser in the *S. pombe* homologue (fig. 2*B*).

In addition, there are two heterozygous changes for which the linkage relationship to the other mutations has not been determined. These are: (1) a silent A-to-C transversion at position 546 and (2) a G-to-A transition at position 1012, resulting in an Asp312-to-Asn substitution. The equivalent amino acid position for Asp312 is also Asp in the hamster and fish homologues but is Gln in the *S. cerevisiae* homologue and Glu in the *S. pombe* homologue (fig. 2*D*).

The mutation at 2050 results in loss of a *Hha*I site. Restriction-site analysis shows loss of this site in the patient and mother but not the father, indicating that this allele is inherited from the mother (data not shown). Direct-sequence analysis confirms that the 2215 mutation is inherited from the father.

A summary of the TTD and XP-D cell strains in which each of these mutant alleles has been identified and the associated characteristics predicted for these alleles are presented in table 3. The UDS, cell-survival, and repair data for these cell strains are presented in table 2.

Discussion

In this report, we identify the causative mutations in the *ERCC2/XPD* gene underlying the photosensitivity of three TTD cell strains. In addition, we identify correlations between the particular *ERCC2* mutations and the various clinical presentations and DNA repair characteristics of the cell strains.

The C-to-T transitions at 2211, 2242, and 2050 and the G-to-A transitions at 1012 and 2051 (C to T on the noncoding strand) are at CpG sites and probably arise from deamination of 5-methylcytosine. In allele 2 of TTD1VI and of TTD3VI, the single altered amino acids are at positions that are identical in all five homologues, indicating that these are the causative mutations. Allele 1 of TTD1VI and TTD3VI has both a conservative Leu461-to-Val substitution in helicase domain III and a deletion of amino acids 716–730. The Leu461-to-Val substitution is also present in the XP group D cell strain XP1NE, and an expression construct with the Leu461-to-Val mutation is unable to correct the UV-sensitivity of mutant cells (Frederick et al. 1994). The deletion of amino acids 716–730 includes a 10–amino acid region with very high evolutionary conservation. Additional evidence that this region is functionally important is the Arg722-to-Trp mutation found in both TTD1VI and TTD1BEL (Broughton et al. 1994). It is very likely that such a drastic change in a highly conserved region of the gene will have a dramatic effect on protein function. Thus, both alterations in this allele are likely to be functionally important, causative mutations. In TTD1RO, the Asp312-to-Asn and the Lys751-to-Gln changes are at nonconserved amino acid positions and have been found in the homozygous state in normal individuals (Broughton et al., in press). Thus, these changes are unlikely to affect the protein function. The remaining amino acid change in TTD1RO allele 1 (Arg658 to Cys) is at a position that is identical in all five homologues, indicating that it is the causative mutation. In TTD1RO allele 2, the remaining altered amino acid is a nonconservative Gly-to-Arg substitution at a position that is identical or has conservative substitutions (on the basis of the functional group and polarity of the residues) among

Am. J. Hum. Genet. 58:263–270, 1996

Table 3

Mutant Alleles Found in XP-D and TTD and the Associated Characteristics

	L461→V Δ716-730	R616→P	R658→H or C	R683→W[a]	G713→R	R722→W
TTD1VI[b]	+	...	...	...	...	+
TTD1BEL[c]	...	+	...	...	...	+
TTD3VI[d]	+	...	+ (H)	...	...	...
TTD1RO[d]	...	...	+ (C)	...	+	...
XP102LO[e]	+	...	...	+	...	...
XP17PV[e]	...	+	...	+	...	...
Associated characteristic	Any	Any	TTD with intermediate UV sensitivity	XP-D	?	TTD with high UV sensitivity

NOTE.—A plus sign (+) indicates the presence of a mutant allele.

[a] Mutation also found in XP6BE(SV40) (Takayama et al. 1995) and XP1DU (A. R. Lehmann and B. C. Broughton, unpublished data).

[b] TTD with high UV sensitivity; mutation data from this study.

[c] TTD with high UV sensitivity; mutation data from study by Broughton et al. (1994).

[d] TTD with intermediate UV sensitivity; mutation data from this study.

[e] XP-D; mutation data from study by Takayama et al. (1995).

the homologues (Gly, Ala, and Ser), again indicating that this is the causative mutation.

Although there is no apparent common ancestor, patients TTD1VI, TTD3VI, and XP102LO share a complex allele (table 3). Therefore, the differences in the repair characteristics of the cell strains from these three patients are likely to result from the different alterations in the second allele. If the hypothesis that mutations in *ERCC2* are the sole genetic determinant for this TTD group is correct, then the differences in the clinical manifestations of these patients, including the difference in cancer-proneness, are also likely to result from the differing alterations in the second allele. In XP102LO, the second allele has an Arg683-to-Trp mutation in the putative nuclear location signal (NLS; Takayama et al. 1995). Consistent with the hypothesis that the second allele is determining the different clinical manifestations for XP102LO, TTD1VI, and TTD3VI, this putative NLS mutation is also found in three other XP group D strains, XP6BE(SV40), XP17PV, and XP1DU (Takayama et al. 1995; A. R. Lehmann and B. C. Broughton, unpublished data) but not in any of the 15 TTD or 2 XP-D/CS strains we have studied (Broughton et al. 1994, 1995; Takayama et al. 1995; K. Takayama and C. A. Weber, unpublished data).

In TTD1VI, a highly UV-sensitive strain, the second allele has an Arg722-to-Trp mutation. This same Arg722 mutation is also found in TTD1BEL, also a highly UV-sensitive strain derived from a severely affected child who died at age 3 years (Broughton et al. 1994). The other allele in TTD1BEL has an Arg616-to-Pro mutation, which is common with one XP17PV allele (the other having the putative NLS mutation). Thus, TTD1VI and TTD1BEL have one differing allele, each common to an XP group D strain, and a common second allele. Again, these findings are consistent with the hypothesis that it is the second allele that determines the different clinical manifestations for XP102LO, TTD1VI, and TTD3VI; in the case of TTD1VI, the Arg722-to-Trp mutation results in highly UV-sensitive TTD.

In TTD3VI, a cell strain with intermediate UV sensitivity (sensitive only at doses $>\sim 10$ J/m^2; Stefanini et al. 1993*a*), the second allele has an Arg658-to-His mutation. In TTD1RO, also a cell strain with intermediate UV sensitivity (sensitive only at doses $>\sim 10$ J/m^2; Stefanini et al. 1993*a*), one allele has an Arg658-to-Cys mutation. These data suggest that the Arg658 mutation is responsible for determining the different characteristics of TTD3VI relative to XP102LO and TTD1VI.

If the initial findings that TTD1RO is deficient in (6-4)photoproduct repair and proficient in cyclobutane dimer repair (Broughton et al. 1990) are correct, then the mutation data suggest that Arg658 (helicase domain VI) is involved in the recognition of these different types of DNA damage, either directly or by interaction with damage-recognition factors. In contrast, the findings of Eveno et al. (1995) show near-wild-type repair of (6-4)photoproducts and deficient repair of cyclobutane dimers for both TTD1VI (high UV sensitivity) and TTD1RO (intermediate UV sensitivity; sensitive only at doses $>\sim 10$ J/m^2). Although the two studies include the same cell strains, they used different antibodies, different UV-doses, and different protocols. Thus, while we find correlations between the *ERCC2* mutations and the UV-survival patterns, it is unclear how they relate to the repair of the two major classes of UV-induced DNA damage.

The C-terminal region of ERCC2 appears to be critical to the function that is deficient in TTD patients. Five of the 13 reported TTD alleles have changes in the region from 713 to 730, and an additional 2 of the 13 alleles

have a frameshift causing amino acids 731–760 to be out of frame and producing a stop codon 14 amino acids downstream of the mutation. We suggest, as have others (Bootsma and Hoeijmakers 1994; Broughton et al. 1994), that TTD is due to mutations that primarily alter the transcriptional role of ERCC2, while XP-D is due to mutations that primarily alter the repair role of ERCC2. This suggestion is supported by recent findings that ERCC2 proteins with the Arg722-to-Trp mutation and the frameshift after amino acid 730 found in some TTD patients are unable to rescue the inviability of a *S. cerevisiae rad3* deletion mutant, while normal ERCC2 and ERCC2 with a Lys48-to-Arg mutation in the ATP-binding site that is predicted to eliminate helicase activity are able to rescue the same inviability (Guzder et al. 1995). The temperature-sensitive conditional lethality of the *rad3-ts*$_{14}$ mutation in *S. cerevisiae* has been shown to be due to the role of Rad3 in RNA polymerase II transcription (Guzder et al. 1994). Future studies of the biochemical and structural characteristics of mutant ERCC2 proteins may provide further insights into the complex roles of ERCC2 in DNA metabolism.

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