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A New Nucleotide-Excision-Repair Gene Associated with the Disorder Trichothiodystrophy

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Summary

The sun-sensitive, cancer-prone genetic disorder xeroderma pigmentosum (XP) is associated in most cases with a defect in the ability to carry out excision repair of UV damage. Seven genetically distinct complementation groups (i.e., A–G) have been identified. A large proportion of patients with the unrelated disorder trichothiodystrophy (TTD), which is characterized by hair-shaft abnormalities, as well as by physical and mental retardation, are also deficient in excision repair of UV damage. In most of these cases the repair deficiency is in the same complementation group as is XP group D. We report here on cells from a patient, TTD1BR, in which the repair defect complements all known XP groups (including XP-D). Furthermore, microinjection of various cloned human repair genes fails to correct the repair defect in this cell strain. The defect in TTD1BR cells is therefore in a new gene involved in excision repair in human cells. The finding of a second DNA repair gene that is associated with the clinical features of TTD argues strongly for an involvement of repair proteins in hair-shaft development.

Introduction

Nucleotide excision repair of UV-induced damage in DNA is a complex process involving 6 genes in *Escherichia coli,* 10 or more genes in *Saccharomyces cerevisiae,* and at least 11 genes in mammals (Hoeijmakers and Bootsma 1990; Lehmann et al. 1992; Riboni et al. 1992). In mammals the different genes are defined by two sets of complementation groups—namely, (*a*) the UV-sensitive rodent mutants (11 groups) and (*b*) patients with XP (7 groups) and Cockayne syndrome (CS) (2 groups). The recent cloning of some of these human DNA repair genes has demonstrated significant overlap in the two sets of complementation groups. The *ERCC3* gene, cloned by its ability to correct the UV sensitivity of certain rodent mutants, has been shown to correct the defect in a patient with clinical symptoms of both XP and CS from XP complementation group B (Weeda et al. 1990). Similarly, the *ERCC2* gene corrects the UV sensitivities of several XP-D cell strains (Flejter et al. 1992; C. A. Weber, cited in Lehmann et al. 1992, pp. 6–7), and *ERCC6* has recently been shown to correct the deficiency in CS patients from CS complementation group B (Troelstra et al. 1992).

Trichothiodystrophy (TTD) is a rare genetic disorder whose clinical symptoms are quite different from those of XP and CS. Sulfur-deficient brittle hair is associated with mental and physical retardation, ichthyosis, an unusual facies, and, in many but not all patients, sun sensitivity. Unlike in XP, there are no reports of skin cancer associated with this disorder. Nevertheless, cells from photosensitive patients with TTD are, like XP cells, deficient in excision repair of UV damage. The extent of this deficiency is very heterogeneous between cells from different patients (Stefanini et al. 1986, 1992; Lehmann et al. 1988; Broughton et al. 1990). Cell fusion experiments have shown that, in all but three TTD cell strains examined so far, the repair deficiency is in

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the same complementation group as is the defect in XP group D (Stefanini et al. 1986, 1992, 1993; Lehmann et al. 1988). In the three exceptional cases (two from related patients), complementation was observed with XP-D cells. In the present paper we show that one of these, TTD1BR, from a 16-year-old boy with a severe deficiency in excision repair, was able to complement the excision-repair defect in all XP complementation groups, and we show that complementation was not intragenic. This cell strain is therefore a representative of a new excision-repair complementation group.

Clinical Description

The patient (described 11 years ago in Jorizzo et al. 1982) had typical symptoms of TTD (characteristic hair-shaft abnormalities with reduced sulfur content, collodion baby, short stature, ichthyosis, bilateral congenital cataracts, and asthmatic attacks). Material for the current study was taken in 1988. A recent examination at age 20 years showed that he had had recurrent infective exacerbations of his asthma and that he remains severely growth retarded (height and weight below the 3d centile) and of limited intelligence but not severely mentally retarded (IQ 70–80). His ichthyosiform erythroderma continues. He has developed limited joint contractures of the hands that are due to the severe ichthyosiform involvement of the palms, and he has limited mobility. Despite all his problems, he retains a friendly personality with pleasingly good humor.

He has been sensitive to sunlight since early childhood, but, apart from sun sensitivity, his clinical features are quite distinct from those associated with XP patients. There is no significant freckling or other pigmentary changes. There are no telangiectases or actinic keratoses, nor have there been any skin tumors. There is no conjunctivitis or keratitis in the eyes, nor is there any sign of mental deterioration.

His immune function has been reported by Norris et al. (1990), who found that his $CD3^+$ cells were at the lower end of the normal range, with a $CD4^+/CD8^+$ ratio in the normal range. His lymphocytes showed a reduced response to phytohemogglutinin. Natural killer cell activity was in the normal range.

Methods

The procedures used in these studies have all been described in earlier work. Complementation studies were carried out as described elsewhere (Vermeulen et al. 1991; Stefanini et al. 1992). Microinjection experiments using cloned DNA repair genes were as described by Van Duin et al. (1989). An *ERCC2* cDNA product was synthesized by using reverse transcriptase (RT)-PCR and then was used to screen a human cDNA library. A full-length *ERCC2* cDNA clone was identified, and the *ERCC2* cDNA subsequently was inserted into the mammalian expression vector pSLM (Koken et al. 1992) for use in the microinjection experiments.

Table 1

Response of TTD1BR Fibroblasts to UV Irradiation

	Normal	TTD1BR	TTD2GL
Cell survival (D_{37}-Jm^{-2}) ...	6.6	1.6	1.1
UDS (% of normal at 10 Jm^{-2})	100	15	8
RNA synthesis (% of normal at 15 Jm^{-2})	100	12	9
Cyclobutane dimer excision (% removed in 24 h)	55	2	15
6-4 Photoproduct excision (% removed in 3 h)	75	18	18

NOTE.—Data from Broughton et al. (1990).

Results

UV Response of Fibroblasts

Table 1 summarizes our previously published results on the response to UV irradiation of TTD1BR cells, which are compared with a normal cell strain and with TTD2GL, a TTD cell strain with a severe DNA repair defect in the XP-D complementation group (see Lehmann et al. 1988, in which this cell strain was designated "P2"). Both cell strains have very pronounced defects in excision repair. The defect in TTD2GL is slightly more severe, TTD2GL being one of the most sensitive cell strains of the many TTD strains that we have examined (Stefanini et al. 1986, 1992, 1993). (The measurement of cyclobutane dimer excision is subject to large experimental errors, and the difference between TTD1BR and TTD2GL in table 1 is not significant.)

Complementation

The severe deficiency in unscheduled DNA synthesis (UDS) following UV irradiation of TTD1BR (table 1) enabled us to carry out complementation studies by fusing these cells with other TTD cell strains and with XP cells from complementation group D, followed by measurement of UDS in binucleate heterokaryons. Results of these experiments are shown in figure 1 (TTD8PV, XP17PV, and XP3NE). The level of UDS in the nuclei of heterokaryons was, in all cases, consider-

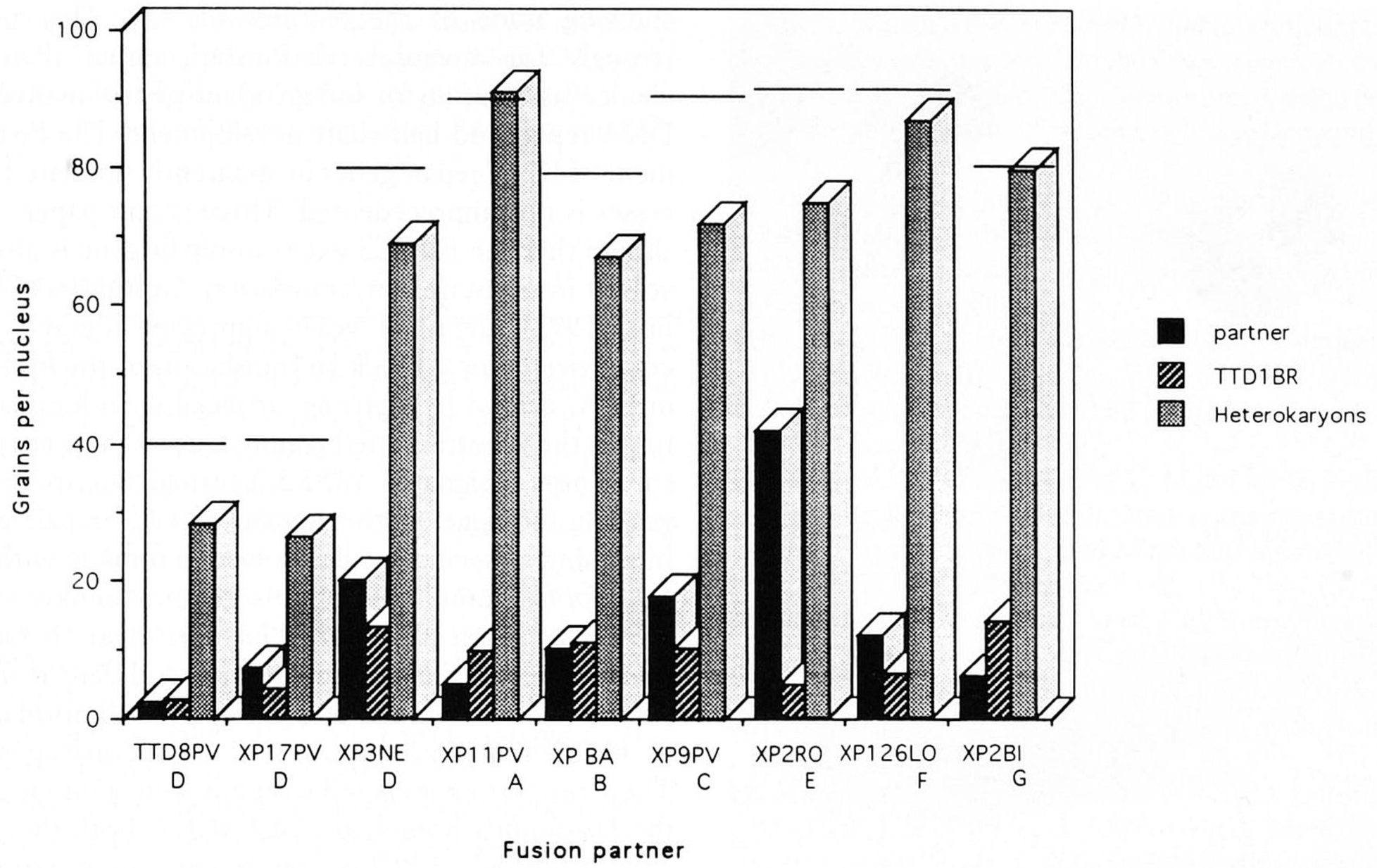

Figure 1 Complementation of TTD1BR. TTD1BR cells were fused with the indicated cell strains, following labeling of each fusion partner with different-sized latex beads. The fused cells were UV irradiated (10–20 Jm^{-2}) and UDS measured by autoradiography following ^{3}H-thymidine incorporation. The horizontal lines show the grains per nucleus in normal cells in the same experiment.

ably higher than that in homokaryons of either fusion partner in the same culture, and it approached that in normal controls. Thus TTD1BR cells clearly complement the repair defects in TTD8PV, XP3NE, and XP17PV, which have all previously been shown, by complementation analysis, to fall into the XP-D complementation group. This confirms our preliminary result reported elsewhere (Stefanini et al., 1993).

These observations suggest that the defect in TTD1BR is not in the XP-D gene, but they do not exclude the possibility of intragenic complementation. In order to evaluate this possibility, microneedle injection experiments were performed with the XP-D gene. It has recently been shown that the previously cloned *ERCC2* gene (Weber et al. 1990) is in fact the XP-D gene (Flejter et al. 1992; C. A. Weber, cited in Lehmann et al. 1992, pp. 6–7). As anticipated, microinjection of *ERCC2* cDNA, cloned into a mammalian expression vector, into various XP and TTD representatives of group D restored UV-induced UDS to normal levels (fig. 2*A*). In contrast, no increase in UDS was found in injected TTD1BR cells (fig. 2*B*), ruling out the possibility of intragenic complementation. This finding was further substantiated by direct sequencing of *ERCC2* cDNA after amplification by PCR. No sequence alterations were found in TTD1BR, whereas in other TTD patients various mutations were found in the *ERCC2* gene (A. R. Lehmann and B. C. Broughton, unpublished observations).

We next fused TTD1BR cells with XP cells from the other six XP complementation groups (i.e., A–C and E–G). Complementation was observed in all cases (fig. 1). To examine intragenic complementation for other XP and CS groups and to assess whether TTD1BR might be the human equivalent of some excision-deficient rodent complementation groups, three cloned human excision-repair genes that we have available—*ERCC1, ERCC3* (XPBC), and *ERCC6* (CSBC)—were microinjected into TTD1BR cells. In no case was UDS restored. These results show that TTD1BR is a representative of a new excision-repair complementation group.

Discussion

We have shown that the severe defect in excision repair of UV damage in TTD1BR cells is complemented

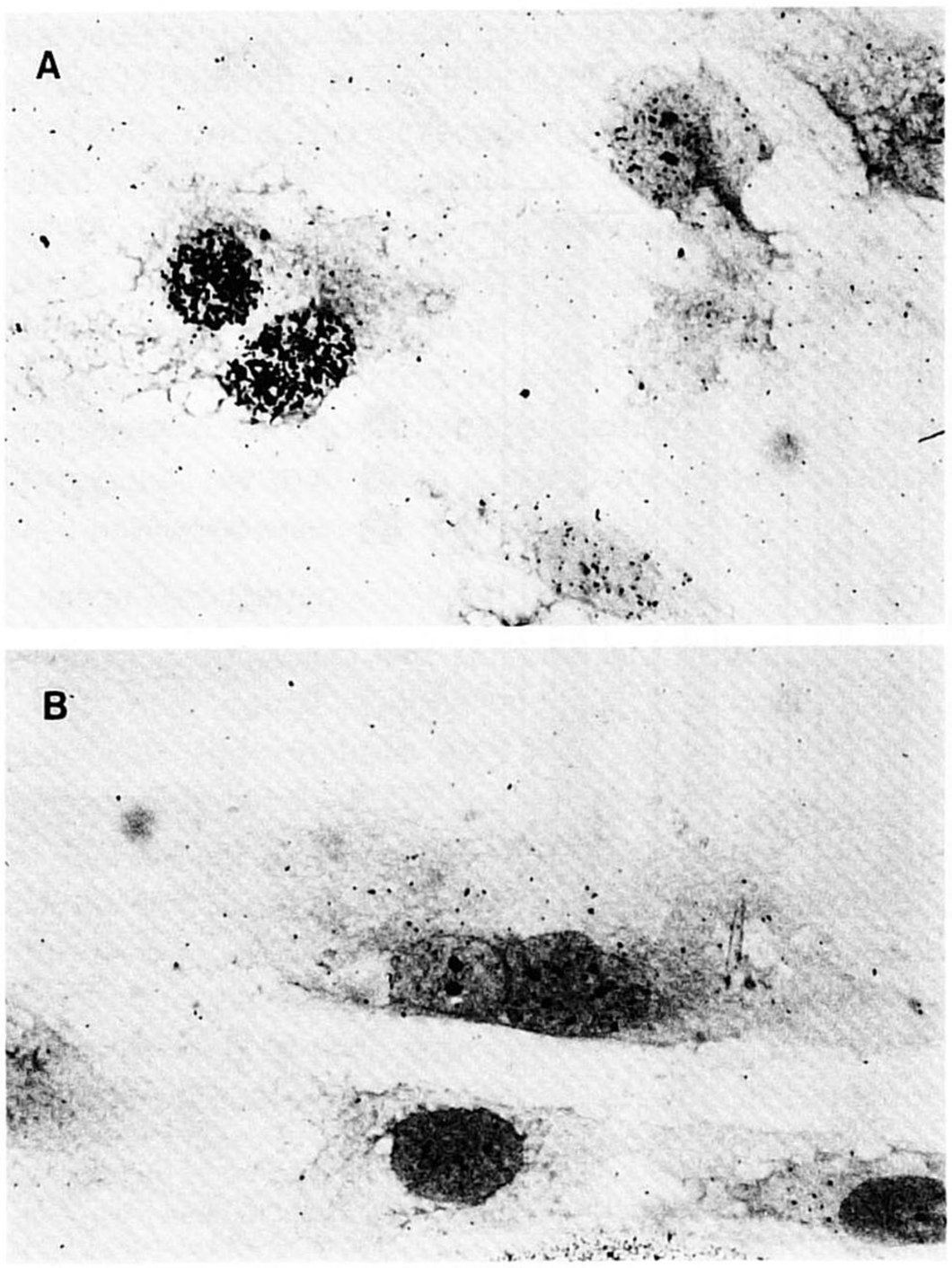

Figure 2 Microinjection of *ERCC2* into TTD1BR cells. The *ERCC2* cDNA inserted into a mammalian expression vector was introduced into homopolykaryons of XP1BR (a representative cell line of XP-D) (*A*) and TTD1BR (*B*) by microneedle injection into one of the nuclei. To permit expression of the injected DNA, cells were incubated for 24 h after injection. Subsequently, the fibroblasts were UV irradiated (15 J/m^2), incubated in the presence of ^{3}H-thymidine for 2 h to label repair patches, and were fixed and processed for autoradiography. The nuclei of the injected XP1BR polykaryon (the binuclear cell) (*A*) shows a complete correction of UDS (apparent from the high number of autoradiographic grains above both nuclei), in contrast to the neighboring, noninjected monokaryons. No induction of UDS is visible in the injected polykaryon of TTD1BR (*B*).

by all known XP complementation groups and is not corrected by several cloned human DNA repair genes. TTD1BR is therefore a representative of a new excision-repair complementation group not yet found in the population of XP patients, in contrast to previously reported TTD cell strains deficient in excision repair, which fall into the XP-D complementation group (Stefanini et al. 1986, 1992; Lehmann et al. 1988). TTD1BR therefore represents the second group in which defective DNA repair is associated with hair-shaft abnormalities, ichthyosis, and the other features of TTD (while showing none of the features of XP). This argues strongly for a causal relationship, rather than the chance association of independent genes involved in DNA repair and hair-shaft development. The involvement of DNA repair genes in apparently unrelated processes is not unprecedented. Three recent papers have shown that the *ERCC3* excision-repair gene is also involved in transcription/translation. Gulyas and Donahue (1992) identified yeast-suppressor mutants that could overcome a block to translation of the *his4-316* mRNA, caused by a strong, artificial stem-loop structure in the 5′ untranslated region. One of these suppressor genes, designated "*SSL2*," turned out to be the yeast homologue of the human *ERCC3* repair gene. Intriguing observations have also been made with the *Drosophila* homologue of this gene (Mounkes et al. 1992). A mutant designated "*haywire*" was characterized by male sterility, UV sensitivity, and defects in the central nervous system, and it displayed abnormalities in microtubule-based processes of spermatogenesis. These properties appeared to result from a mutation in the *Drosophila* homologue of *ERCC3*. Both the yeast and *Drosophila ERCC3* counterparts (and probably also the human gene) were shown to have an undefined function essential for cell viability, a function distinct from their role in excision repair. These observations suggest that the *ERCC3* gene product has two different functions (one involved with excision repair and the other involved with control of transcription/translation), which are not obviously related. This has been confirmed very recently by Schaeffer et al. (1993), who demonstrated that the 89-kD subunit of the transcription factor TFIIH (BTF2) corresponds to the *ERCC3* gene product. It is thus plausible to suggest that both the *ERCC2* gene product (which has several features in common with the ERCC3 protein) and the new gene defective in TTD1BR cells may also be involved in two apparently unrelated processes—namely, excision repair and another process involved in hair-shaft development. The clinical phenotype of the patients (e.g., XP or TTD) may depend on the way in which the causative mutation affects one or the other or both of the hypothesized functions. Further experiments should provide evidence supporting or disproving these speculative ideas on the relationship of excision-repair defects to hair-shaft abnormalities.

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References

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

Flejter WL, McDaniel LD, Johns D, Friedberg EC, Schultz RA (1992) Correction of xeroderma pigmentosum complementation group D mutant cell phenotypes by chromosome and gene transfer: involvement of the human *ERCC2* DNA repair gene. Proc Natl Acad Sci USA 89:261–265

Gulyas KD, Donahue TF (1992) *SSL2*, a suppressor of a stem-loop mutation in the *HIS4* leader encodes the yeast homolog of human *ERCC-3*. Cell 69:1031–1042

Hoeijmakers JHJ, Bootsma D (1990) Molecular genetics of eukaryotic DNA excision repair. Cancer Cells 2:311–320

Jorizzo JL, Atherton DJ, Crounse RG, Wells RS (1982) Ichthyosis, brittle hair, impaired intelligence, decreased fertility and short stature (IBIDS syndrome). Br J Dermatol 106:705–710

Koken MHM, Vreeken C, Bol SAM, Cheng NC, Jaspars-Dekker I, Hoeijmakers JHJ, Eeken JCJ, et al (1992) Cloning and characterization of the Drosophila homolog of the xeroderma-pigmentosum complementation-group-B correcting gene, *ERCC3*. Nucleic Acids Res 20:5541–5548

Lehmann AR, Arlett CF, Broughton BC, Harcourt SA, Steingrimsdottir H, Stefanini M, Taylor AMR, et al (1988) Trichothiodystrophy, a human DNA repair disorder with heterogeneity in the cellular response to ultraviolet light. Cancer Res 48:6090–6096

Lehmann AR, Hoeijmakers JHJ, Van Zeeland AA, Backendorf CMP, Bridges BA, Collins A, Fuchs RPD, et al (1992) Workshop on DNA repair. Mutat Res 273:1–28

Mounkes LC, Jones RS, Liang B-C, Gelbart W, Fuller MT (1992) A *Drosophila* model for xeroderma pigmentosum and Cockayne's Syndrome: *haywire* encodes the fly homolog of *ERCC3*, a human excision repair gene. Cell 71:925–937

Norris PG, Limb GA, Hamblin AS, Lehmann AR, Arlett CF, Cole J, Waugh APW, et al (1990) Immune function, mutant frequency and cancer risk in the DNA repair defective genodermatoses xeroderma pigmentosum, Cockayne's syndrome and trichothiodystrophy. J Invest Dermatol 94:94–100

Riboni R, Botta E, Stefanini M, Numata M, Yasui A (1992) Identification of the eleventh complementation group of UV-sensitive excision repair-defective rodent mutants. Cancer Res 52:6690–6691

Schaeffer L, Roy R, Humbert S, Moncollin V, Vermeulen W, Hoeijmakers JHJ, Chambon P, et al (1993) DNA repair helicase: a component of BTF2 (TFIIH) basic transcription factor. Science 260:58–63

Stefanini M, Giliani S, Nardo T, Marinoni S, Nazzaro R, Rizzo R, Trevisan G (1992) DNA repair investigations in nine Italian patients affected by trichothiodystrophy. Mutat Res 273:119–125

Stefanini M, Lagomarsini P, Arlett CF, Marinoni S, Borrone C, Crovato F, Trevisan G, et al (1986) Xeroderma pigmentosum (complementation group D) mutation is present in patients affected by trichothiodystrophy with photosensitivity. Hum Genet 74:107–112

Stefanini M, Lagomarsini P, Giliani S, Nardo T, Botta E, Peserico A, Kleijer WJ, et al (1993). Genetic heterogeneity of the excision repair defect associated with trichothiodystrophy. Carcinogenesis 14:1101–1105.

Troelstra C, Van Gool A, De Wit J, Vermeulen W, Bootsma D, Hoeijmakers JHJ (1992) *ERCC6*, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. Cell 71:1–15

Van Duin M, Vredeveldt G, Mayne LV, Odijk H, Vermeulen W, Klein B, Weeda G, et al (1989) The cloned human DNA excision repair gene ERCC-1 fails to correct xeroderma pigmentosum complementation groups A through I. Mutat Res 217:83–92

Vermeulen W, Stefanini M, Giliani S, Hoeijmakers JHJ, Bootsma D (1991) Xeroderma pigmentosum complementation group H falls into complementation group D. Mutat Res 255:201–208

Weber CA, Salazar EP, Stewart SA, Thompson LH (1990) *ERCC-2:* cDNA cloning and molecular characterization of a human nucleotide excision repair gene with high homology to yeast RAD3. EMBO J 9:1437–1448

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