

First Reported Patient with Human ERCC1 Deficiency Has Cerebro-Oculo-Facio-Skeletal Syndrome with a Mild Defect in Nucleotide Excision Repair and Severe Developmental Failure

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Nucleotide excision repair (NER) is a genome caretaker mechanism responsible for removing helix-distorting DNA lesions, most notably ultraviolet photodimers. Inherited defects in NER result in profound photosensitivity and the cancer-prone syndrome xeroderma pigmentosum (XP) or two progeroid syndromes: Cockayne and trichothiodystrophy syndromes. The heterodimer ERCC1-XPF is one of two endonucleases required for NER. Mutations in *XPF* are associated with mild XP and rarely with progeria. Mutations in *ERCC1* have not been reported. Here, we describe the first case of human inherited ERCC1 deficiency. Patient cells showed moderate hypersensitivity to ultraviolet rays and mitomycin C, yet the clinical features were very severe and, unexpectedly, were compatible with a diagnosis of cerebro-oculo-facio-skeletal syndrome. This discovery represents a novel complementation group of patients with defective NER. Further, the clinical severity, coupled with a relatively mild repair defect, suggests novel functions for ERCC1.

Helix-distorting DNA lesions, such as those produced by UV light, are repaired by nucleotide excision repair (NER), a multistep cut-and-paste mechanism requiring >30 proteins.¹ Helical distortion is recognized throughout the genome by the protein complexes XPC-HR23B and DDB. As an alternative to this global-genome NER (GG-NER), DNA damage blocking RNAPol II progression on transcribed strands is rapidly repaired by transcription-coupled NER (TC-NER). This subpathway of NER specifically requires CSA, CSB, and UVS proteins. Once the damage is recognized, the repair reactions for GG-NER and TC-NER are identical. The helix is locally unwound by the TFIIH complex and bound by XPA and RPA proteins, which ensure correct positioning of two endonucleases, ERCC1-XPF and XPG. These enzymes incise the damaged strand on either side of the lesion, to remove the damaged oligonucleotide and to allow resynthesis and restoration of the phosphate backbone by DNA polymerase and ligase.

Among patients with defective NER, 11 genetic complementation groups are known.² Defects in GG-NER cause the cancer-prone syndrome xeroderma pigmentosum (XP [MIM 278700, MIM 610651, MIM 278720, MIM 278730, MIM 278740, MIM 278760, MIM 278780, and MIM 133530]), characterized by severe photosensitivity; a 1,000-fold increased risk of skin cancer, which appears as early as the 1st decade of life; a 10-fold increased risk of other tumors; and, in severe cases, neurodegeneration.² Selective impairment of TC-NER, due to mutation of *CSA* or *CSB*, causes Cockayne syndrome (CS [MIM 133540 and

MIM 216400]), characterized by UV-sensitivity—but not cancer—cachectic dwarfism, and progeroid symptoms, including profound neurodegeneration.² Several of the NER proteins have functions distinct from NER, leading to complex and pleiomorphic disease phenotypes. A notable example is TFIIH, which is essential for both NER and basal transcription. Specific hypomorphic mutations in various subunits of TFIIH (*XPB*, *XPD*, and *TFB5/TTD-A*), give rise to a heterogeneous syndrome, trichothiodystrophy (TTD [MIM 601675]), characterized by photosensitivity and hair and skin abnormalities.^{3–5} The developmental and degenerative features of TTD and CS are attributed to reduced transcriptional capacity rather than defective DNA repair.⁴

ERCC1-XPF is a structure-specific endonuclease that is required to incise the damaged strand of DNA 5' to the lesion.⁶ In addition, the heteroduplex participates in the repair of DNA interstrand crosslinks^{7,8} through a mechanism distinct from NER.⁹ In yeast and *Drosophila*, ERCC1-XPF orthologs participate in meiotic and mitotic recombination, including homology-mediated DNA double-strand break repair.^{10–13} In mammals, the heterodimer is essential for homology-dependent gene targeting in hamster¹⁴ and mouse embryonic stem cells,¹⁵ but its physiological role in recombination is not known. ERCC1-XPF is also associated with telomeres, where it modulates recombination of telomeric sequences and loss of telomeres from deprotected chromosome ends.^{16,17} Numerous reports have suggested ERCC1 expression in tumors as a

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predictor of DNA-crosslinker-based chemotherapy efficacy (for review, see work of Reed¹⁸).

Despite the involvement of ERCC1-XPF in multiple genome maintenance mechanisms, patients with defective XPF typically display only very mild XP, rarely developing skin tumors before adulthood.^{19,20} In all cases, the mutations are hypomorphic and cause significant residual protein and NER. Although *ERCC1* (MIM 126380) was the first mammalian repair gene to be cloned²¹ and targeted in mice,²² not a single case of an *ERCC1* defect has been identified, despite exhaustive screens in photosensitive patients for 3 decades. Here, we describe the very first case of human *ERCC1* deficiency. Unexpectedly, the patient displayed a relatively mild impairment of NER, similar to that seen in XPF cases, but very severe symptoms, including pre- and postnatal developmental failure and death in early infancy.

Material and Methods

The Patient

Patient 165TOR was born from nonconsanguineous Italian white parents, after a 37-wk pregnancy complicated by intrauterine growth retardation. Amniocyte analysis did not reveal any karyotypic abnormality. At birth, the patient's weight, length, and occipitofrontal circumference were <3rd percentile. The infant had microcephaly (fig. 1A and 1B) with premature closure of fontanels, bilateral microphthalmia, blepharophimosis, high nasal bridge, short filtrum, micrognathia, low-set and posterior-rotated ears (fig. 1A), arthrogryposis with rocker-bottom feet (fig. 1B), flexion contractures of the hands (fig. 1B), and bilateral congenital hip dislocation. On x-rays, there was no evidence for spine abnormalities. Nuclear magnetic resonance (fig. 1C and 1D) revealed a simplified gyral pattern and cerebellar hypoplasia. There was mild hypoplasia of the kidneys, with normal structure and function. Echography did not reveal any congenital heart defects. These combined symptoms were compatible with a clinical diagnosis of a severe form of cerebro-oculo-facial-skeletal syndrome (COFS [MIM 214150]). In the differential diagnosis, other dysmorphology syndromes, such as Warburg Micro syndrome (MIM 600118) and CS type II, were considered. Because of the absence of genital abnormalities and retinopathy, COFS was the preferred diagnosis, with the caveat that a definitive diagnosis for these progressive disorders is hard to make in an infant.

The child showed failure to thrive, was tube fed, and did not pass any developmental milestone. Respiratory failure due to bilateral pneumonia was the cause of death at age 14 mo. Weight was 4.5 kg, height was 56 cm, and occipitofrontal circumference was 38 cm, at the time of death. A postmortem study was not performed.

Cell Cultures

Dermal fibroblasts were grown in Ham's F10 medium (without thymidine) containing 15% fetal bovine serum and antibiotics. Cell strains 165TOR, 173TOR, and 174TOR were obtained from the proband, his father, and his mother, respectively. Other NER-deficient standard strains and their defective genes (HUGO name, best-known alias) are XP25RO (*XPA* [MIM 278700]), XP131MA (*ERCC3*, *XPB* [MIM 610651]), XP21RO (*XPC* [MIM 278720]),

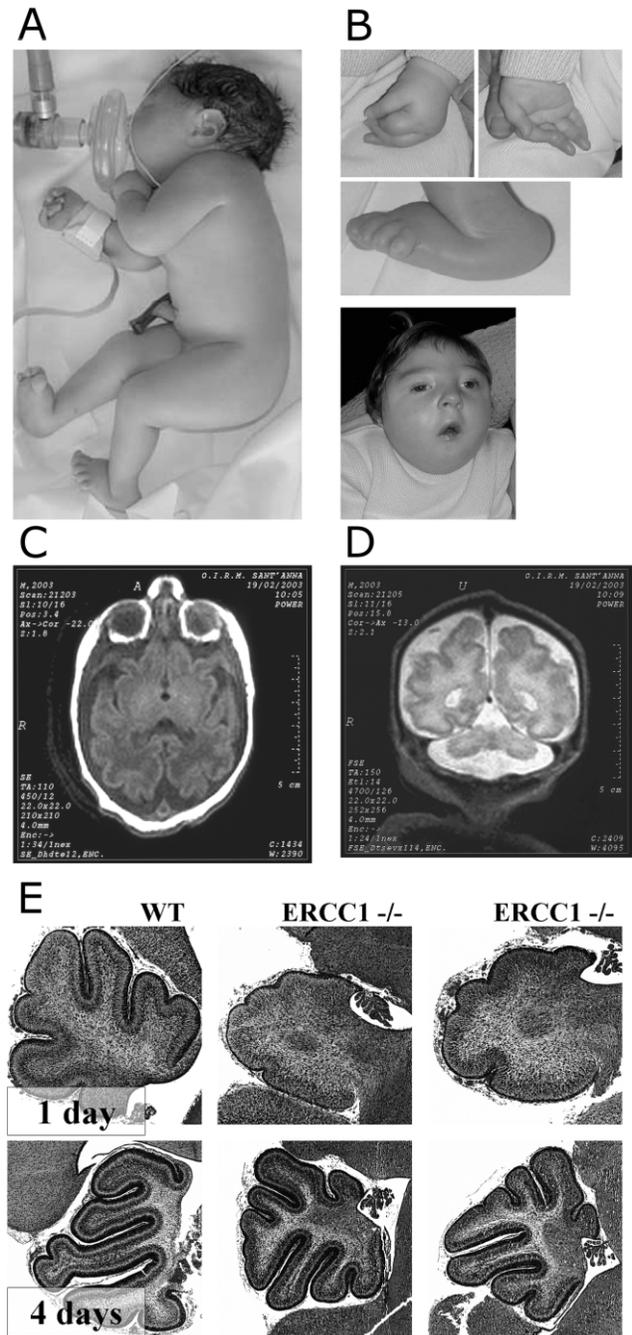


Figure 1. Characteristics of patient 165TOR and *Ercc1*^{-/-} mice. *A*, Image of patient at birth. *B*, Arthrogryposis of hands and rocker-bottom feet and frontal face appearance. Note microcephaly. *C* and *D*, Nuclear magnetic resonance scans of head, demonstrating simplified gyral pattern and cerebellar hypoplasia. *E*, Hematoxylin-eosin stained sections of 1-d-old mice (top row) and 4-d-old mice (bottom row), demonstrating delayed development of *Ercc1*^{-/-} mouse cerebellum (including hypoplasia and blunted gyri) compared with that of wild-type (WT) littermates. Images are representative of those obtained for at least three mice of each genotype.

XP6BE (*ERCC2*, *XPD* [MIM 278730]), XP42RO and XP51RO (both *ERCC4*, *XPF* [MIM 278760]), XPCS1RO and XP3BR (both *ERCC5*, *XPG* [MIM 133530]), CS3BE (*ERCC8*, *CSB* [MIM 133540]), CS1AN (*ERCC6*, *CSA* [MIM 216400]), TTD1BR (*GTF2H5* [MIM 608780], *TTDA*), and Kps3 (*UVS* [MIM 600630]). XPF patient strain XP51RO is the same as the one called "XFE" elsewhere.²³ NER deficiency of embryonic fibroblasts derived from *Ercc1*^{-/-} mice was described elsewhere.²⁴ Chinese hamster CHO mutant cells 43-3B,²⁵ transfectant cells, and normal AA8 or CHO9 cells were cultured in HamF10 and Dulbecco's modified Eagle medium (1:1) plus 10% serum.

Preloading of human fibroblasts with cytoplasmic beads was performed as described elsewhere.^{4,26} In short, subconfluent cultures were kept in the presence of polystyrene microspheres of either 0.8 or 2.2 μm for 3 d. Cultures were trypsinized, extensively washed, mixed, and seeded for use in other procedures mentioned below.

UV Exposures and Repair Assays

UV light of predominantly 254 nm was emitted from a germicidal low-pressure mercury lamp, at a fluence of 0.6 W/m². Local exposure of subcellular areas through Millipore filters was done as described elsewhere.²⁷ For global genome NER activity, unscheduled DNA synthesis (UDS) was measured by exposure of coverslip cultures to 16 J/m² of UV light and subsequent incubation for 3 h in medium containing 10 $\mu\text{Ci}/\text{mL}$ [3H-1,2]-thymidine (120 Ci/mmol [Amersham TRK565]). Cells were fixed, coverslips were dipped in Ilford K2 photographic emulsion, and slides were developed after 3 d of exposure and were stained with Giemsa. Nuclear silver grains were counted in 50 cells. TC-NER was measured as the ability to recover from UV-induced inhibition of transcription. After 16 h of exposure to 16 J/m² of UV, cells were cultured for 1 h in medium containing [3H-5',6]-uridine (40 Ci/mmol [Amersham TRK410]) and were processed for autoradiography as described above. Survival of fibroblasts (exposed to UV) and Chinese hamster cells (exposed to UV and mitomycin C) was measured as described elsewhere,²⁸ by exposing 48-h-old sparse cultures to graded UV or mitomycin C doses. After further growth for 3–4 d, cells were labeled for 1–3 h in medium containing 2.5 $\mu\text{Ci}/\text{ml}$ [methyl-³H]-thymidine (50 Ci/mmol [Amersham TRK418]), were chased for 1 h, were lysed in 0.25-M NaOH, and were scintillation counted. Fibroblasts' colony-forming ability (after mitomycin C) was measured by seeding fibroblasts on 24-h-old thin fibroblast feeder layers (exposed to 40 Gy of gamma-rays) and by counting the colonies after 13–18 d.

Complementation Analysis and Sequencing

For classical complementation analysis of mutant human fibroblasts,²⁹ two fibroblast strains were preloaded with distinct types of cytoplasmic beads, mixed 1:1 and fused using inactivated Sendai virus. At 24–48 h after reseeding and attachment, these cultures, containing a mixture of unfused cells and fused multikaryons, were exposed to UV and were assayed for GG-NER and TC-NER activities. In the slides, hetero- and homokaryons were distinguished on the basis of the beads.

For sequencing, total RNA was isolated from the patient fibroblasts and was reverse transcribed using random hexamer primers. *ERCC1* was amplified in two overlapping fragments from the cDNA and was directly sequenced by standard protocols and after cloning. Coding stretches of *ERCC1* were also amplified from

fibroblast genomic DNA by use of exon-specific primers and were sequenced.

Microinjection

Procedures for microinjection were as described elsewhere.³⁰ Before seeding was done, fibroblast fusion was induced using inactivated Sendai virus, to generate scattered multikaryons. In 3-d-old cultures, recognizable multikaryons were microinjected with concentrated proteins (in the cytoplasm) or with cDNA-expression vector (in one of the nuclei). At 24–48 h after injection, UDS was assayed in the cultures as described above.

Immunodetection

Expression of XPF and ERCC1 proteins was detected by immunoblotting 10 μg of crude cell extracts from human fibroblasts and CHO cells, as well as by immunofluorescence of paraformaldehyde-fixed cultures, by use of mouse monoclonal anti-hXPF (1:1,000) and affinity-purified rabbit polyclonal anti-hERCC1 antibodies.³¹ The latter detects the human protein only. Rabbit anti-XPC (1:500),³² mouse anti-p62 (3C7) (donated by J. M. Egly) (1:2,000), and anti-XPA²⁷ (1:500) were used for immunofluorescence.

Mouse Histology

Ercc1^{-/-} and wild-type littermates were bred from heterozygous crossings in a mixed (C57Bl/6:FVB/n) genetic background. Mice were euthanized by decapitation on postnatal day 1 or 4. Heads were fixed in neutral buffered saline containing 20% sucrose for 48 h, were embedded in paraffin, were sectioned, and were stained with hematoxylin and eosin. Cerebellar sections from three mice of each genotype were examined at each time point. Genomic DNA was isolated from tissue of each pup for PCR-based genotyping.

Results

A Defective NER Core Factor in 165TOR

Since some patients with COFS were described as having defective NER caused by mutations in *ERCC6/CSB*, *ERCC2/XPD*, or *ERCC5/XPG*,^{33–35} we assessed NER in the skin fibroblasts of the patient (165TOR). UV-induced UDS, a measure of GG-NER activity, was reduced to 15% of normal (fig. 2A and 2C). RNA synthesis recovery after UV exposure, a measure of TC-NER, was reduced to 13% of normal, which, while low, was not as severe as in cells from a patient with CS-B or a patient with XP from complementation group A (XP-A) (fig. 2B and 2C). Similarly, the patient's cells were fourfold more sensitive to UV than were normal fibroblasts (fig. 2D), which is intermediate compared with XP-A (10 \times hypersensitive) and is similar to XP-C cells, missing only GG-NER or CS-B cells, missing only TC-NER. The compromise of both GG-NER and TC-NER in 165TOR strongly implies that a core NER factor (TFIIH, XPA, RPA, XPG, or XPF/ERCC1) is defective.

This NER defect was used for prenatal diagnosis in a next at-risk pregnancy in the family, as described elsewhere.³⁴ A fully normal level of NER was observed in cultured chorionic villus cells from the fetus (data not

shown), and a healthy child was born after an uneventful pregnancy.

Patient 165TOR, Representing a Novel NER Complementation Group

In an attempt to identify the defective NER factor, genetic complementation was performed by somatic cell fusion of the patient cells with a well-defined panel of cells from patients with defective NER. All the strains tested were able to fully complement the low UV-induced UDS or RNA synthesis recovery of 165TOR (data not shown). The results excluded not only the three COFS candidate genes but also *XPA*, *XPC*, and *XPF*; the recently identified gene *TTDA*⁵; and the TC-NER factors *CSA*, *CSB*, and *UVS*. The data demonstrate that this patient represents a new NER complementation group and carries a recessive gene defect that is distinct from those carried by all known patients with defective NER.

Identification of the Genetic Defect

Of the 10 subunits of TFIIH, only XPB, XPD, and TTDA are linked with genetic diseases. Since an *XPD* mutation has been associated with severe COFS,³⁴ we next considered the other subunits of TFIIH as candidates. The TFIIH complex, purified from HeLa cells, was microinjected into 165TOR homokaryons, and UV-induced UDS was measured. TFIIH corrected the XPB cells but not the 165TOR cells (data not shown). We concluded that the patient's NER defect was not caused by a defect in any of the TFIIH subunits.

Since the steps of NER are well defined,³⁶ it is possible to functionally map the NER defect in 165TOR cells by use of immunofluorescence. Sequential recruitment of NER proteins XPC, XPA, and TFIIH to subnuclear domains of local UV damage²⁷ was found to be undisturbed in 165TOR cells (fig. 2E and 2F). So, the defect in 165TOR cells is at a late stage of NER, which leaves *RPA*, *ERCC1*, and DNA replication factors as candidate genes.

To test these possibilities, 165TOR cells were microinjected with recombinant ERCC1-XPF or with RPA heterotrimer. The latter failed to correct the low UV-induced UDS in 165TOR cells (not shown). In contrast, purified ERCC1-XPF protein (fig. 3A) as well as *ERCC1* cDNA (fig. 3B) did correct UDS. *ERCC1* cDNA is known not to correct any other NER complementation group,^{37,38} and two independent XP-F cell lines fully complement 165TOR, so these results strongly implicate *ERCC1* as the gene affected in 165TOR.

We next sequenced both the *ERCC1* cDNA and genomic locus from cells from the patient (165TOR) and his parents. This revealed two point mutations in the coding region of *ERCC1* (fig. 3E) and no abnormalities in the splice signals of all nine introns. A C→T transition predicted to convert codon Gln¹⁵⁸ into an amber translational stop signal was inherited from the mother (fig. 3C). The truncated polypeptide encoded by this allele lacks the en-

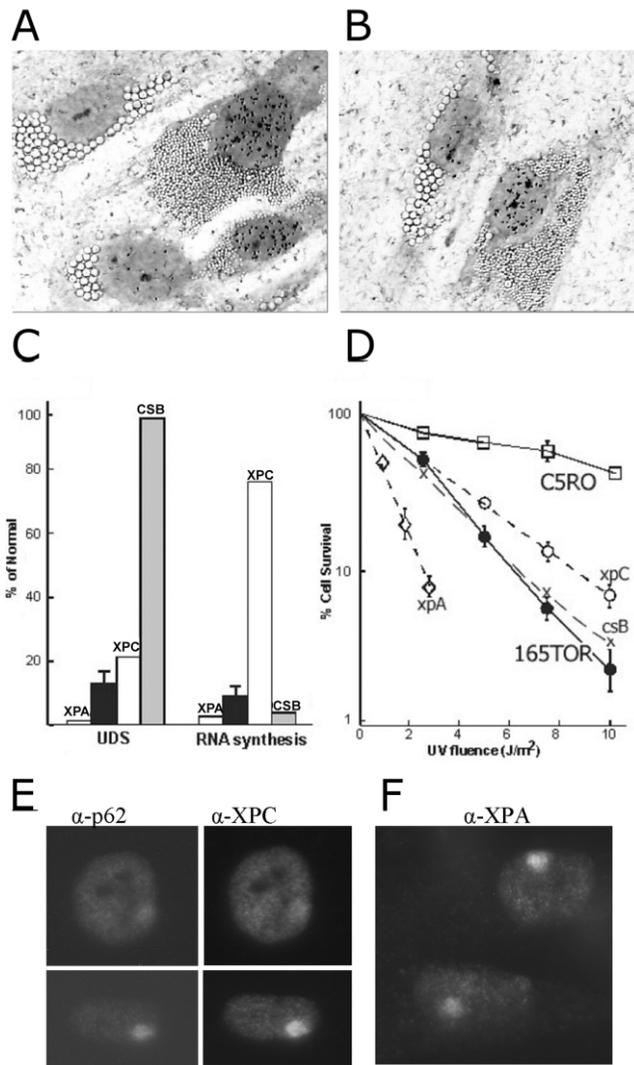


Figure 2. NER in 165TOR cells. *A* and *B*, Comparative autoradiography of a mixed culture of normal C5RO (small cytoplasmic beads) and 165TOR patient fibroblasts (large beads). *A*, GG-NER was measured as UDS by pulse labeling the cells with ³H-thymidine after exposure to 16 J/m² UV-C, as described elsewhere.²⁰ *B*, TC-NER was measured as RNA synthesis recovery (RSR) after UV by pulse labeling the cells with ³H-uridine 16 h after they were exposed to 16 J/m² UV. *C*, Quantification of UDS and RSR by counting the silver grains in 50 nuclei of 165TOR (black) compared with in normal cells on the same slide. Data are shown in comparison with XP-A, XP-C, and CS-B standard strains measured in separate experiments. *D*, Cell survival assay to measure UV sensitivity of 165TOR (dots) relative to that of normal (C5RO), XP-A (diamonds), XP-C (unblacked circles), and CS-B (crosses) standard strains. *E* and *F*, Immunofluorescence detection of NER proteins to sites of UV-induced DNA damage. 165TOR cells were exposed to local UV radiation as described elsewhere,²⁷ were fixed after 1 h, and were stained with antibodies against TFIIH core component p62 and XPC (*E*) or with anti-XPA antibodies (*F*).

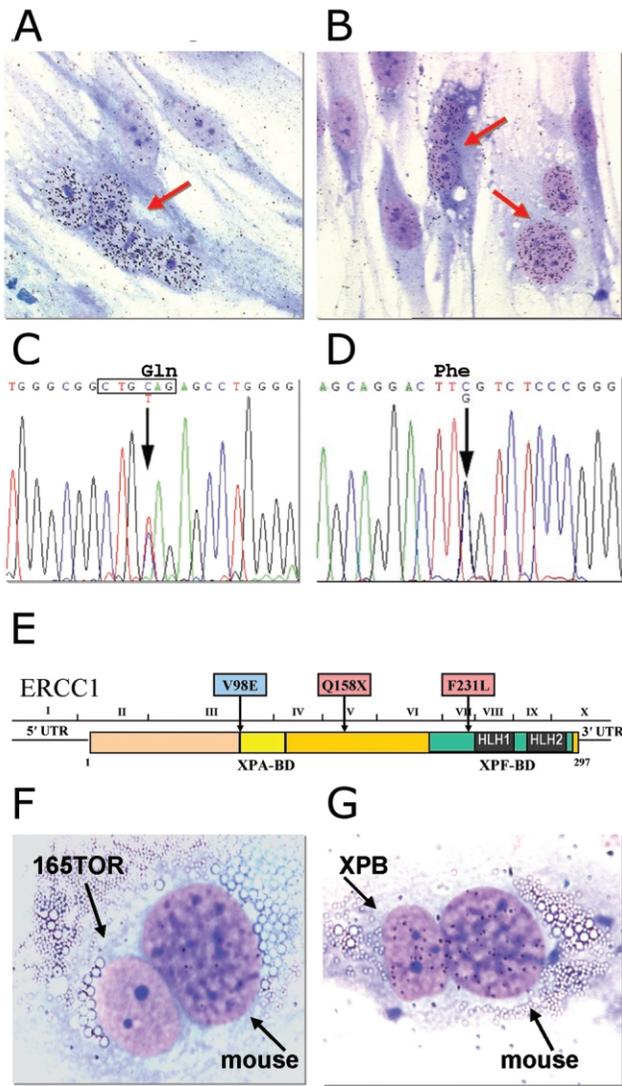


Figure 3. ERCC1 defect in patient 165TOR. *A* and *B*, UV-induced UDS measured after microinjection of purified ERCC1-XPF protein complex (*A*) or ERCC1-GFP expression vector (*B*) into 165TOR multikaryons (arrows). Note the low number of grains in single cells from the same patient that were not injected. *C* and *D*, Partial genomic sequences of ERCC1 exon 5 (Q¹⁵⁸X) and exon 7 (F²³¹L). The presence of Q¹⁵⁸X was verified by restriction analysis of the destroyed *Pst*I site (boxed). *E*, Schematic diagram of ERCC1, indicating exons (Roman numerals), known protein domains (XPA and XPF binding domain [BD] and helix-hairpin-helix [HLH] DNA binding motifs), and the inactivating mutations found in patient 165TOR (red-shaded boxes). The mutation identified in 43-3B CHO cells, which have undetectable levels of ERCC1-XPF, is also indicated (blue-shaded box). *F* and *G*, Fusion of mouse *Erc1*^{-/-} embryonic fibroblasts with human fibroblasts (165TOR [*F*] and XPB [*G*]), followed by UDS assay. Note noncomplementation in panel *E* and complementation in panel *G*. Heterokaryons were identified on the basis of cytoplasmic beads as well as by nucleolar morphology.

tire C-terminal domain, which is essential for interaction with XPF.³⁹ Heterodimerization of ERCC1-XPF is required for complex stability and endonuclease activity.^{6,40–42} Therefore, this Q¹⁵⁸amber allele is expected to be a functional null. The paternal allele carried a C→G transversion predicted to change Phe²³¹ to a leucine (fig. 3D), a sequence variant not described in the public SNP database (NCBI: SNPs Linked to Gene *ERCC1*). This residue lies within the XPF binding domain of ERCC1 and is fully conserved among mammals and in *Xenopus laevis*. Fish, plants, and yeast or fungi have physicochemically similar aromatic tyrosine residues at this position, whereas insects have proline. In none of the sequences from 38 widely divergent species was a leucine or leucine-like amino acid (valine, isoleucine, alanine, or methionine) found there (table 1), although these residues together represent almost 30% of the available codons. Both mutated mRNAs were normally expressed, and the parents are both heterozygous for their respective mutations.

Low Levels of ERCC1-XPF Heterodimer in 165TOR

To determine whether these mutations affected the level of ERCC1 protein in 165TOR cells, we used comparative immunofluorescence, by labeling normal fibroblasts with cytoplasmic beads and then coculturing them with 165TOR cells before immunodetection of ERCC1.⁴³ In comparison with normal and parent fibroblasts, 165TOR cells had significantly reduced levels of ERCC1 in the nucleus, with concomitant intense immunoreactivity in the cytoplasm (fig. 4A and 4C). Nuclear XPF was similarly reduced, but cytoplasmic staining was not apparent (fig. 4B). The average fluorescence intensities of ERCC1 and XPF were estimated at 15% and 30% of neighboring normal fibroblasts, respectively. These results were confirmed by immunoblotting (fig. 4E and 4F), yielding 14% and 20%. With this antibody,⁴¹ truncated protein from the Q¹⁵⁸ allele was not readily detected on immunoblot. Cytoplasmic ERCC1 immunoreactivity was also observed in cells from the mother only, who carries the Q¹⁵⁸X allele (fig. 4F and 4G). ERCC1 levels in 165TOR cells were comparable to a typical XP-F patient with mild XP and very late neurodegeneration (XP42RO)²⁰ and a unique XP-F patient with juvenile progeroid symptoms (XP51RO)²³; remarkably, XPF levels were somewhat higher in 165TOR cells (for immunoblot, see fig. 4C and 4D; comparative immunofluorescence data not shown). The residual level of ERCC1-XPF in each of these cell lines is predictive of moderate sensitivities to UV and the crosslinking agent mitomycin C (165TOR: 4 × UV and 2–3 × mitomycin C; XP51RO: 5 × UV and mitomycin C; fig. 5A).²³ Together, these data indicate that the ERCC1 mutations observed in 165TOR

Table 1. Conservation of Phenylalanine at Position 231 in ERCC1

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

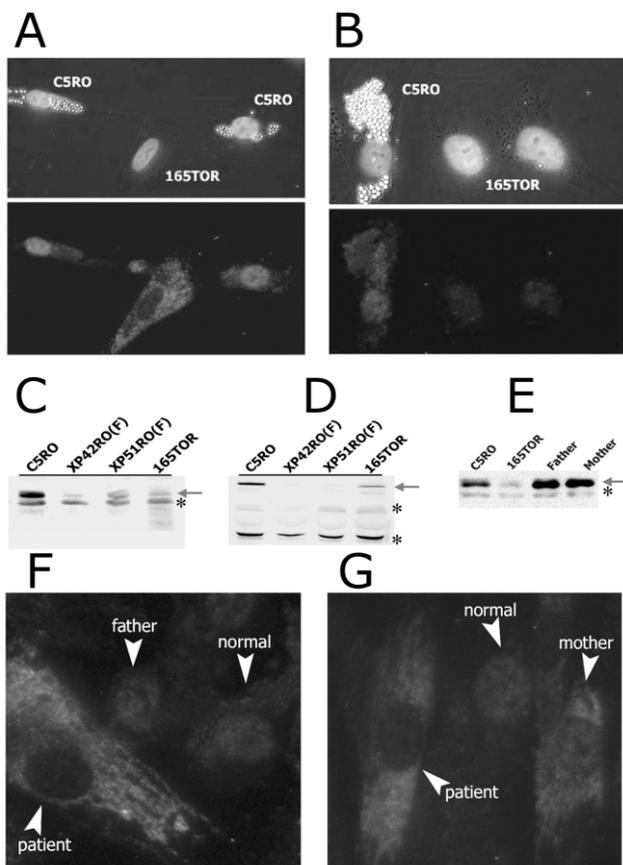


Figure 4. ERCC1/XPF protein levels in the patient and his parents. *A* and *B*, Comparative immunofluorescence with anti-ERCC1 (*A*) or anti-XPF (*B*) antibodies. Upper panels are mixed phase contrast and 4'-6 diamidino-2-phenylindole images, to demonstrate the presence of cytoplasmic beads in the normal control fibroblasts (C5RO), mixed 1:1 with 165TOR cells (which have no beads). Note cytoplasmic pseudofluorescence caused by the optic effects of beads. *C–E*, Immunoblots with anti-ERCC1 (*C* and *E*) or anti-XPF (*D*) on whole-cell extracts from C5RO (normal), two XP-F patient cell lines (XP42RO(F) and XP51RO(F)), 165TOR, and the mother and father of 165TOR. The relevant bands are indicated with arrows; cross-reacting bands (indicated by an asterisk [*]) served as loading controls. Note the presence of degraded ERCC1, specifically in 165TOR (*C*). *F* and *G*, Comparative immunodetection of ERCC1 on cells from the parents, compared with 165TOR and normal. Mixed cultures contained C5RO (large beads) and 165TOR cells.

cause reduced but not absent nuclear ERCC1-XPF and a partial NER and crosslink-repair defect. In addition, loss of the XPF-binding domain of ERCC1 prevents nuclear localization of the protein.

Correcting Activities of the Two ERCC1 Alleles

To definitively demonstrate ERCC1's involvement in the NER defect, we fused 165TOR cells to cultured embryonic fibroblasts derived from *Ercc1*^{-/-} mice. In the heterokaryons, the ERCC1 F²³¹L mRNA is expressed at physiological

levels and specifies the only full-length ERCC1 species available. UV-induced UDS was not complemented (fig. 3F), whereas NER was fully restored by fusion of NER-defective human XPB with mouse *Ercc1*^{-/-} fibroblasts (fig. 3G). In addition, we cloned and expressed both mutant human cDNAs in Chinese hamster 43-3B cells. These cells harbor a V⁹⁸E point mutation in *Ercc1* (fig. 3E),⁴⁴ which results in undetectable levels of the protein and its binding partner XPF.⁴¹ CHO transfectants typically carry numerous copies of their transgene, resulting in strong overexpression.^{21,38} XPF protein levels were restored in 43-3B cells expressing wild-type ERCC1 or ERCC1-F²³¹L cDNA (fig. 5B). Although the Q¹⁵⁸X mutation causes truncation of ERCC1 before the XPF binding domain, overexpression of the mutant cDNA resulted in stabilization of XPF protein to a limited extent (fig. 5B). This was attributed to a low-level read-through of the amber codon, resulting in a small amount of full-length ERCC1 protein, both with and without a C-terminal green fluorescent protein (GFP) tag (fig. 5E), as a consequence of very high mRNA levels.

Expression of wild-type ERCC1 cDNA in 43-3B cells resulted in near-complete correction of UV and mitomycin C hypersensitivity (fig. 5C and 5D). Stable expression of ERCC1-F²³¹L cDNA corrected UV and mitomycin C sensitivity to a similar extent as in wild-type cells (fig. 5C and 5D). This is consistent with a "leaky" mutation, in which overexpression leads to complementation. A low but significant correction of UV sensitivity was also detected in cells stably expressing the Q¹⁵⁸ truncation mutation (fig. 5B). Remarkably, the small amount of full-length ERCC1 generated from the nonsense allele was sufficient to almost completely correct mitomycin C hypersensitivity of the parental cell line (fig. 5D). This supports previous work indicating that very low levels of ERCC1-XPF are sufficient for crosslinker resistance in CHO cells.⁴⁵

Finally, to fulfil a formal requirement by this journal's editor, we checked the presence of ERCC1-F²³¹L in a matched human control population. Among 364 chromosomes 19 tested, we found none.

Discussion

COFS Due to Unstable ERCC1-XPF

With the reservation that we describe only a single case patient here, genetic and functional data support the conclusion that the severe congenital and developmental abnormalities in this patient were caused by biallelic mutation of ERCC1. On the basis of the independent functional analysis of each mutation, we conclude that the Q¹⁵⁸ mutation results in a null allele, whereas the ERCC1 F²³¹L, in combination with XPF, has enzymatic activity. The clinical phenotype of the patient and the DNA repair defect in 165TOR cells are a direct consequence of low levels of ERCC1-XPF rather than loss of enzymatic activity. This is analogous to patients with XP-F. In all cases in which the mutations are well characterized, point mutations are found, which occur outside of the essential

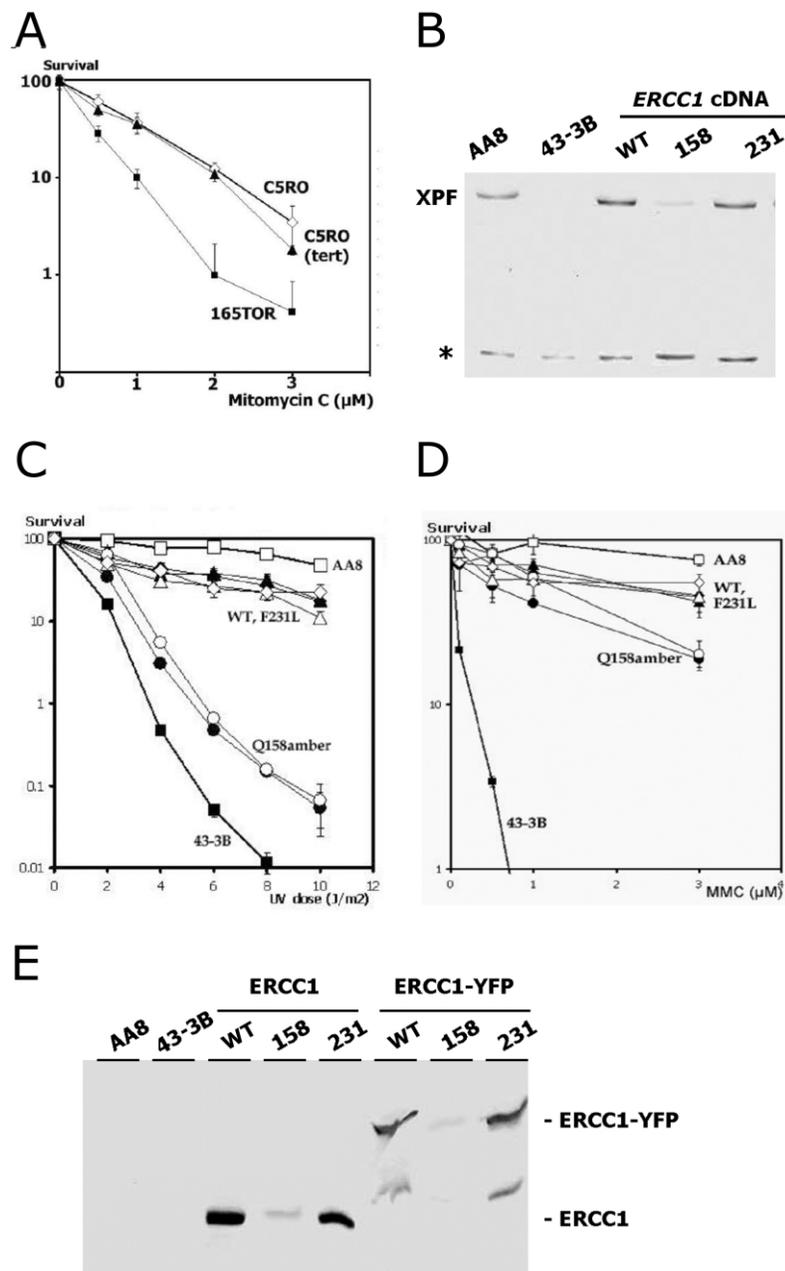


Figure 5. Functionality of mutant *ERCC1* cDNA. *A*, Clonogenic survival assay to measure sensitivity of fibroblasts to the crosslinking agent mitomycin C. 165TOR cells are compared with normal primary and human tert-immortalized fibroblasts (C5RO(tert)). For panels B–E, *ERCC1*-deficient 43-3B cells were stably transfected with expression plasmids carrying human *ERCC1* or human *ERCC1*-GFP driven by a cytomegalovirus promoter and a neo^R selection marker. G⁴¹⁸-resistant mass populations were checked for survival after exposure to UV (*C*) or mitomycin C (*D*), compared with untransfected 43-3B cells (blackened squares) and wild-type AA8 cells (unblackened squares). Vectors were *ERCC1* wild type (WT) (diamonds), *ERCC1* F²³¹L (triangles), and *ERCC1* Q¹⁵⁸X (circles). Blackened and unblackened symbols represent *ERCC1* and *ERCC1*-GFP constructs, respectively. *B* and *E*, Western blots of transfected mass populations by use of antibodies against XPF (*B*) or *ERCC1* (*E*). Cross-reacting bands (serving as loading controls) are indicated by an asterisk (*).

functional domains of XPF (i.e., the catalytic domain, the helicase motifs, and the *ERCC1*-binding domain).²³ Also, in all cases, there is significant residual repair activity (15%–30%) and very moderate (2–3 times) UV sensitivity.^{19,20} Remarkably, even though these mutations are

outside the protein interaction domain, both XPF and *ERCC1* levels are strongly reduced in fibroblasts from each of these XP-F patients (fig. 4A and 4B).^{20,42} The CHO 43-3B mutation V⁹⁸E recapitulates this. Moreover, whereas the N-terminal 92 aa of *ERCC1* are dispensable for DNA

repair,⁴⁵ two missense mutations within the same domain abolish repair and heterodimer expression.⁴⁶ This absolute concordance between point mutations in either *ERCC1* or *XPF* and the instability of the entire heteroduplex implies that, in living cells, the stability of the heterodimer is under strict regulation, likely via careful scrutiny of the conformation of the complex, which explains the difficulties in its overproduction.⁴⁷

Genotype-Phenotype Relationships of ERCC1-XPF Deficiency

This unique patient with mutated *ERCC1* genes represents the clinically severest case of NER deficiency described so far and contrasts with the very mild symptoms of XP-F patients, who may easily escape clinical diagnosis. Both *Ercc1* and *Xpf* have been genetically deleted in the mouse resulting in seemingly identical phenotypes, including defective DNA repair, impaired intrauterine and postnatal growth failure, life-limiting liver and kidney disease, and early death.^{22,48–50} This phenotype, being much more severe than that of *Xpa*^{-/-} mice, which are completely deficient in NER,⁵¹ underscores the importance of ERCC1-XPF functions distinct from NER. The symptoms are further complicated by neuronal dysfunction, such as cerebellar ataxia in later life.²³ Premature replicative senescence of *Ercc1*^{-/-} primary fibroblasts, hepatocytes, and bone marrow progenitors are consistent with growth failure and has been attributed to an inability to repair endogenous DNA damage.⁵² Furthermore, recent evidence indicates that genotoxic stress or poor genome maintenance causes suppression of the somatotroph axis, a self-protective mechanism that focuses energy stores on maintenance rather than on growth.²³ The clinical course of patient 165TOR bears striking resemblance to these ERCC1-XPF-deficient mice. Unfortunately, a postmortem examination was not done, making it impossible to extend the comparison beyond clinical data. The simplified gyral pattern and cerebellar hypoplasia seen in patient 165TOR, which were not previously described in patients with defective NER, are indicative of postmitotic neuronal migration defects⁵³ and a probable cause of impaired fetal movement, which is consistent with the joint deformities of the patient at birth. A closer histologic examination of the cerebellum of 1-d-old *Ercc1*^{-/-} mice revealed hypoplasia and simplified gyri as well (fig. 1E). By day 4, there was significantly less difference between *Ercc1*^{-/-} and wild-type littermates, which establishes this phenomenon as delayed cerebellar development. Remarkably, similar arrested development of the cerebellum was observed in *Xpa*^{-/-}; *Csb*^{-/-} mice,⁵⁴ which are NER deficient, suggesting that cells of the cerebellum, including Purkinje cells, must be particularly vulnerable to DNA damage during development. Patient 165TOR demonstrates that impaired DNA repair compromises embryonic postmitotic neuronal function in humans as well.

In contrast to the null mice, 165TOR has reduced but clearly detectable levels of ERCC1-XPF protein and resid-

ual NER and crosslink repair. In fact, these levels are similar to that of a typical XP-F patient with mild XP without developmental defects²⁰ and are even higher than those of an exceptional XP-F case patient with a more severe mutation that caused growth failure at a juvenile age.²³ This discrepancy in phenotype can be interpreted to mean that ERCC1 and XPF have distinct functions in vivo. However, on the basis of the similarity of the *Ercc1*^{-/-} and *Xpf*^{-/-} mouse phenotypes and the requirement for ERCC1 and XPF proteins to stabilize one another in vivo, other interpretations seem plausible as well. First, processing of ERCC1/XPF, as seen in cultured fibroblasts here, may differ in target tissues. Nothing is known about tissue-specific ERCC1-XPF levels yet, but known differences in proteasomic activity could be involved. Second, ERCC1 has been reported to interact with the TFIIH subunit XPB.⁵⁵ Failure of interaction may mimic other genetic defects that cause severe COFS, such as the TFIIH subunit XPD³⁴ or the TFIIH-binding NER endonuclease XPG.^{35,56,57} On the basis of the severe mouse phenotype, the rarity of a human ERCC1 deficiency was attributed to embryonic lethality.²⁴ This is further supported by the extremely severe phenotype of patient 165TOR despite residual protein and measurable DNA repair. Alternatively, the possibility of ERCC1 deficiency in a neonate with growth retardation may be frequently overlooked because sun sensitivity is classically required for diagnosis of an NER defect.

In conclusion, we identified the first case of human ERCC1 deficiency, resulting in severe embryonic and postnatal growth failure and COFS, which represents the clinically severest NER deficiency published so far and is consistent with mouse models of ERCC1-XPF deficiency. The phenotype is also distinct and more severe than that of NER deficiency alone. This case reveals the importance of ERCC1-XPF during human fetal development, in particular for the CNS.

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Web Resources

The URLs for data presented herein are as follows:

NCBI: SNPs Linked to Gene *ERCC1*, http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=2067

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for XP, CS, TTD, *ERCC1*, COFS, Warburg

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