Supporting Information

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SI Text

Plasmid Preparation and Isolation of Transfectants from SV40-Transformed CS3BE Cells. Full-length *CSA* cDNA from UV^{S}S1VI cells was amplified by PCR using Pfu DNA polymerase and primers: 5′-CATTCAGAATTCTATGCTGGGGTTTTTGTCCGC-3′ (CSA forward) and 5′-GGCAGGCATTCAGTCGAC CCTCCTTCTTCATCACTGCTGCTC-3′ (CSA reverse). EcoRl and SalI restriction sites for cloning in frame are underlined. The PCR product was digested with EcoRI and SalI and inserted into the EcoRI-SalI sites of pEGFP-C1 downstream of the EGFP-cDNA (Clontech), to produce the plasmid pEGFP-CSA expressing the EGFP-CSAtrp361cys fusion protein. The identity of the *CSA* cDNA was confirmed by DNA sequencing.

SV40-CS3BE cells (2×10^5) were plated on 3-cm Petri dishes (day 1), transfected by using the Lipofectamine LTX method (Invitrogen Life Technologies) with 2.5 μg of the pEGFP-CSAtrp361cys construct (day 2), transferred to 10-cm dishes (day 3), and incubated in medium supplemented with 300 μg/mL G418-sulfate (Invitrogen) on day 4. Three weeks later, 36 G418-resistant clones were trypsinized within cloning rings, transferred to 35-mm dishes, and analyzed for the expression of the ectopic protein by immunofluorescence. Three GFP-positive clones were expanded, analyzed for the expression of the ectopic protein by Western blot using anti-GFP antibodies (1:500; Roche Applied Science), and tested for their sensitivity to menadione and UV by clonogenic assays. Isogenic CS3BE cell lines stably expressing either the WT CSA tagged with EGFP or EGFP alone were analyzed in parallel.

DNA Repair Investigations. Cell cycle analysis after UVC irradiation was performed on a FACSCalibur (Becton Dickinson). Briefly, exponentially growing cells were exposed to 2.5, 5, 10, or 15 J/m^2 UV, pulse-labeled 24 h later with 30 μM BrdU (Sigma/Aldrich) for 3 h, harvested, and stained for replicative DNA synthesis with a monoclonal mouse anti-BrdU antibody (1:50; DakoCytomation) and a FITC mouse antibody (1:100; AbCys) and for DNA content with propidium iodide.

To test the cells' sensitivity of UV in the presence of caffeine, cells were seeded in triplicate in 6-well plates at a density of 2×10^4 per well, and allowed to attach overnight. The cells were irradiated with UV as described above and incubated in medium containing 2 mM caffeine for 3 days. Then the cells were trypsinized and counted after Trypan blue staining.

Cell survival after $KBrO_3$ treatment was determined by standard clonogenic assays. Briefly, primary fibroblasts were seeded at a density of $8–10 \times 10^2$ cells in 6-cm dishes. After overnight incubation in standard medium, dishes in duplicate were treated with increasing doses of $KBrO_3$ in PBS with 20 mM Hepes for 30 min at 37 °C and incubated again in standard medium. Colonies were fixed 15 days later, and the number of clones was counted. The number of colonies in treated cultures was expressed as a percentage of that in untreated cultures. For assessing clonogenic survival in transformed cell lines after exposure to UV or menadione, cells were seeded at a density of $1.5–2 \times 10^3$ cells per 10-cm dish, incubated in complete medium for 24 h, rinsed with PBS, UV-irradiated or treated with menadione diluted in PBS or with PBS alone for 1 h at room temperature, and incubated again with medium containing 10% serum and fixed after 10–15 days. Triplicate sets of dishes were used for each dose.

Characterization of the Gene Responsible for the Disease. Complementation analysis was performed by measuring RRS in hybrids obtained by fusing patient's cells with CS reference strains as described (1). Briefly, fibroblast strains used as partners in the fusion were grown for 3 days in medium containing latex beads of different sizes (0.8 and 1.7 μm) that were incorporated into the cytoplasm as markers. The cells were fused by using PEG-4000 (Merck), incubated for 48 h at 37 °C, UV-irradiated (20 J/m^2), incubated again for a further 24 h at 37 °C, labeled for 1 h with ^{3}H-Urd, and processed for autoradiography. One sample was treated in parallel but without UV irradiation. The grains over nuclei in 25 homodikaryons (identified as binuclear cells containing beads of 1 size) and in 25 heterodikaryons (identified as binuclear cells containing beads of different sizes) were counted. Two cell strains were classified in the same complementation group if the heterodikaryons failed to recover normal RNA synthesis levels after UV irradiation.

Complementation of DNA repair capacity in vivo after transfection of primary fibroblasts with a plasmid expressing either the normal or mutated (trp361cys) CSA protein tagged with EGFP was assessed by measuring RRS after UV irradiation. Briefly, cells were grown for 3 days in Ham F10 medium with 0.5% FCS and transfected with 3 μg of the plasmids pEGFP-CSA or pEGFP-CSAtrp361cys by using an Amaxa Nucleofector apparatus, according to the manufacturer's instructions (Program U23). Cells were then seeded onto microscope slides, incubated again in medium with low serum for 24 h, and irradiated with a UV dose of 20 J/m^2. After incubation for 24 h, cells were labeled for 1 h with ^{3}H-Urd and then washed, fixed with 4% paraformaldeyde, and scored under a fluorescence microscope to detect cells expressing the EGFP-CSA fusion protein. Then the slides were fixed in cold methanol and processed for autoradiography. RRS was measured by counting the number of grains on the nuclei of at least 50 cells. Photographs were taken with a digital camera (Olympus C-5060) and images were processed with Adobe Photoshop software.

Molecular Analysis of the *CSA* Gene. Total RNA was extracted from 4×10^6 fibroblasts by using the RNeasy Mini Kit (Qiagen). Two micrograms of RNA were reverse-transcribed into cDNA by using Moloney murine leukemia virus reverse transcriptase (Promega) according to the manufacturer's instructions. The coding region of *CSA* was amplified in a single fragment by using the primers M1 (5′-gccggtgtgaggacacgaTA-3′) and M4 (5′-aaagtttcagcagagacaaa-3′) corresponding to nucleotides 52–71 and 1300–1281 of *CSA* mRNA (GenBank accession no. NM_000082.3). PCR amplification was carried out as follows: 20 μL of cDNA was used in 50-μL reactions containing 1× PCR Gold buffer (Applied Biosystems), 2 mM $MgCl_2$, 0.4 mM dNTPs, 50 pmol each of the required primers, and 2 units of AmpliTaq Gold (Applied Biosystems). Cycling conditions were initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 95 °C for 1 min and annealing/extension at 53 °C for 10 min, and a final extension step of 72 °C for 11 min. Gel-purified PCR products were directly sequenced by using the primers U227 (5′-TACATGTTATCAGGTGGTTC-3′), L229 (5′-TAAAGTACAATCACACCATC-3′), U460 (5′-AGATGTATTTAATTTTGAGG-3′), U743 (5′-AATGGGAAAAAGTCACAAGC-3′), U1057 (5′-TAAAACTGTTGACTGCTGTG-3′), and L1103 (5′-AACCCAAGCCAGAATGTTGC-3′), corresponding to nucleotides 242–261, 282–263, 475–494, 758–777, 1072–1091, and 1156–1137 of *CSA* mRNA.

Genomic DNA was isolated from 5×10^6 fibroblasts by using the blood and cell culture DNA mini kit (Qiagen), followed by isopropanol precipitation. Genomic DNA amplification was carried out with 2-μg DNA samples by using the primer pairs AS1 (5′-TCACACCATTAGTAAGCTCTGACA-3′) and AS2 (5′-GGCAAGAAATGCTTTAAAAGTCTC-3′) corresponding to nucleotides 151.563–151.586 and 151.968–151.945 of the genomic sequence of the *CSA* gene (GenBank accession no. AC022445.6). Amplification was performed by using the AmpliTaq Gold PCR kit (Applied Biosystems) and the following cycling conditions: initial denaturation at 95 °C for 10 min followed by 40 cycles of 95 °C for 1 min and 61 °C for 10 min, and a final extension step of 72 °C for 11 min. Gel-purified PCR products were directly sequenced with the primer AS2.

1. Stefanini M, Fawcett H, Botta E, Nardo T, Lehmann AR (1996) Genetic analysis of UV hypersensitivity in twenty-two patients with Cockayne syndrome. *Hum Genet* 97:418–423.

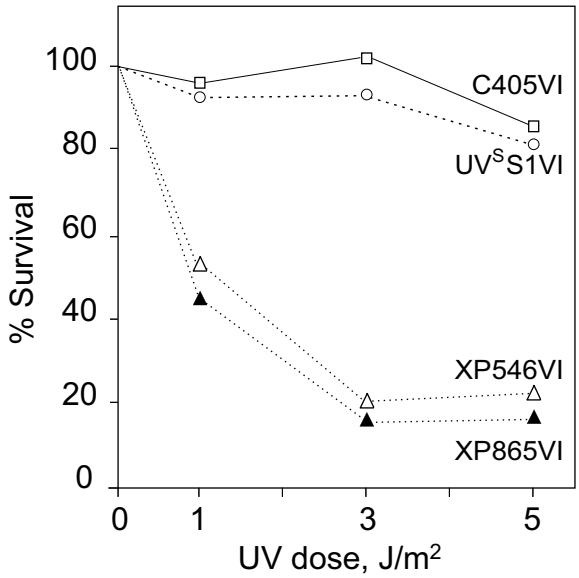

Fig. S1. Response to UV radiation in the presence of caffeine. Cells were primary fibroblasts from normal (C405VI, □), UV^{S}S1VI (○), and 2 XP variant (XP546VI, △; XP865VI, ▲) donors. Survival values in samples incubated in the presence of caffeine are given as percent of the corresponding samples incubated without caffeine. The experiment was done twice; SD were ≈10% for all cell strains.

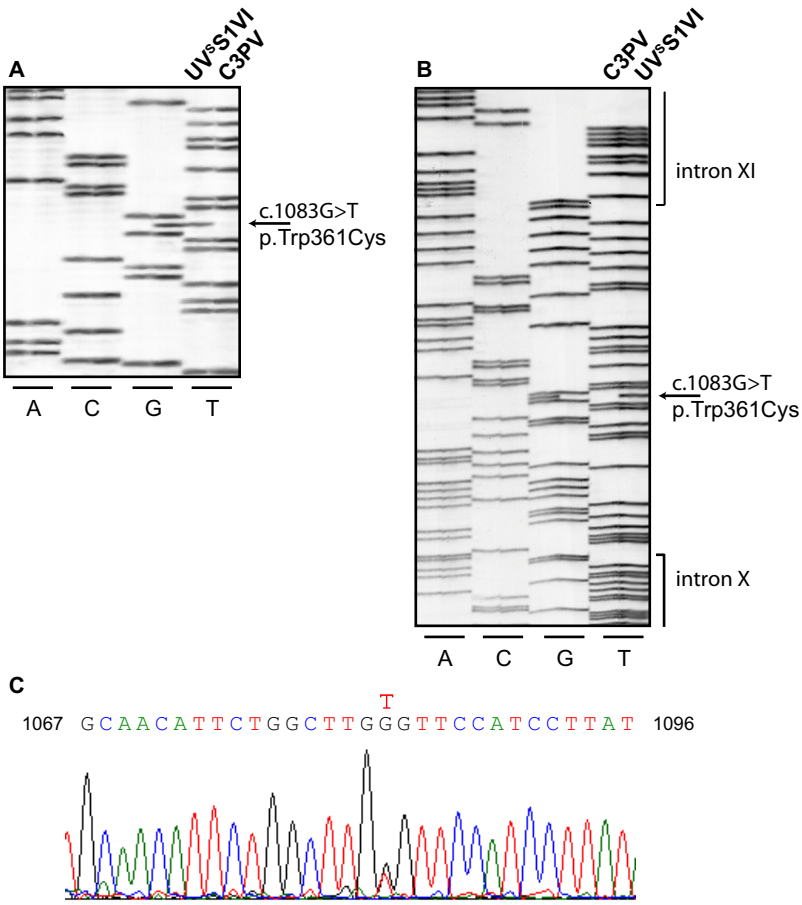

Fig. S2. Mutations in the *CSA* gene of patient UV^{S}S1VI and her mother. Autoradiographs (*A* and *B*) and electropherogram (*C*) of sequencing gels, showing a G-to-T transversion at position 1083 (c.1083G>T) on the cDNA (*A*) and genomic DNA (*B*) of the patient and on genomic DNA (*C*) of her mother. This change results in a trp361cys substitution. Mutation nomenclature follows the format indicated at www.hgvs.org/mutnomen and refers to the cDNA sequence NM_000082.3 and protein sequence NP_000073.1. For cDNA numbering, +1 corresponds to the A of the ATG translation initiation codon in the reference sequence. The initiation codon is codon 1.