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Molecular Analysis of Mutations in the *CSB* (*ERCC6*) Gene in Patients with Cockayne Syndrome

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Summary

Cockayne syndrome is a multisystem sun-sensitive genetic disorder associated with a specific defect in the ability to perform transcription-coupled repair of active genes after UV irradiation. Two complementation groups (CS-A and CS-B) have been identified, and 80% of patients have been assigned to the CS-B complementation group. We have analyzed the sites of the mutations in the *CSB* gene in 16 patients, to determine the spectrum of mutations in this gene and to see whether the nature of the mutation correlates with the type and severity of the clinical symptoms. In nine of the patients, the mutations resulted in truncated products in both alleles, whereas, in the other seven, at least one allele contained a single amino acid change. The latter mutations were confined to the C-terminal two-thirds of the protein and were shown to be inactivating by their failure to restore UV-irradiation resistance to hamster UV61 cells, which are known to be defective in the *CSB* gene. Neither the site nor the nature of the mutation correlated with the severity of the clinical features. Severe truncations were found in different patients with either classical or early-onset forms of the disease.

Introduction

The process of nucleotide excision repair (NER) protects cells from damage produced in cellular DNA by a wide variety of carcinogens, including UV light. Three genetic disorders—xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD)—are associated with defects in NER (Lehmann 1995). Most patients with XP are deficient in NER and contain mutations in one of seven genes (*XPA–XPG*) whose products are directly involved in NER. The recent discovery that the products of the *XPB* and *XPD* genes are components of the basal transcription factor TFIIH—which has a dual role, in transcription and as a component of NER—revealed an unexpected link between DNA repair and transcription (reviewed in Lehmann 1995; Hoeijmakers et al. 1996).

The clinical features of XP—namely, multiple skin abnormalities, including a greatly elevated skin-cancer incidence caused by exposure to sunlight—are consistent with predictions based on the hypersensitivity and hypermutability of cultured XP cells to UV light. In contrast, CS patients do not have an increased frequency of skin cancers. CS is a multisystem disorder characterized by severe physical and mental retardation, microcephaly, progressive neurological and retinal degeneration, skeletal abnormalities, gait defects, and sun sensitivity but no increased frequency of cancer (Nance and Berry 1992). Like XP, CS cells are hypersensitive to the lethal effects of UV light, but NER of the bulk of genomic DNA is unaffected. CS cells are, however, defective in a subpathway of NER, known as "transcription-coupled repair" (TCR), whereby damage in the transcribed strand of active genes is rapidly and preferentially repaired. In CS cells, damage in active genes is repaired at the same relatively slow rate as the bulk DNA (van Hoffen et al. 1993). This correlates with earlier findings that RNA synthesis recovers rapidly after UV irradiation of normal cells, whereas this recovery does not occur in CS cells (Mayne and Lehmann 1982). Cell-fusion studies have assigned some 30 CS patients to two complementation groups, CS-A and CS-B (Tanaka et al. 1981; Lehmann 1982; Stefanini et al. 1996); 80% of them are in the CS-B group. In addition, there are a few rare cases of individuals with the clinical and cellular features of

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Table 1

Primers Used for Reverse Transcription and PCR

Primer	Sequence	Position[a]	Annealing Temperature
1F	5′-TCTCTGTTTCCTTGTGGGCGCTCG-3′	31–54	
1R	5′-CTCTGGCCTCATGTCTGACTCCCA-3′	1142–1165	65°C
2F	5′-CATGCTTAATGAAGCATCAGGCTTC-3′	868–892	
2R	5′-TTGAACCACGAGTCCTGATCTTGC-3′	1734–1757	63°C
3F	5′-GGTGTTAGGTGGCTGTGGGAATT-3′	1619–1641	
3R	5′-GGTATCTCGTAAGACACATGCACAC-3′	2266–2290	67°C
4F	5′-CCTCATCGGATCATTCTGTCTGGC-3′	2081–2104	
4R	5′-CATCATGGTCTGCTCCAAAGGCTGGTT-3′	3201–3227	67°C
5F	5′-ATTACGAGATACAATGAGGACACATCC-3′	2771–2805	
5R	5′-TAGCCAAGAGTGAGGAGGAAGCGA-3′	4237–4253	67°C
6F	5′-GTGAAAACAAGAGTGAGGCCAAGG-3′	3786–3809	
6R	5′-CCTTCCTTAAAGTTTTAATTCTGAGG-3′	4688–4714	60°C

[a] The ORF starts at nucleotide 79 and ends at nucleotide 4558 (Troelstra et al. 1992).

both CS and XP. The defects in these individuals have been assigned to the XP-B, XP-D, and XP-G groups (Hoeijmakers 1993).

The genes defective in the CS-A (Henning et al. 1995) and CS-B (Troelstra et al. 1992) complementation groups have both been cloned recently. A gene designated "*ERCC6*" was cloned by its ability to correct the UV sensitivity of a mutant hamster cell line, UV61 (Troelstra et al. 1990). When the *ERCC6* cDNA was introduced into different human cell lines, it was able to correct the UV sensitivity and post–UV-irradiation RNA-synthesis defect in a CS-B cell line, CS1AN (Troelstra et al. 1992), and this cell strain was found to contain mutations in the *ERCC6* gene. *ERCC6* was therefore subsequently renamed "*CSB*" (Lehmann et al. 1994). *CSB* encodes a 1,493-amino-acid protein, which contains seven domains characteristic of DNA helicases (Troelstra et al. 1992). However, the CSB protein is a member of the SWI2/SNF2 family of ATPases, and, like other members of this family, CSB does not appear to be a helicase (Selby and Sancar 1997). The function of the SWI/SNF protein family is thought to involve remodeling of protein-DNA interactions—such as chromatin structure—in different circumstances (Peterson and Tamkun 1995; Cairns et al. 1996; Pazin and Kadonaga 1997). The *CSA* gene has been cloned directly by its ability to correct the UV sensitivity of a CS-A cell line, CS3BE, and encodes a "WD-repeat" protein (Henning et al. 1995). In order to gain more insight into genotype-phenotype relationships in CS, we have identified mutations in the *CSB* gene in 16 CS patients whom we previously had assigned to this complementation group.

Material and Methods

Cell Culture

All CS cells were primary fibroblast cultures derived from patients with CS. Cultures were grown in Eagle's minimal essential medium supplemented with 15% FCS. The UV-sensitive Chinese hamster cell line UV61 was grown in Eagle's minimal essential medium with 10% FCS.

cDNA Synthesis and PCR

RNA was extracted from ~10^7 cells, by use of lysis either (*a*) in NP40, by spinning out the nuclei and phenol extraction of the cytoplasmic fraction, followed by ethanol precipitation, or (*b*) with guanidinium isothiocyanate, followed by phenol extraction and isopropanol precipitation. cDNA synthesis was performed in three parts (A–C), by use of primers 2R, 4R, and 6R (see table 1). Reactions were performed by use of 1–5 μg RNA and Moloney murine-leukemia-virus reverse transcriptase in a total volume of 20 μl. After incubation for 1 h, the mixture was diluted to 40 μl, and 5 μl was used in PCR. Each of the cDNA samples was amplified in two parts, by the primers shown in table 1, so that the whole open reading frame (ORF) was amplified as six fragments. PCR mixtures contained 5 μl appropriate cDNA, 20 pmol primers, and 0.125 mM each dNTP, in a total volume of 50 μl. PCR conditions were 35 cycles at 94°C for 1 min, annealing (at temperatures indicated in table 1) for 1.5 min, and elongation at 72°C for 3 min. The products were reamplified by means of the same 3′ primer and a nested biotinylated 5′ primer, under conditions identical to those described above. PCR products were captured on streptavidin-coated Dynabeads and were sequenced by means of the dideoxy termination procedure with T7 DNA polymerase and ^{35}S-dATP, as described elsewhere (Steingrimsdottir et al. 1993).

In compound-heterozygote patients, in whom the mutations in the two alleles were different, the sequencing gel had both normal and mutant bands at the site of the mutation. In many of these cases, we confirmed the mutation by cloning the PCR products into a "T vector" and sequencing several clones.

Table 2

Mutations in CS-B Patients

Cell Line	Inactivating Mutation(s)[a]	Amino Acid Change	Location
CS2TAN	G1630A	Trp517stop	Exon 7
CS1TAN	C2282T	Arg735stop	Exon 11
CS8BR	C2639T	Gln854stop	Exon 13
25627	C2282T	Arg735stop	Exon 11
	C1436T	Arg453stop	Exon 5
CS4BR	C629T	Gln184stop	Exon 4
	C2087T	Arg670Trp	Exon 10
CS1BO	−G 1597 and C2918T	Frameshift 506→542stop and Arg947stop	Exon 6 and 16
	G3363C	Pro1095Arg	Exon 18
CS1BE	C2087T	Arg670Trp	Exon 10
	−A 3615	Frameshift 1179→1200stop	Exon 18
CS10BR	+26nt 3686	Frameshift 1203→1235stop	Exon 18
CS1ABR	−G 2223	Frameshift 715→738stop	Exon 10
CS10LO	+T 1359	Frameshift 427→435stop	Exon 5
CS2BE	−T 3614	Frameshift 1179→1200stop	Exon 18
	Deletion of exon 10	Deletion of 665−723	Exon 10
CS2BI	C2087T	Arg670Trp	Exon 10
	C3204T	Pro1042Leu	Exon 18
CS3TAN	T2630A	Trp851Arg	Exon 13
CS7TAN	A3716G	Arg1213Gly	Exon 18
CS1IAF	T2949G	Val957Gly	Exon 16
CS1MA	Deletion of exon 10	Del 665–723	Exon 10

[a] Missense mutations shown to be inactivating by failure to restore UV resistance to UV61 cells.

Functional Complementation

Mutations were introduced into the *CSB* gene by means of two different procedures. PCR products containing the mutation of interest were cloned into a T vector and were sequenced to check for the absence of any PCR-induced spurious mutations. An appropriate restriction fragment containing the mutation was excised and used to replace the corresponding wild-type fragment in the intact *CSB* gene in BlueScript. Alternatively, the mutation was introduced directly into the gene by site-directed mutagenesis using the procedure of Kunkel et al. (1987). The entire ORF was checked for the absence of spurious mutations. The *CSB* gene was then transferred as a *Bam*HI fragment into the mammalian expression vectors pCDNA3 or pCIneo (Invitrogen). For transfection experiments, 5×10^5 UV61 cells were plated on 9-cm dishes. Two days later, 40 μl polybrene was added to the plates, followed by 5 μg DNA. After 6 h, the cells were treated with 20% dimethyl sulfoxide in serum-free medium for 4 min. Two days later, the cells were trypsinized, split into three aliquots, and replated in the presence of 1 mg G418/ml. The cells on one dish were grown and frozen as a mass population, and the second dish was subjected to UV-irradiation selection with three doses of 4 Jm^{-2} with 2-day intervals between; individual colonies were isolated from the third plate and were grown for UV-irradiation–survival experiments.

Results

The 16 patients whom we have analyzed all failed to restore RNA synthesis to normal levels after UV irradiation, a phenotype that we regard as diagnostic for CS (Lehmann et al. 1993). Cell-fusion studies have assigned these patients to the CS-B group (Lehmann 1982; Stefanini et al. 1996). For molecular analysis, total cellular RNA was reverse transcribed by use of three *CSB*-specific primers. Each of the three cDNA samples was then amplified in two parts by PCR, so that the whole *CSB* cDNA was amplified in a total of six overlapping fragments of ~1 kb each, which were then sequenced directly. Results of our analyses are summarized in table 2 and figure 1.

Nonsense Mutations

Four cell strains contained nonsense mutations in both alleles, which resulted in truncation of >40% of the protein. Two Turkish patients, CS2TAN and CS1TAN, and one British patient of Asian origin, CS8BR, all the offspring of consanguineous marriages, were homozygous for G1630A, C2282T, and C2639T, respectively, which convert Trp517, Arg735, and Gln854, respectively, to stop codons. In 1978 we reported on a sun-sensitive patient, designated "11961" (Arlett et al. 1978), who was subsequently diagnosed as having CS and who was assigned to group B (Lehmann 1982). We have deter-

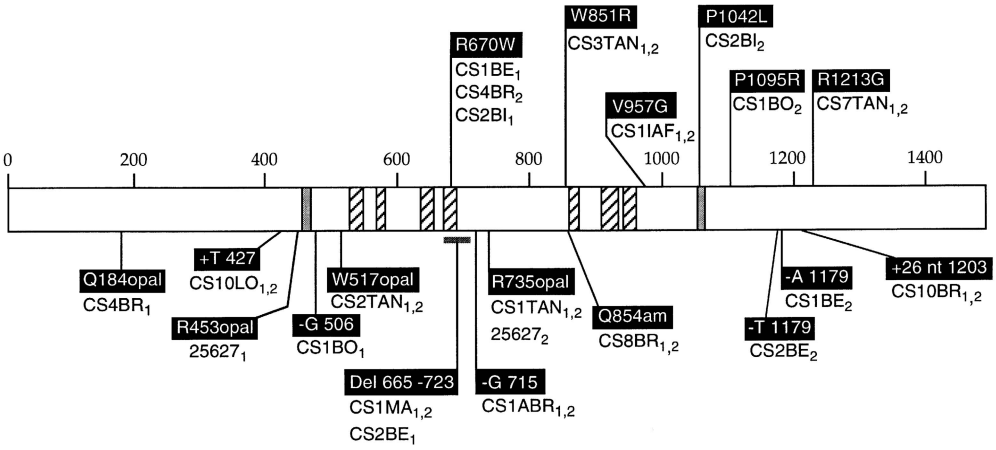

Figure 1 Inactivating amino acid changes caused by mutations in the *CSB* gene. The CSB protein is shown with the seven domains conserved in DNA helicases (*hatched boxes*) and with the putative nuclear-localization signals (*gray-shaded boxes*). Amino acid changes resulting from mutations are shown boxed, with the change indicated by white letters on a black background, and the cell-line designations are shown below them. Subscripts "1" and "2" denote the different alleles. Mutations shown below the depicted protein result in protein truncations, whereas those above the depicted protein are all single-amino-acid changes.

mined the sites of the mutations in cells from his similarly affected brother, coded as "25627." The patient is a compound heterozygote: one allele contains the Arg735opal mutation found in CS1TAN, and the other allele contains the mutation C1436T, resulting in Arg453opal. CS4BR, derived from a patient in the United Kingdom, is a compound heterozygote with the mutation C629T, resulting in Gln184opal in one allele. CS1BO is a rare example of a black patient with CS. She is a compound heterozygote with C2918T in one allele, resulting in Arg947opal; this mutation is, however, not relevant for the pathological phenotype, since it is 1,321 bases downstream of a frameshift mutation in the same allele (see below).

Frameshifts and Insertions

Single-base frameshifts, all resulting in stop codons 7–35 amino acids downstream, were detected in six patients and are shown in table 3. Three of these were single-base deletions or insertions in runs of five or six identical bases, presumably the result of replication slippage, which could also account for the loss of an A residue sandwiched between a run of 5 T's and a single T in CS1BE. In CS1BO there was a loss of a G residue from the center of a 12 bp inverted repeat. In CS10BR there was an insertion of 26 bases at position 3686, in the middle of exon 18. The origin of this insertion is not known. At the time of the writing of this report, this sequence was not present in any of the publicly available sequence databases.

Missense Mutations

A missense mutation, C2087T, causing an Arg→Trp change at amino acid 670, has been found in one allele of three unrelated patients, CS4BR and CS2BI from the United Kingdom and CS1BE (GM1629) from the United States. Arg670 is in helicase domain III and is part of a stretch of 34 amino acids that are 97% identical or conserved in CSB and the *Saccharomyces cerevisiae* homologue, Rad26 (van Gool et al. 1994). Two other mis-

Table 3

Frameshifts and Insertions

Cell Strain	Status	Mutation	First Mutated Codon	Sequence	Size of Truncated Protein (amino acids)
CS10LO	Homozygous	+T 1355–1359	427	ACTTTTTCC	434
CS1ABR	Homozygous	−G 2218–2223	715	ATGGGGGGAT	737
CS2BE	Heterozygous	−T 3610–3614	1179	AATTTTTAT	1,199
CS1BE	Heterozygous	−A 3615	1179	TTTTTATAAGC	1,199
CS1BO	Heterozygous	−G 1597	506	AAAAAGCTTTTTT	541
CS10BR	Homozygous	+26nt[a] 3686	1203	ACCAA +26 nt[a] +AGCAA	1,234

[a] Insertion of GG GCT GGC TGC TTA AGG TCC ACC TTA.

sense homozygous mutations were present in highly conserved regions. Trp851Arg, found in CS3TAN from Turkey, is close to helicase domain IV in a run of 37 amino acids that are 83% identical or conserved between CSB and Rad26, and Val957Gly in CS1IAF from Israel is in a run of 79 amino acids that are 97% conserved between yeast and man. The other missense mutations (Pro1042Leu, heterozygous in CS2BI; Pro1095Arg, heterozygous in CS1BO; and Arg1213Gly, homozygous in CS7TAN) were in less-conserved regions of the protein, but Pro1042Leu is located in the putative nuclear-localization sequence (Troelstra et al. 1992).

It is of interest that all six of the missense mutations are in the C-terminal two-thirds of the protein. This could indicate that the N-terminal third of the protein is less important for the repair function of the CSB protein and that this domain is involved in some other function (for other possible roles of the CSB protein, see the Discussion section). Preliminary results from site-directed mutagenesis of the N-terminal part of the gene are consistent with this possibility.

Splice Mutations

In two patients the *CSB* mRNA was abnormally spliced. Exon 10 was absent from the cDNA of CS1MA, suggesting that the mutation in both alleles affected the splicing of this exon. This same exon was missing in one allele of CS2BE. We have sequenced the regions around the splice-donor site of intron 10 and the splice-acceptor site of intron 9, in genomic DNA of CS1MA, but this did not reveal the cause of the abnormal splicing. The loss of exon 10 results in an in-frame deletion of 59 amino acids encompassing the whole of helicase domain III.

Polymorphisms

In addition to the inactivating mutations, we have detected the following changes in at least two patients: G214C (Leu45), C2830T (Gly917), G1275A (Gly399Asp), A3368G (Met1097Val), and A4317G (Gln1413Arg). We presume that Gly399Asp and Met1097Val are silent. Residue 399 is Gly in the human gene but is Asp in the mouse gene, and Met1097Val is a conservative change. We show below that Gln1413Arg is not inactivating. In patient CS7TAN, we found three of the above-mentioned polymorphic changes, as well as Arg1213Gly, which we show to be inactivating (see below), and Lys255Thr, all mutations being homozygous. Since Arg1213Gly is sufficient to account for the repair-deficient phenotype, we presume that Lys255Thr does not contribute to the phenotype.

Functional Studies

Our mutation analyses have identified several different types of mutation in the *CSB* gene. The nonsense, frameshift, and splice mutations have sufficiently severe effects on the protein structure for us to be confident that they are indeed the inactivating mutations. This contention is supported by our recent results (to be presented elsewhere) showing that deletion of only 25 amino acids from the C-terminus of the protein completely abolishes its repair function. In order to confirm that the missense mutations that we identified were causative, we have constructed intact *CSB* cDNAs containing these mutations in Bluescript, either by subcloning fragments used in our sequence analysis or by site-directed mutagenesis. All cloned PCR products or mutated plasmids were sequenced in their entirety, to ensure that the DNA contained only the desired mutations. The *CSB* cDNA was then transferred, as a *Bam*HI fragment, from Bluescript into one of the mammalian expression vectors pCDNA3 or pCIneo. Normal and mutant plasmids were transfected into the UV-sensitive CHO cell line UV61 (from the same complementation group as the CS-B cells), and selection for the *neoR* gene was applied by use of G418. Clones were picked and expanded, and the remaining clones in the dishes were trypsinized, respread, and subjected to UV-irradiation selection with three doses, each of 4 Jm^{-2}. This gave a crude measure of UV sensitivity of the transfectants. Whereas a confluent layer of cells resulted from the culture of UV61 cells that were transfected with the wild-type *CSB* gene and then UV irradiated, very few cells survived after transfection with either empty vector or the *CSB* gene containing the mutations R670W, W851R, V957G, P1042L, or R1213G. In contrast, when the cDNA contained mutation Q1413R, which we presumed to be a silent polymorphism, the cells survived the UV-irradiation selection, as did the wild-type transfectants.

The clones that were picked and expanded without UV irradiation were examined by PCR, to ensure that the transfected human *CSB* cDNA was intact. For each transfection, two clones in which PCR indicated that the whole of the cDNA was present were examined quantitatively for the effects that UV irradiation had on cell survival. Results are shown in figure 2. These results confirmed the findings seen with the batch cultures described above. In early experiments, in which the pCDNA3 expression vector was used, clones from cells transfected with the wild-type gene were much more resistant than were untransfected UV61 cells, although the survival did not reach that of the wild-type parental cell line AA8. In later experiments, with the pCIneo vector, the wild-type *CSB* gene restored the survival of UV61 cells up to the level of the parental AA8 line. Irrespective of which vector was used, the survival of

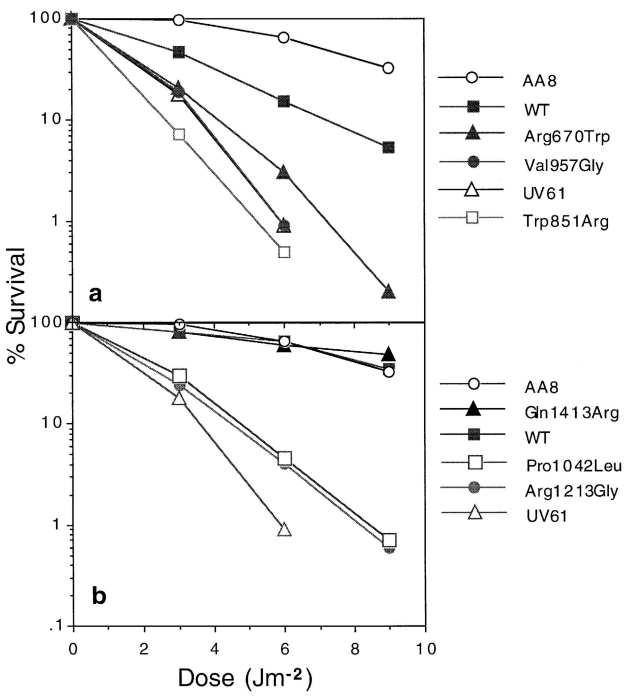

Figure 2 UV-irradiation survival of UV61 cells transfected with the *CSB* gene containing different mutations. Transfectants are from *CSB* constructs made in pCDNA3 (*a*) or in pCIneo (*b*). "AA8" denotes the normal parental hamster cell line from which UV61 was derived, and all other cell lines are UV61 cells transfected with *CSB* constructs containing the indicated mutations.

UV61 cells transfected with DNA containing the mutations that we had identified as inactivating was indistinguishable from that of cells transfected with the empty vector. In contrast, the survival of transfectants with the Q1413R DNA approached that of the normal transfectant.

Discussion

We have identified the inactivating mutations in the *CSB* gene in 16 affected patients. The results are summarized in figure 1 and tables 2 and 4. A variety of different types and positions of mutations result in the CS phenotype. Of the 18 identified inactivating mutations, four were CG→TA transitions at CpG sites, resulting from deamination of 5-methylcytosine, a proportion similar to that found in other human genetic disorders; and a further five were transitions at other sites, two were transversions, six were frameshifts, and one was an aberrant splicing.

Twelve of the 18 mutations resulted in severely truncated products, because of either stop codons, frameshifts, or splice abnormalities. In nine patients (table 4, top), both alleles were affected in this way, and it is highly unlikely that the CSB protein in these individuals would have any functional ability. This was also found in the single patient analyzed by Troelstra et al. (1992). This demonstrates that *CSB* is not an essential gene, as also has been found for the yeast *RAD26* homologue (van Gool et al. 1994) . Further evidence to support this comes from the generation of a *CSB* knockout mouse, which showed near-normal development (van der Horst et al. 1997). Of the six missense mutations, three were located in regions that were highly conserved not only in the mouse CSB protein but also in the homologous yeast Rad26 protein. These mutations lie either within or very close to the helicase domains of the protein and are likely to abolish any function associated with these domains. Of the three other mutated sites, Arg1213 is conserved in the human and mouse proteins. Pro1042, which is mutated to leucine in one allele of CS2BI, is not present in the mouse protein (G. T. J. van der Horst and J. H. J. Hoeijmakers, personal communication), but it is located in a putative nuclear-localization signal, and we have shown that this alteration, like the other amino acid changes indicated in figure 1, fails to restore UV-irradiation resistance to UV61 cells.

The CS patients studied come from a wide range of racial backgrounds, including Caucasian, Turkish, Indo-Pakistani, Israeli Jewish, and Black patients. The clinical features of CS are fairly heterogeneous, as can be seen in table 4, in our earlier clinical survey (Lehmann et al. 1993), and in reviews in the literature (Nance and Berry 1992). We previously had found that, in those patients in whom RNA synthesis failed to recover after UV irradiation (a feature that we use as a diagnostic marker for CS), the magnitude of this defect was broadly similar, irrespective of the clinical features (Lehmann et al. 1993). Similar observations have been made in Japanese patients, by Sugita et al. (1991). Lowry (1982 [also see Nance and Berry 1992]) has suggested that CS can be divided clinically into type I, with classical CS symptoms that become manifest within the first few years of life, and type II, with more-severe symptoms already manifest prenatally. Cellular defects in the response to UV irradiation appear to be similar for type I patients and type II patients (Sugita et al. 1991). We do not have full clinical histories of all the patients in our study, but at least four of them—CS1MA, CS1ABR, CS8BR, and CS1BO—had the more-severe, type II features. Three of these are homozygous for mutations resulting in severely truncated proteins, whereas CS1BO is a compound heterozygote and has the Pro1095Arg change in one allele. However, six other patients also produced truncated products from both alleles but had classical, type I features. It thus seems unlikely that the assignment into one of the two clinical types can be based on the position or type of mutation; the clinical disparity is more likely

Table 4

CS Mutations and Symptoms

Patient[a]	Amino Acid Change[b]	Racial Group	Age at Biopsy (years)	Age at Onset (years)	Pigmentary Retinopathy Symptoms	Gait Defect[c]	Cataracts	Caries	Reference[d]
Truncated protein in both alleles:									
CS10LO	+1 nt 427	Black	4	.5	No	Yes	No	?	
CS2TAN	Trp517stop	Turkish	9	1.5	Yes	Yes	No	No	Lehmann et al. (1993); 88
CS1MA[e]	Deleton of 665–723	Caucasian	4 (died)	0	?	Yes	Yes	Yes	Lehmann et al. (1993); 34
CS1ABR[e]	−1 nt 715	Caucasian	6	0			Yes		Stefanini et al. (1996)
CS1TAN	Arg735stop	Turkish	8	1.5	Yes	Yes	No	Yes	Lehmann et al. (1993); 77
25627	Arg735stop Arg453stop	Caucasian	5		Yes	Yes			
CS8BR[e]	Gln854stop	Indo-Pakistani	.5	0					
CS2BE	−1 nt 1178 Deletion of 665–723	Caucasian	21	1	Yes	Yes			Brumback et al. (1978)
CS10BR	+26 nt 1203	Caucasian	5	.2	No	Yes	Yes	No	
Missense mutation in at least one allele:									
CS4BR	Arg670Trp Gln184stop	Caucasian	24	3	Yes	Yes	Yes	Yes	Lehmann et al. (1993); 39
CS2BI	Arg670Trp Pro1042Leu	Caucasian	16	.7	Yes	Yes			Lehmann et al. (1993); 9
CS1BE	Arg670Trp −1 nt 1179	Caucasian	11	1.5	Yes	Yes	No		Brumback et al. (1978)
CS3TAN	Trp851Arg	Turkish	1.7	.3	No	Yes	No	No	Lehmann et al. (1993); 98
CS1IAF	Val957Gly	Israeli Jewish	7	2	Yes			Yes	Lehmann et al. (1993); 67
CS1BO[e]	Pro1095Arg −1 nt 506	Black	.75	0	Yes	NA	Yes	Yes	Stefanini et al. (1996)
CS7TAN	Arg1213Gly	Turkish	18	2	Yes	Yes	No	Yes	

[a] Listed in order of amino acid position of truncation or frameshift.

[b] The mutation responsible for the pathological phenotype is underlined; for compound heterozygotes, the less severe mutation is underlined. This is assumed to be the more C-terminal of two truncating mutations—or a missense mutation, if one allele is a truncation and the other is a missense mutation.

[c] NA = not applicable.

[d] Numbers refer to the designations of the patients in Lehmann et al. (1993).

[e] Type II patient with symptoms at birth.

to result from other differences in genetic background or in the intrauterine environment.

CS-B (and CS-A) cells are sensitive to the lethal effects of UV irradiation and of a variety of chemical carcinogens (Wade and Chu 1979). This is associated, in all cases, with a failure of RNA synthesis to recover after DNA damage (Mayne and Lehmann 1982). Until recently this had been attributed to a deficiency in the ability of CS to perform TCR—that is, the rapid removal of damage from the transcribed strand of active genes (van Hoffen et al. 1993). These observations, in turn, have led to the suggestion that the function of the CS gene products is to recruit the NER machinery to actively transcribed regions of DNA, a role analogous to that of the transcription-repair coupling factor Mfd protein in *Escherichia coli* (Selby and Sancar 1993). Although this model has been widely accepted, it has recently been questioned by some of its original proponents, who found that, after damage to CS cells by the carcinogen N-acetoxy-acetylaminofluorene, TCR occurs at normal levels, although RNA synthesis fails to recover (van Oosterwijk et al. 1996). Those authors proposed that, after UV irradiation, the dual-function transcription/repair factor TFIIH is converted into a form utilized in NER and that the function of the CS proteins is subsequently to revert TFIIH from repair mode to transcription mode, so that transcription can recommence. It has been proposed that, in CS patients, TFIIH is locked in the repair mode and transcription fails to recover (van Oosterwijk et al. 1996). In an alternative model, the features of CS have been suggested to result from defects in base excision repair of oxidative base damage (Cooper et al. 1997).

Except for sun sensitivity, the clinical features of CS cannot be obviously attributed to defects in DNA repair. This is in contrast to xeroderma pigmentosum, in which most of the symptoms are readily explicable in terms of a repair deficiency. It has therefore been suggested that the CSB protein has a second function, possibly an involvement with transcription, as has been found to be the case with TFIIH (Bootsma and Hoeijmakers 1993; Friedberg 1996). Although this is an attractive hypothesis, *CSB* is not an essential gene, and therefore the CSB protein cannot be an integral part of the transcriptional machinery. Evidence in support of a role for the CS proteins in general transcription comes both from data suggesting that transcription rates are reduced in CS cells (Balajee et al. 1997) and from data showing that 10%–15% of RNA polymerase II is strongly associated with CSB protein in cell extracts (van Gool et al. 1997*a*). This has led to the suggestion that the role of CSB might be to release stalled transcription, by direct action on the RNA polymerase II (van Gool et al. 1997*b*). An alternative possibility is that CS genes are involved in tissue-specific transcription. A gene that has been designated "*ATR-X* " is another member of the SWI/SNF superfamily. It is an X-linked gene that, when mutated, specifically down-regulates expression of several genes, including the α-globin gene on chromosome 16 (Gibbons et al. 1995; Picketts et al. 1996). One can envisage that CSB might act in a similar manner on the expression of a set of tissue-specific genes involved in aspects of development that are abnormal in CS patients.

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