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ARTICLE

Deletion of 5′ sequences of the *CSB* gene provides insight into the pathophysiology of Cockayne syndrome

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Cockayne syndrome is an autosomal recessive neurodegenerative disorder characterized by a specific defect in the repair of UV-induced DNA lesions. Most cases of Cockayne syndrome are caused by mutations in the *CSB* gene but the pathophysiological mechanisms are poorly understood. We report the clinical and molecular data of two severely affected Cockayne patients with undetectable CSB protein and mRNA. Both patients showed severe growth failure, microcephaly, mental retardation, congenital cataracts, retinal pigmentary degeneration, photosensitivity and died at the ages of 6 and 8 years. UV irradiation assays demonstrated that both patients had the classical DNA repair defect. Genomic DNA sequencing of the *CSB* gene showed a homozygous deletion involving non-coding exon 1 and upstream regulatory sequences, but none of the coding exons. Functional complementation using a wild-type *CSB* expression plasmid fully corrected the DNA repair defect in transfected fibroblasts. Horibata *et al* recently proposed that all type of *CSB* mutations result in a defect in UV damage repair that is responsible for the photosensitivity observed in the syndrome, but that only truncated CSB polypeptides generated by nonsense mutations have some additional inhibitory functions in transcription or in oxidative damage repair, which are necessary to lead to the other features of the phenotype. Our patients do not fit the proposed paradigm and new hypotheses are required to account for the pathophysiology of Cockayne syndrome, at the crossroads between DNA repair and transcription.
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Introduction

Cockayne syndrome (CS) (MIM 133540-216400) is an autosomal recessive neurodegenerative disorder characterized by progressive growth failure, microcephaly, mental retardation, retinal pigmentary degeneration, sensorineural deafness and photosensitivity.[1] CS belongs to the family of DNA repair disorders together with xeroderma pigmentosum (XP) (MIM 278700-780) and trichothiodystrophy (MIM 601675). Fibroblasts from CS patients show a specific defect in transcription-coupled DNA repair (TCR), a subpathway of nucleotide excision repair (NER) involved in the removal of UV-induced DNA lesions of actively

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transcribed genes.[2–4] The other subpathway of NER (global genome repair or GGR), which is responsible for the removal of UV-induced lesions in the rest of the genome, is active in CS cells but defective in XP cells. CS has been mainly associated with mutations in the *CSB* and *CSA* genes.[5,6] The pathogenesis of CS and the exact role of CSB and CSA proteins remain poorly understood. Additional functions of these proteins in general transcription, in chromatin remodeling and/or in oxidative damage repair have been recently put forward, especially to account for the neurological features of CS patients.[7–10]

No obvious genotype–phenotype correlation has ever been documented in classical CS patients,[11] but the recent description of a severe *CSB* mutation in a milder NER disorder named UV-sensitive syndrome (UVSS) (MIM 600630) appeared as a potential breakthrough in the understanding of the molecular basis of CS.[12] UVSS is clinically characterized by cutaneous photosensitivity including freckling and pigmentary anomalies of sun-exposed skin, but does not involve cancer proneness or neurological impairment.[13,14] At the cellular level, fibroblasts from UVSS patients show the same TCR-restricted defect following UV exposure as CS cells. In the only known case of UVSS related to a *CSB* mutation, the very mild clinical presentation of UVSS is due to a homozygous truncating mutation in the 5′ end of the *CSB* coding sequence inducing the absence of detectable CSB protein on western blot,[12] whereas the much more severe phenotype of classical CS patients is usually due to apparently milder mutations.[11,15] The following hypothesis was proposed to resolve this paradox: UVSS and CS patients may be equally defective in TCR of UV-induced lesions, which are responsible for the clinical photosensitivity observed in both syndromes, but CSB polypeptides produced in CS-B patients (and completely absent in the reported UVSS patient) may have some inhibitory functions in transcription or in oxidative damage repair that could specifically lead to the severe CS phenotype.[12] In contrast to this hypothesis, we report the clinical and molecular characterization of two severely affected CS patients with undetectable CSB protein. Our results do not fit the proposed paradigm and possible explanations for this discrepancy are discussed.

Material and methods

Cell culture

Primary fibroblast cultures were established from skin biopsies from the patients. Cells were grown at 37°C in 5% CO_2 humidified atmosphere in Eagle's minimal essential medium (GIBCO, Inchinnan, UK) and supplemented with 15% fetal bovine serum (Dominique Dutscher SA, Strasbourg, France), 1% antibiotics and fungicides and L-glutamine. Four different cell lines were used and were numbered as follows: CS548VI corresponds to patient 1, CS539VI to patient 2, CS548VI#1 and CS539VI#1 to their *CSB* wild-type-complemented counterparts, 405VI to a healthy control, CS177VI and CS543VI to unrelated CS-B patients with documented mutations in the *CSB* gene, XP202VI to an XP patient with a known mutation in the *XPC* gene.

UV irradiation assays

Recovery of RNA synthesis (RRS) and unscheduled DNA synthesis (UDS) were evaluated as described previously[16,17] and were considered to reflect the efficiency of the TCR and GGR pathways respectively. For RRS measurement, coverslip-grown fibroblasts were exposed to different doses of 254 nm UV light (5–20 J/m^2), allowed to recover for 23 h, labeled with [^{3}H]uridine for 1 h and processed to autoradiography. The next day, the number of grain was counted for at least 30 nuclei per sample. The relative rate of RRS was expressed as (number of grains in UV-exposed nuclei)/(number of grains in non-exposed nuclei). For the UDS assay, coverslip-grown fibroblasts were exposed to different doses of UV light, pulse-labeled with [^{3}H]thymidine, post-incubated with cold thymidine and subjected to autoradiography. After 1 week, the average number of grains was determined for at least 30 non-S-phase nuclei per sample.

Western blot

Whole-cell extracts were separated on SDS/8% polyacrylamide gel and transferred to Hybond nitrocellulose membrane (Amersham Biosciences) in blotting buffer (Tris 25 mM, glycine 0.2 mM, methanol 20%). After blocking (60 min in 5% milk), the blot was incubated overnight with the anti-CSB antibody (dilution 1:1000) purchased from Santa Cruz Biotechnology (CA, USA) and 45 min with anti-goat secondary antibody (dilution 1:5000), then revealed by ECL Western blotting detection system (Amersham Biosciences).

Analysis of CSB mRNA

Total RNA was prepared from human fibroblast cell lines using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. One microgram of RNA and 100 ng of hexamer were heated to 65°C for 10 min. The solution was immediately chilled on ice and mixed with a premix that contained 8 μl 5 × reverse transcription buffer, 0.4 μl 100 mM dNTP, 4 μl DTT 100 mM, 40 U RNAguard and 400 U Moloney murine leukemia reverse transcriptase (Gibco BRL). cDNA synthesis was performed at 37°C for 60 min.

PCR amplifications were performed on 1 μl of cDNA using Mastercycler (Eppendorf, Hamburg, Germany). CSB mRNA was amplified by RT-PCR in nine overlapping fragments covering the whole CSB mRNA sequence (see Supplementary Information).

Sequencing of genomic DNA

Cells were harvested and incubated in TE 20.5/SDS 1%, protease K (10 mg/ml) overnight at 42°C. NaCl 5 M and absolute ethanol were added to the samples before centrifugation at 3500 r.p.m. for 15 min. The pellets were recovered and homogenized in TE 20.5/NaCl 0.2 M overnight at 42°C. Precipitation step was performed with absolute ethanol and the suspension was pelleted at 3500 r.p.m. for 15 min. The pellets were finally resuspended in TE 20.1 O/N at 42°C. DNA concentration was determined using $OD_{260\,nm}$ measurement and 50 ng was used for PCR amplifications, performed on Mastercycler (Eppendorf, Hamburg, Germany).

We amplified the 21 exons from *CSB* gene, using primers encompassing each exon and their flanking sequences. Eleven pairs of primers were also designed to cover the whole first intron of the *CSB* gene (see Supplementary Information). The PCR products were purified and sequenced on ABI PRISM®3100 sequencer (Applied Biosystems, Foster, CA). Sequence analyses were performed using SeqScape (Applied Biosystems).

Microsatellite analysis

We used three microsatellites on each side of the *CSB* gene (D10S1793, D10S2216, D10S1609, D10S1724, D10S1447 and D10S196) and one intragenic marker (D10S1766). Fifty nanograms of DNA was amplified by fluorescent PCR in a total volume of 25 μl using 0.2 μl of Taq DNA polymerase. PCR products were then pooled, diluted and analyzed on CEQ8800 genetic analysis system (Beckman Coulter).

Functional complementation

Functional complementation was performed using a pcDNA3.1 plasmid containing the CSB cDNA driven by the cytomegalovirus promoter. This plasmid was electroporated into the cells according to the Nucleofector™ protocol (Amaxa biosystems, Germany): 5×10^5 pelleted cells were mixed with 3 μg of the plasmid and subjected to electroporation according to the manufacturer's instructions (Amaxa). The cells were then cultured in a standard medium with 20% fetal bovine serum for 48 h, before neomycin (400 μg/l) was added to the medium to select the transfected clones. The neomycin-resistant clones were pooled together after 3 weeks of culture. The RRS assay and western blot analysis were then performed on this pooled population.

All molecular and clinical studies were approved by the Local Ethics Committee at the Strasbourg University Hospital.

Results

Clinical reports

Patient 1 was a girl born at 39 weeks gestation by vaginal delivery. There was no known consanguinity in recent familial history but both healthy parents originated from the highly inbred population of Reunion Island (French Overseas Department in the Indian Ocean). Pregnancy was marked by moderate intrauterine growth failure in the third trimester and dilated cardiomyopathy was noted in the fetus from the 30th week. Birth weight was 2225 g (−2.5 SD), height was 43 cm (−3 SD) and head circumference was 31 cm (−2 SD). In the first months of life, the infant showed axial hypotonia, feeding difficulties and poor visual contact. Ophthalmological examination showed bilateral congenital cataracts, surgically removed at 6 months. The patient progressively showed severe mental retardation and extreme growth failure. She was never able to sit unassisted and never developed any language. She never presented any epileptic seizure. At the age of 2 years, her body weight was 6.5 kg (−4.5 SD), height was 67 cm (−5 SD) and head circumference was 38 cm (−6 SD). She displayed a livedo pattern of the skin and had very frequent sunburns. Auditory assessment revealed progressive sensorineural deafness and electroretinogram showed pigmentary retinal degeneration.

High-resolution karyotype analysis and extensive screening for metabolic disorders were normal. Brain MRI showed white matter hypersignal in T2-weighted images. CT scan did not reveal intracranial calcifications at 2 years. She later developed the classical cachectic facial appearance of CS with sunken eyes and a beaked nose. She showed spastic hypertonia of the lower limbs and progressive joint contractures. In spite of gastrostomy feeding from the age of 3 years, her body weight still remained extremely low, around −5 SD. She died at the age of 6 years, from cachexia and pneumonia.

Patient 2 (Figure 1) was a girl born at term to apparently non-consanguineous parents also originating from Reunion Island, but the family was not known to be related to the family of patient 1. Birth weight was 3200 g (mean), height was 49 cm (−0.5 SD) and head circumference was 34.5 cm (mean). In her first 3 months, she showed poor visual contact, strabismus, nystagmus, axial hypotonia and delayed developmental milestones. Ophthalmological examination revealed bilateral congenital cataracts, surgically removed at 8 months. In her first year, she suffered from recurrent epileptic seizures. Progressive microcephaly appeared from the third month of life, followed by progressive inflection in growth curves for height and weight below the −2 SD threshold (in the second half of the first year). Neurological examination at 2 years revealed cerebellar ataxia, decreased deep tendon reflexes, progressive spastic hypertonia of the lower limbs, progressive joint contractures of the ankles and knees, kyphoscoliosis, persistent pendular nystagmus and severe mental retardation in spite of relatively preserved social interactions. Sitting position was possible with support at the age of 2 years but walking and articulate language could never been acquired. The patient also showed severe eczematous lesions on the face and forearms strongly

enhanced by sun exposure and had to be protected by total sunblock cream. Pigmentary and atrophic lesions progressively appeared on sun-exposed skin only. She also showed marked enophthalmia and numerous dental caries. Electroretinogram and fundus examination showed severe pigmentary retinal degeneration. Nerve conduction studies showed a severe demyelinating peripheral neuropathy. Brain MRI at 18 months showed global cerebral atrophy with progressive white matter dysmyelination (Figure 2a). CT scan showed bilateral calcifications of the basal ganglia at the age of 2.5 years (Figure 2b). Cerebrospinal fluid protein was elevated (0.7 and 0.9 g/l) with normal cell count. Extensive metabolic investigations were normal. At the age of 3.5 years, body weight was 9 kg (−5 SD), height was 82 cm (−5 SD) and head circumference was 43 cm (−6 SD). Gastrostomy feeding was initiated in her fourth year but was unable to significantly improve her nutritional status. She eventually died at age 8 from extreme cachexia and generalized edema caused by severe hypoproteinemia.

Figure 1 Patient 2 at 5 years of age.

Severe and specific TCR defect in CS539VI and CS548VI cells

To demonstrate the DNA repair defect and the diagnosis of CS in these patients, we performed UV irradiation studies in CS539VI and CS548VI fibroblasts. The RRS level was 20% of the normal control at 20 J/m^2 in CS539VI and CS548VI cells (Figure 3a). In contrast, the UDS level was normal in both cell lines (Figure 3b). These results indicate a specific and severe defect in the TCR subpathway of the NER, which is characteristic of CS and similar to the defect shown by classical CS patients and by the UVSS patient described by Horibata *et al.*[12]

Absence of CSB mRNA and protein in CS539VI and CS548VI cells

To explore the expression of CSB mRNA in the patients, we performed RT-PCR on RNA extracts from cultured fibroblasts. None of the nine fragments of the CSB cDNA could be detected in patients 1 and 2, whereas these fragments were readily amplified in healthy controls in the same

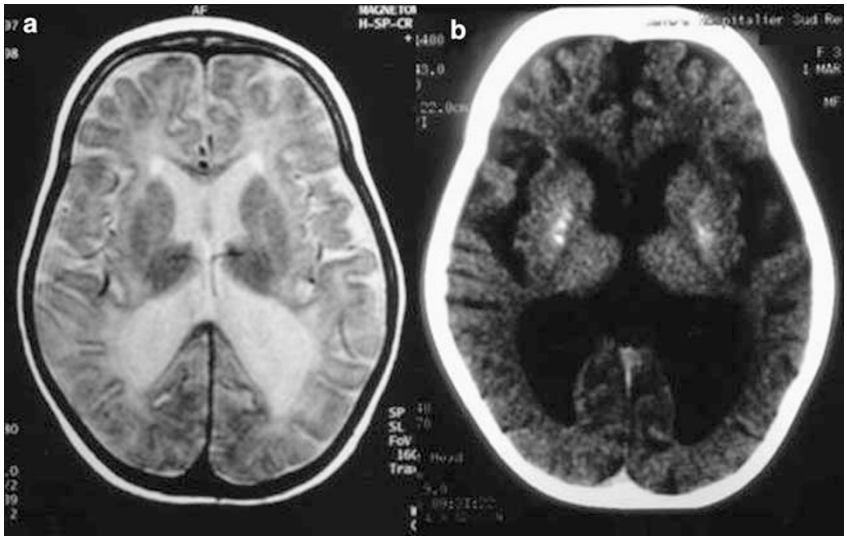

Figure 2 (**a**) T2-weighted brain MRI of patient 2 at 18 months showing diffuse hypersignal of the white matter, moderate cerebral atrophy and dilated ventricles. (**b**) CT scan of patient 2 at 3 years of age, showing bilateral calcifications of the putamina and severe cerebral atrophy with enlarged ventricles.

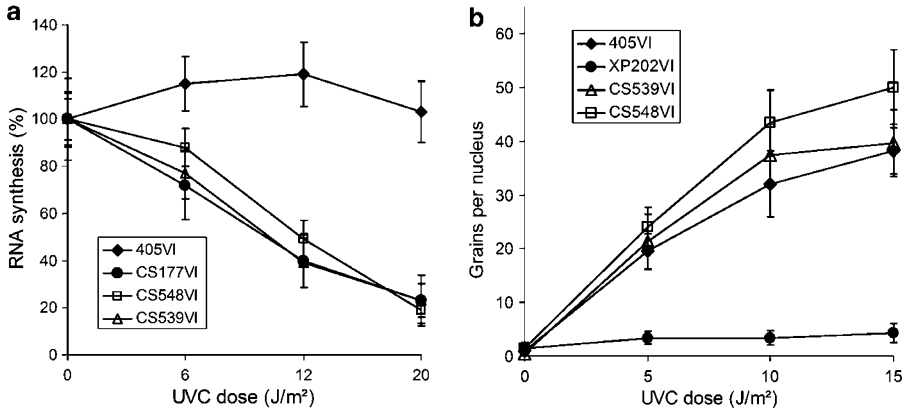

Figure 3 (**a**) Recovery of RNA synthesis (RRS) after UV irradiation in fibroblasts from patients 1 (CS548VI) and 2 (CS539VI), compared to normal control (405VI) and *CSB*-mutated cell line (CS177VI). (**b**) Unscheduled DNA synthesis (UDS) in fibroblasts from patients 1 (CS548VI) and 2 (CS539VI), compared to normal control (405VI) and xeroderma pigmentosum group C cell line (XP202VI).

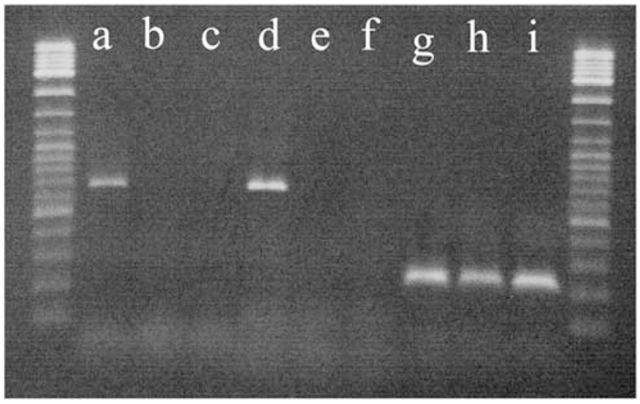

Figure 4 (**a**–**f**) PCR products of the CSB cDNA in normal control (**a** and **d**), patient 1 (**b** and **e**) and patient 2 (**c** and **f**). Lanes a–c correspond to the first fragment (5′) of the CSB cDNA and lanes d–f correspond to the last (9th) fragment (3′) of the cDNA (fragments 2–8 show the same pattern; data not shown). (**g**–**i**) PCR products of the β-actin cDNA in normal control (**g**), patient 1 (**h**) and patient 2 (**i**).

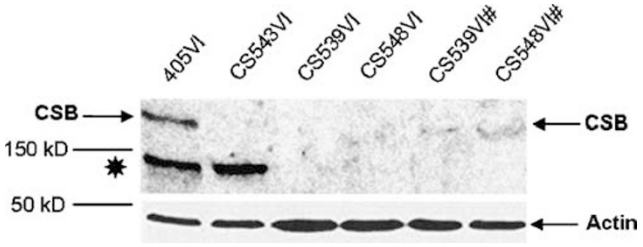

Figure 5 Western blot analysis of CSB protein in normal control (405VI), *CSB*-mutated cell line (CS543VI), patient 1 and patient 2 cell lines (CS539VI and CS548VI) and their complemented counterparts (CS539VI#1 and CS548VI#1). The lower band (✱) probably corresponds to the protein derived from a fusion transcript of the first five exons of *CSB* and the *PGBD3* transposon located in intron 5 of the *CSB* gene; this band is present in the normal control and in the CS-B patients with mutations downstream to intron 5, but is absent in the patients' cell lines and in their complemented counterparts. Actin is used as a loading control.

experiments. β-Actin was used as control to check the integrity of the total mRNA and could be normally amplified both in healthy controls and in patients 1 and 2 (Figure 4). CSB cDNA could also be normally amplified and sequenced in three other CS-B patients CS177VI, CS493VI and CS789VI in which nonsense and frameshift mutations were detected (data not shown).

Using an antibody directed toward the N-terminal portion of the CSB protein, we showed that the upper band migrating to 180 kD and corresponding to the CSB full-length protein (Ensembl peptide ENSP00000348089) was present in the normal control but was not detectable in patients 1 and 2 as well as in other CS-B patients (Figure 5). Another shorter band of 140 kD is likely to correspond to a reported protein (Ensembl peptide ENSP00000363240) derived from a fusion transcript of the first five exons of *CSB* and the *PGBD3* transposon located in intron 5 of the *CSB* gene. This band is present in the normal control and in other CS-B patients with mutations downstream to intron 5, but is absent in patients 1 and 2.

CSB genomic sequencing and microsatellite mapping in patients 1 and 2

All coding exons (exons 2–21) could be amplified in both patients and healthy controls and no mutation could be detected in the patients (Figure 6d–i show exons 1 and 21; data not shown for other exons). Non-coding exon 1 however could not be detected in patients 1 and 2 but was normally amplified in healthy controls (Figure 6a–c). We then amplified the totality of intron 1 using overlapping fragments. None of these fragments were present in CS548VI and CS539VI (data not shown). Thus, exon 1 and intron 1 at least are deleted in the *CSB* genomic sequence of these patients, but exon 2 is not involved in

the deletion. In the patients, the 5′ UTR of the *CSB* gene could not be amplified by PCR at least up to 516 nucleotides upstream of exon 1, that is 6.5 kb upstream of exon 2, which contains the ATG codon (data not shown).

To confirm the length of this large deletion, we then used microsatellite mapping of the *CSB* locus (Figure 7). We showed that an intragenic marker located in intron 1 (D10S1766) was absent in patient 1 and patient 2 but present in the healthy controls (data not shown), confirming that intron 1 is involved in the deletion. We also showed that the D10S1724 marker located upstream of the *CSB* gene could be normally amplified in the patients and was not involved in the deletion. Other markers located upstream of the D10S1724 marker and downstream of the *CSB* gene were normally present in both patients and healthy controls.

To further determine the deletion upstream of the *CSB* gene, we explored the expression of the three genes reported between the *CSB* gene and the D10S1724 microsatellite: (i) *CHAT*, a choline acetyl transferase, (ii) *C10orf53*, 'chromosome 10 open reading frame 53 isoform a' and (iii) *OGDHL*, an oxoglutarate dehydrogenase-like. The first exon of each of these genes was present in the patients and healthy controls (data not shown).

These data altogether lead us to the conclusion that both patients bear a homozygous deletion involving the 5′ UTR of the *CSB* gene, responsible for the absence of detectable CSB mRNA and protein.

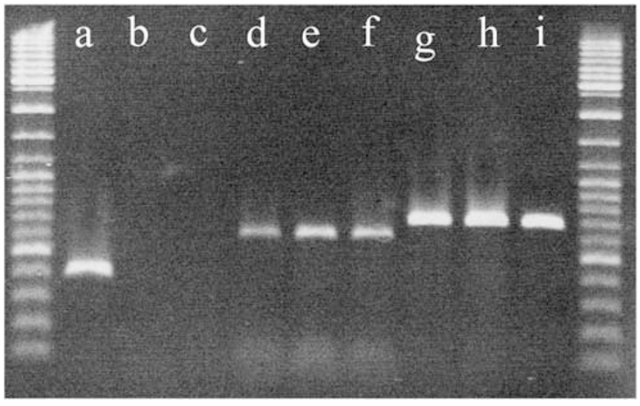

Figure 6 PCR amplification products of exons 1 (lanes **a**–**c**), 2 (**d**–**f**) and 21 (**g**–**i**) of the *CSB* gene in normal control (**a**, **d**, **g**), patient 1 (**b**, **e**, **h**) and patient 2 (**c**, **f**, **i**).

Correction of the TCR defect in CS539VI and CS548VI cells by the introduction of wild-type CSB cDNA

To confirm that the TCR defect was due to the deficiency of the CSB protein, we performed a complementation assay by transfecting the patients' cell lines with a plasmid expressing the wild-type CSB cDNA. The full-length CSB protein, which was undetectable in the parental cell lines, was expressed in the transfected cell lines, even if the expression level of the protein was much lower in the transfected cell lines than in the normal control (Figure 5). However, this low amount of full-length CSB protein was sufficient to fully restore the DNA repair capacity of the cells, as both complemented cell lines exhibited a normal RRS level (Figure 8).

Discussion

We describe two patients who present the clinical criteria for CS as defined by Nance and Berry.[11] They both show microcephaly, extreme growth failure, mental retardation, retinal pigmentary degeneration, enophthalmia and cutaneous photosensitivity. The presence of congenital cataracts, early failure to thrive and death before the end of the first decade further classify these patients in the severe type

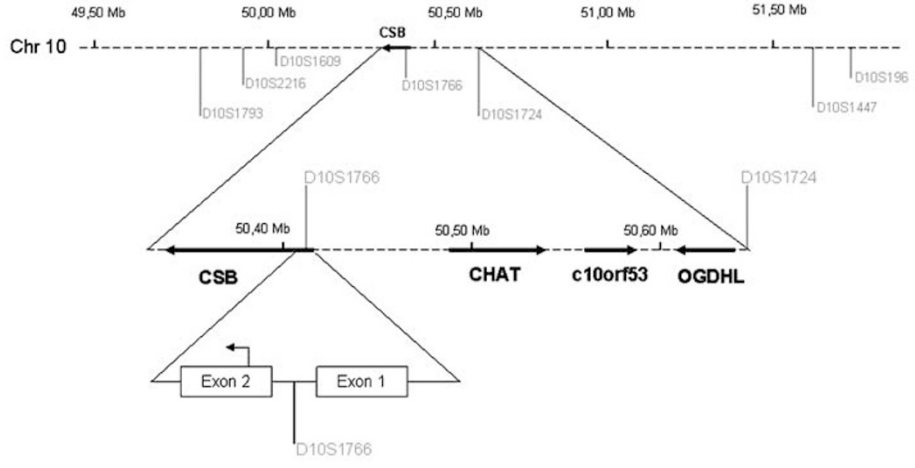

Figure 7 Microsatellite mapping of the genomic region of the *CSB* gene.

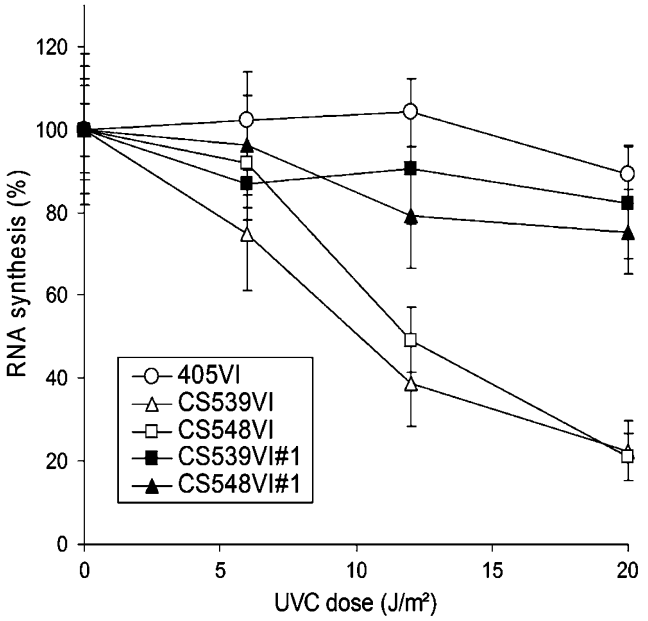

Figure 8 Recovery of RNA synthesis (RRS) after UV irradiation in complemented cell lines derived from patients 1 and 2 (CS548VI#1 and CS539VI#1), compared to their non-transfected counterparts (CS548VI and CS539VI) and to a normal control (405VI).

of CS. Fibroblasts from both patients show exactly the same cellular defect in the NER pathway, which is similar to the classical cellular phenotype of CS and UVSS patients. In both patients, this defect is caused by the absence of detectable CSB protein and mRNA, which appears to be linked to a homozygous deletion in the 5′-UTR region of the *CSB* gene. This homozygous mutation found in two apparently unrelated and non-consanguineous families is consistent with a likely founder effect in the inbred population of Reunion Island.

All coding exons of the *CSB* gene are present at the genomic level in both patients but no mRNA and full-length protein could be detected. No smaller CSB polypeptide either could be detected by immunoblotting using an antibody directed toward the N-terminal end of the protein. Interestingly, an isoform encompassing the first five exons of *CSB* and a transposon, which can be detected by the same antibody in normal controls and in all CS-B patients with mutations downstream to intron 5, was not present in both the patients. This finding suggests that the deletion involving the 5′-UTR region of the *CSB* gene may prevent any transcription of the gene. Alternatively, the absence of exon 1 in the CSB mRNA could be sufficient to induce the decay of all alternative transcripts using exon 1. The upstream end of the deletion could not be defined accurately, but near-by genes or open-reading frames were all present at the genomic level, which argues against the hypothesis that the severe phenotype shown by the two patients could be linked to a contiguous gene syndrome. Very little is known about the regulatory sequences in CS genes and this is the first report of a pathogenic mutation located outside the coding sequence or intron/exon borders. It also indicates that the sequencing of all coding exons of the *CSB* gene from genomic DNA may overlook pathogenic mutations in CS patients and that cDNA sequencing may be a more reliable way to search for mutations in this gene.

To the best of our knowledge, the absence of detectable CSB mRNA and protein has not been described previously in CS patients. A similar absence of the CSB full-length protein has only been reported in one UVSS patient who showed the same NER defect after UV exposure as our patients but displayed a much milder phenotype.[12,14,18] In this case, the absence of detectable CSB full-length protein was secondary to a homozygous stop mutation at amino-acid position 77. This molecular finding was in sharp contrast with the mild clinical picture presented by the patient, who had only moderate cutaneous photosensitivity and no sign of neurological or growth defect. Comparatively, all CS-B patients described previously had 'milder' *CSB* mutations, either missense mutations or truncating mutations downstream to position 77,[11] and paradoxically presented a much more severe clinical picture. It has then been hypothesized that the truncated CSB polypeptides produced in CS-B patients (and not in the reported UVSS patient) may have some additional inhibitory functions in transcription or in oxidative damage repair, which could specifically lead to the severe CS phenotype. This hypothesis, however, would probably imply that a mutated or truncated CSB protein has a dominant negative effect, which is hardly compatible with the totally normal clinical and biochemical phenotype of heterozygous carriers. Direct experimental evidence for this inhibitory effect has not been provided yet; truncated or mutated CSB protein has not been shown to actively inhibit normal transcription processes or oxidative damage repair. Moreover, small amounts of CSB protein, which would not be detectable by the immunoblot, might still be produced in the Japanese UVSS patient (for instance, by readthrough or by restart of the initiation of translation behind the stop codon in the N-terminal part of the protein). As shown in Figure 5, small quantities of CSB might still have substantial biological function and be responsible for the mild phenotype.

Our data do not imply that UVSS and CS cannot be distinguished by a differential effect on transcription or oxidative damage repair. Our report confirms that the same NER defect can be observed in a broad spectrum of conditions ranging from UVSS to severe CS and is consistent with the absence of correlation between the level of residual RRS and the severity of the clinical presentation in CS patients.[19] This suggests that the NER pathway is probably not the most relevant mechanism to account for the clinical severity of the diseases, outside

photosensitivity. Spivak *et al*[8,20,21] have recently confirmed that UVSS and CS share a common defect in TCR of photoproducts in expressed genes, but that CS cells are the only ones to be defective in transcription reactivation of genes containing oxidative lesions, whereas UVSS cells are perfectly proficient in oxidative damage repair. Our data only indicate that this distinctive feature between UVSS and CS is not mediated by inhibitory properties of truncated CSB protein and probably involve other processes to reconcile our data with previous reports. The UVSS Japanese patient and our two CS patients from Reunion Island all originate from a highly consanguineous background. As Horibata *et al*[12] had already mentioned for his case, these patients could well have another mutation in a separate gene which could modify – that is compensate or worsen – the effects of the null mutation in the *CSB* gene.

The fact that a complete lack of CSB protein is still viable suggests the existence of redundant functions and parallel cellular pathways. The $Csb^{-/-}$ knockout mouse displays cancer proneness after UV irradiation but only very mild developmental and neurological impairment whereas the double knockout mouse $Xpa^{-/-}Csb^{-/-}$ exhibits a much more severe phenotype, much closer to the human CS clinical picture.[22,23] The functional redundancy between CSB and other proteins involved in repair or transcription pathways is probably different in mice and humans but remains largely unknown. The apparent absence of genotype–phenotype correlation for *CSB* mutations may be explained by other genes acting as confounding factors. Further molecular and clinical studies will be needed to test these alternative hypotheses and elucidate the intimate mechanisms underlying CS.

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