

## **Functional and clinical relevance of novel mutations in a large cohort of patients with Cockayne syndrome**

Nadege Calmels<sup>1\*</sup>, Elena Botta<sup>2\*</sup>, Nan Jia<sup>3\*</sup>, Heather Fawcett<sup>4</sup>, Tiziana Nardo<sup>2</sup>, Yuka Nakazawa<sup>3,5,6</sup>, Manuela Lanzafame<sup>2</sup>, Shinichi Moriwaki<sup>7</sup>, Katsuo Sugita<sup>8</sup>, Masaya Kubota<sup>9</sup>, Cathy Obringer<sup>10</sup>, Marie-Aude Spitz<sup>11</sup>, Miria Stefanini<sup>2</sup>, Vincent Laugel<sup>10,11+</sup>, Donata Orioli<sup>2+</sup>, Tomoo Ogi<sup>3,5,6+</sup>, Alan Lehmann<sup>4+</sup>

<sup>1</sup>Laboratoire de Diagnostic Génétique, Nouvel Hôpital Civil, F-67091 Strasbourg, France;

<sup>2</sup>Istituto di Genetica Molecolare, Consiglio Nazionale delle Ricerche, Via Abbiategrosso 207, 27100 Pavia, Italy; <sup>3</sup>Department of Genetics, Research Institute of Environmental Medicine

(RIEM), Nagoya University, Furo-cho, Chikusa-ku, Nagoya, Japan; <sup>4</sup>Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton, UK; <sup>5</sup>Nagasaki University Research

Centre for Genomic Instability and Carcinogenesis (NRGIC); <sup>6</sup>Department of Genome

Repair, Atomic Bomb Disease Institute, Nagasaki University, Japan; <sup>7</sup>Department of Dermatology, Osaka Medical College, 2-7 Daigaku-machi, Takatsuki, Osaka 569-8686,

Japan; <sup>8</sup>Division of Child Health, Faculty of Education, Chiba University, Chiba,

Japan; <sup>9</sup>Division of Neurology, National Center for Child Health and Development, 2-10-1, Ohkura, Setagaya Ku, Tokyo, Japan; <sup>10</sup>Laboratoire de Génétique Médicale, INSERM 1112,

Faculté de Médecine, F-67085 Strasbourg, France; <sup>11</sup>Département de Pédiatrie, Hôpitaux Universitaires de Strasbourg, France.

\*These authors contributed equally; +Joint corresponding authors

### **Running Title: Molecular analysis of Cockayne Syndrome**

The authors declare that there are no conflicts of interest.

Correspondence to: A.R.Lehmann, Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton BN1 9RQ, UK; tel +44 1273 678120; Fax +44 1273 678121; e-

mail a.r.lehmann@sussex.ac.uk

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## **ABSTRACT**

**Background** Cockayne syndrome (CS) is a rare, autosomal recessive multi-system disorder characterised by pre- or post-natal growth failure, progressive neurological dysfunction, ocular and skeletal abnormalities and premature ageing. About half of the patients with symptoms diagnostic for CS show cutaneous photosensitivity and an abnormal cellular response to UV light due to mutations in either the *ERCC8/CSA* or *ERCC6/CSB* gene. Studies performed thus far have failed to delineate clear genotype-phenotype relationships. We have carried out a four-centre clinical, molecular and cellular analysis of 124 patients with CS.

**Methods and Results,** We assigned 39 patients to the *ERCC8/CSA* and 85 to the *ERCC6/CSB* genes. Most of the genetic variants were truncations. The mis-sense variants were distributed non-randomly with concentrations in relatively short regions of the respective proteins. Our analyses revealed several hotspots and founder mutations in *ERCC6/CSB*. Although no unequivocal genotype-phenotype relationships could be made, patients were more likely to have severe clinical features if the mutation was downstream of the PiggyBac insertion in intron 5 of *ERCC6/CSB* than if it was upstream. Also a higher proportion of severely affected patients was found with mutations in *ERCC6/CSB* than in *ERCC8/CSA*.

**Conclusion** By identifying more than 70 novel homozygous or compound heterozygous genetic variants in 124 CS patients with different disease severity and ethnic backgrounds, we considerably broaden the *CSA* and *CSB* mutation spectrum responsible for CS. Besides providing information relevant for diagnosis of and genetic counselling for this devastating disorder, this study improves the definition of the puzzling genotype-phenotype relationships in CS patients.

**Key Words**

ERCC6, ERCC8, CSA, CSB, Hotspot

## INTRODUCTION

Cockayne Syndrome (CS) (OMIM #216400 and #133540) is a rare autosomal recessive disorder characterised by severe developmental delay, mental retardation, microcephaly, cachexia and a variety of other features, which may include cataracts, retinal degeneration, sensorineural hearing loss, dental anomalies and photosensitivity [1-5]. There is a large variation in severity of the disorder, which has led to categorisation into three types: Type I is associated with normal features at birth, followed by the onset of clinical features starting in the first or second year of life. The clinical features are progressive, usually leading to death in the second or third decade of life. Type II represents a more severe form of the disorder with features present at birth or prenatally. This group typically does not survive beyond the first decade. Type III represents a group with less severe features than those in Type I. They may survive for several decades. Cerebro-oculo-facio-skeletal Syndrome (COFS) has also been used to describe a very severe form of the disorder. This categorisation is quite convenient for a rough description of the patients' severity [2], but in reality there is probably a continuum of severity of features [6].

At the cellular level, a robust diagnostic test is provided by the response of RNA synthesis to UV irradiation of cultured fibroblasts [7]. Whereas RNA synthesis and subsequently DNA synthesis recover rapidly following UV-irradiation of normal fibroblasts, this recovery is much delayed or absent in CS fibroblasts [8]. Nearly all cases in which there is a clear clinical diagnosis of CS are defective in this test, and in almost all patients diagnosed by this test, the causative mutation lies in one of two genes, *ERCC6/CSB* (OMIM 609413) or *ERCC8/CSA* (OMIM 609412). The encoded proteins, CSB and CSA are respectively a DNA-dependent ATPase [9,10] and a WD40 protein component of a large cullin4-mediated E3-ubiquitin ligase complex [11,12]. The ATPase activity of the 1493-aa CSB protein falls into the SWI2/SNF family and is associated with seven so-called helicase domains, even though

CSB does not have helicase activity. Towards the C-terminus there is a ubiquitin-binding domain [13]. CSB can be modified by phosphorylation, ubiquitylation on lys991 [14] and SUMOylation, most likely on lys205 [15,16]. The 396 aa-CSA protein comprises a seven-bladed WD40 propeller attached to the DDB1 protein via a helix-loop helix motif at the N-terminus [12].

The best characterised role of the CS proteins is in the transcription-coupled branch of nucleotide excision repair (NER) of UV-induced DNA damage. This damage, when in the transcribed strand of active genes, results in stalling of RNA polymerase II. The CS proteins are thought to modify the chromatin in the region of the stalled polymerase, enabling the polymerase to back-track and then to assist in the recruitment of TFIIH and other proteins involved in subsequent steps of NER [17,18]. This role of the CS proteins readily explains the failure of RNA synthesis to recover following UV-irradiation of CS cells and the photosensitivity of the patients. However it is not so easy to reconcile with many of the other features of CS. Indeed a few patients, with so-called UV-Sensitive Syndrome (UVSS), have been identified with mutations in *CSA*, *CSB* or a recently identified gene *UVSSA* [19-23]. Cells from these individuals show the same defective recovery of RNA synthesis as CS cells but the patients display only the sun-sensitivity and not the broad spectrum of other features of CS such as neurodegeneration and premature ageing [24]. These observations suggest that the CS proteins have other functions as well, and evidence has been provided for several other roles (reviewed in [5,25]), including the repair of oxidative damage in DNA [26-29] and roles in mitochondrial DNA metabolism [30-33]. A recent elegant study, using both whole brains and cultured cells, identified a crucial role for the CS proteins in expression of neuronal genes and thereby in neuronal differentiation [34]. Similar conclusions have been reached from a study in which iPSC-derived neuronal cells from CS patients had reduced

transcription of many neural-specific genes [35]. This role of CS proteins in neuronal differentiation could account for some of the developmental defects found in CS patients.

In this manuscript, we have gained further insight into the genetics and molecular basis of CS by analysing the clinical features and mutations in 124 CS patients, combining data gathered over several decades from four Centres, in Strasbourg (France); Pavia (Italy); Nagasaki and Nagoya (Japan); and Brighton (UK). Our results have identified many novel genetic variants and provide insights into previously unreported genotype-phenotype relationships and their relevance for clinical diagnosis.

## **MATERIALS AND METHODS**

Samples were obtained as skin biopsies, fibroblast cultures, blood or DNA extracted from blood, all with appropriate informed consent.

Fibroblast cultures and lymphoblastoid cell lines were established from skin biopsies and blood lymphocytes respectively, and grown using standard procedures. In the Pavia, Nagasaki and Brighton labs, cells were first screened on a diagnostic basis using the post-UV recovery of RNA synthesis (RRS) test using liquid scintillation counting [7,36], autoradiography [37] or a fluorescence assay [7,36]. Several cell samples were also analysed for hypersensitivity to the killing effects of UV exposure and levels of UV-induced DNA repair synthesis (UDS). Only cells displaying a defective RRS were characterised further to identify the mutation in the defective CS gene. In Pavia, cell fusion with known CS-A or CS-B cells using polyethylene glycol was used to establish complementation group [37]. In Japan, complementation group was established by transduction with lentivirus expressing either *ERCC6/CSB* or *ERCC8/CSA* cDNA [38]. Finally the appropriate gene was sequenced using genomic DNA (*ERCC6* RefSeq NG\_009442.1; *ERCC8* RefSeq NG\_009289.1) and/or cDNA (*ERCC6* NM\_000124.3; *ERCC8* RefSeq NM\_000082.3). In Strasbourg, RRS and

molecular screening (genomic and/or cDNA sequence) were performed concomitantly. Genomic sequencing was performed either by Sanger or next generation sequencing [39]. Mutation nomenclature follows the format indicated at <http://varnomen.hgvs.org/>. Nucleotide numbering of coding sequences starts with the A of the ATG translation initiation site as nucleotide 1. When appropriate, we consulted the Human Splicing Finder (HSF), a tool to predict the effects of mutations on splicing signals or to identify splicing motifs in any human sequence (<http://www.umd.be/HSF3/HSF.shtml>).

Clinical examination was carried out by VL and colleagues for all patients analysed in Strasbourg. Descriptions of clinical features at the other centres were dependent on clinical notes supplied by the referring clinicians.

## **RESULTS AND DISCUSSION**

Out of the 124 patients identified as having a specific defect in RRS, 39 were mutated in *ERCC8/CSA* (Table 1) and 85 in *ERCC6/CSB* (Table 2), representing 32 and 68% of the population respectively. Homozygous patients (30 CS-A and 43 CS-B) are listed first in order of mutation position. Compound heterozygotes (9 CS-A and 42 CS-B) are listed subsequently in order of the most 5' of the two genetic variants. Tables 1 and 2 also summarise as much clinical data as we have available, including previous reports on 13 CS-A and 5 CS-B cases.

### **CSA mutations**

We have identified 32 pathogenic genetic variants in *CSA*, of which 25 have not been reported previously. Six were mis-sense mutations, all but one previously unreported, and one a small in-frame indel. Mis-sense mutations are indicated below the *CSA* linear structure (Fig 1A) with the previously unreported alterations indicated in bold. *CSA* comprises a seven-bladed WD40 propeller attached to the DDB1 protein via a helix-loop helix motif at



the N-terminus [12]. All the newly identified mis-sense mutations affect residues that are conserved among CSA orthologs and are located in WD40 repeats. Together with previously reported mutations, indicated above the CSA linear structure, there is a particularly high concentration of mis-sense mutations around aa 200 (4 mutations within 12 aa) and aa 270 (3 mutations within 7 aa). All the 13 mis-sense mutations are located in the blades of the beta propeller structure, with 8 of them clustered in blades 4 and 5 (Fig 1B), and are likely to disrupt the structure of the protein [12].

All the mis-sense mutations are predicted to be pathogenic using Polyphen, MutPred2 and SIFT (Supplementary Table 1). We have confirmed their defective function by transducing a CS-A cell line, CS9LO, with virus containing the mutant cDNA (Figure 2A, B). Wild-type *CSB* almost completely restored RRS to the recipient UV-irradiated CS9LO cell line. In contrast, when the cells were transduced with any of the six mutant cDNAs, RRS remained close to the level of the untransduced cells (Figure 2A). The infection efficiency was similar for all transductions (Figure 2B).

We have identified 19 protein-truncating genetic variants, of which 14 are new, that include frameshift, splicing and premature stop mutations. They are predicted to result in 18 distinct truncated proteins (12 new), because we found that the Thr134Leufs\*13 truncation (due to exon 5 deletion, i.e. r.400\_481del) is caused by two distinct mutations affecting either the splice acceptor site of intron 4 (c.400-2A>G in CS261ST) or the splice donor site of intron 5 (c.479C>T in CS133NY). In addition, two mutations resulting in large in-frame deletions (p.Val282\_Gln347del, Val282\_Glu374del and p.Val27\_Arg92del) are respectively predicted from c.966C>A and a previously unreported rearrangement involving part of intron 2 and exon 3, which results in a transcript lacking exons 2 and 3 (patient CS1LE). Interestingly, the genomic mutation c.966C>A (in exon 10), previously described as resulting in a single, full-length normal-spliced transcript (r.966c>a, p.Tyr322\*) [39], was shown to generate also two

abnormally spliced transcripts carrying the deletion of exons 10 and 11 (r.844\_1122del; p.Val282\_Glu374del in CS9IAF) and/or the deletion of exon 10 (r.844\_1041del; p.Val282\_Glu347del in CS5IAF and CS9IAF). Indeed, this mutation is predicted to alter an exonic splicing enhancer (ESE) site, and potentially alter the splicing as indicated by the bioinformatics tool *HSF*.

Finally, at the genomic level we identified three new *CSA* genetic variants located in splice donor sites (c.481+1G>C, c.1041+1G>T and c.1122+1delG) most probably affecting splicing. The resulting transcripts could not be identified because of the unavailability of RNA samples. However, *HSF* analysis indicated potential splicing alterations for the three mutations as well as the activation of an intronic cryptic donor site for the latest two.

### **CSB mutations**

In *CSB*, we identified 73 pathogenic genetic variants of which 46 were previously unreported. 10 of the mutations were mis-sense, nine in-frame deletions (two small and seven large), five null mutations leading to unexpressed transcripts and the rest being truncations resulting from stop (16), frameshifts (20) or splice mutations (13). The 10 mis-sense mutations are indicated in Fig 3A, below the *CSB* linear structure, with the 6 new mutations indicated in bold. Other previously reported mis-sense mutations are shown above the *CSB* linear structure.

All mis-sense mutations are predicted to be pathogenic (Supplementary Table 1). We have confirmed the defective function of several of them by transducing a *CS-B* cell line, CS10LO, with virus containing the mutant cDNA (Figure 2C, D). Similar to the *CSA* data, wild-type *CSB* almost completely restored RRS to the recipient UV-irradiated CS10LO cell line, whereas with the six mutant cDNAs tested, RRS remained close to the level of the untransduced cells (Figure 2C). The infection efficiency was similar for all transductions (Figure 2D).

**Table 1 Mutation and clinical data on CS-A patients**

Code	Country (origin)	Sex	Age at onset, yrs	Age at biopsy (latest report or death*), yrs	Clinical classification	Mutation genomic DNA	Mutation cDNA	Protein Alteration	Growth failure	Low birth weight	Cachexia / bird-like faces	Mental retardation <sup>a</sup>	Microcephaly <sup>b</sup>	Cataracts <sup>b</sup>	Microphthalmia	Retinal degeneration <sup>c</sup>	Hearing loss	Clinical photosensitivity	Fitzpatrick skin type scale	Dental anomalies	Arthrogyposis	Reference <sup>d</sup>			
<b>HOMOZYGOTES</b>																									
CS15PV	Morocco	M	1.5	10 (14)	I	c.37G>T	r.(37g>u)	p.(Glu13*)	+	-	+	+	+	+	+		-	-		+	+				
CS2JE	Israel (Ukraine)	M	0.5	11 (13)	I		r.37g>u	p.Glu13*	+	-	+	+	+	+		OA									
CS218ST	India	M	0.7	13*	I	c.37G>T	r.37g>u	p.Glu13*	+	-	+	S	P	-	-	+	-	+	IV	-	-	[6]			
CS1LE	UK (Pakistan)	F	0.3	9	I	Rearrangement involving part of intron 2 and exon 3	r.78_275del (exons 2-3)	p.Val27_Arg92del	+	-	+	M	+				+								
CS30PV	Italy	F	0.2	0.8	I	c.162delT	r.162delu	p.Glu55Lysfs*13	+	-	-	+	+	-	-	+	+	-			-	[40]			
CS4PV	Italy	M	0.2	3 (6)	II	c.223_227del	r.223_227del	p.Asn75Glnfs*9	+	+	+	S	+	C				+			+				
CS2NG	Japan					c.[275+703_399+347del;399+348_399+2007inv;399+2008_399+2558delins8] (large deletion of exon 4 and inversion in intron 4)	r.276_399 del (exon 4)	p.Asp93Leufs*26																	
CS3NG	Japan																								
CS28NG	Japan																								
CS29NG	Japan																								
CS30NG	Japan																								
CS37NG	Japan																								
CS2OS	Japan																								[41]
CS2AW	Japan																					[41]			
CS263ST	France (Turkey)	M	0.6	6	I	c.316C>T	r.(316c>u)	p.(Gln106*)	+	-	+	S	P	-	-	+	+	-	IV	+	-				
CS261ST	Tunisia	F	0	11	I/II	c.400-2A>G	r.400_481del (exon 5)	p.Thr134Leufs*13	+	+	+	S	C	C	-	+	+	-	IV	+	-				
CS133NY	USA	M	2	(32*)	I/III	c.479C>T (third to last nt of exon 5)	r.400_481del (exon 5)	p.Thr134Leufs*13	+	-	+	+	+	+		+	+	+				[42] [43]			
CS1BR	France	F				c.481G>A	r.[481g>a;481_482ins481+1_481+4 (part of intron 5)]	p.Val161Serfs*5																	
CS165ST	France (Turkey)	M	0	2	II	c.481+1G>C	r.?	p.?	+			S	P	C	-		+	-	IV		-				
CS24PV				5		c.598_600delinsAA	r.598_600delinsaa	p.Tyr200Lysfs*12																	
CS1GLO (CS192ST)	UK (Libya)	M	0	7	I	c.598_600delinsAA	r.598_600delinsaa	p.Tyr200Lysfs*12	+	+	+		+												
CS7PV	Italy	F	0.1	4 (9*)	II	c.719-1G>A	r.719_843del (exon 9)	p.Ala240Glyfs*8	+	+	+	S	+	C				+							
CS276ST	France	M	?	15	III ?	c.730C>T	r.(730c>u)	p.(His244Tyr)	-		+	S	-		-		+	+	II	+		[44]			
CS172ST	France	F	0	14*	II	c.752delT	r.752delu	p.Leu251Tyrfs*18	+	-	+	S	P	C	+	+	+		III	+	-				
CS260ST	France	M	0	28	III ?	c.793A>C	r.793a>c	p.Thr265Pro	+		+	M	+				+	+	II			[44]			
CS240ST	India	F	0	6	I/II	c.802C>T	r.(802c>u)	p.(Arg268*)	+	+	+	S	C	-	-	+	+	-	V	+	-				
CS3BR	UK	M	0.5	2 (6*)	II		r.812u>c	p.Leu271Pro	+	-	+	S	+	+	-	-	-	+		+					
CS9IAF	Israel (Arab)	F		3	I	c.966C>A	r.[966c>a,844_1041del (exon10),844_1122del (exons 10-11)]	p.[Tyr322*,Val282_Gln347del, Val282_Glu374del]	+	-	+		+												

CS5IAF	Israel (Arab)	M	0.5	6	I	c.966C>A	r.[966c>a,844_1041del (exon10)]	p.[Tyr322*,Val282_Gln347del]	+	-	+	+		+			-	+		+		
GM02965	USA	F	13	25	III		r.1049a>g	p.Tyr350Cys	+	-	+	mild					-	+				[45]
COMPOUND HETEROZYGOTES																						
CCS4	Japan	F				c.[2T>A];[275+703_399+347del;399+348_399+2007inv;399+2008_399+2558delins8 (large deletion of exon 4 and inversion in intron 4)]	r.[?];[(276_399 del (exon 4))]	p.[?];[(Asp93Leufs*26)]	+	-	+	mild	+	-	-	-	mild	mild				[46]
CS9LO	UK	F	1	2.5	I/II	c.[282delT];[c.481G>A]	r.[282delu];[481g>a;481_482ins481+1_481+4 (part of intron 5)]	p.[Pro95Leufs*30];[Val161Serfs*5]	+	-	+	S	+	-	-	-	+	+			-	
CS11PV	Italy	M	2.5	7 (12*)	I	c.[300C>G];[c.399+10773_550+837 (deletion spanning from intron 4 to intron 6)]	r.[300C>G];[400_550del (exons 5-6)]	p.[Tyr100*];[Thr134Valfs*7]	+	-	+	+	+	+		OA		+				[47]
CS040ST	France	F	2	16*	I	c.[356C>T(;);618-1G>A]	r.[(356c>u)(;);(618_626del)]	p.[(Ser119Leu)(;);(Ala207_Ser209del)]	+		+	M	P	-	-	+	+	+	II	+	-	[44]
CS6PV	Italy	F	0.8	5 (16*)	I		r.[400_481del (exon 5);[400_550del (exons 5-6)]	p.[Thr134Leufs*13];[Thr134Valfs*7]	+	+		+	+	-		+		+				
CS16PV	Italy	F	0.2	8 (15)	I	c.[594_595insAT];[659C>G]	r.[594_595insau];[659c>g]	p.[Asp199Metfs*14];[Ser220*]	+	+	+	+	+	-	-		+	+			+	+
CS309ST	France	M	0.25	2	I	c.[611C>A];[1122+1delG]	r.[(611c>a)];[?]	p.[(Thr204Lys)];[?]	+	-	+	M	+	-	-	-	-	+	III	-	-	[44]
CS1JE	Israel (Tunisia/Algeria)	F		4.5	I	c.[618-1G>A];[?]	r.[618_626del (first 9 nt of exon 8)];[0]	p.[Ala207_Ser209del];[0]	+	-	+	+	+			+		+				
CS291ST	France	F	0.3	19*	I	c.[927delT];[1041+1G>T]	r.[(927delu)];[?]	p.[Phe309Leufs*19];[?]	+	-	+	S	P	C	-	-	-	+	III	+	-	[44]

Details of 39 CS-A patients are summarised. Nucleotide numbering starts with the A of the ATG translation initiation site as nucleotide 1. Mis-sense and small in-frame deletion mutations are indicated in italics on shaded background. Mutation nomenclature follows the format indicated at <http://varnomen.hgvs.org/>. [Protein](#) alterations are deduced from the DNA changes.

<sup>a</sup>M, moderate; S, severe. <sup>b</sup>C, congenital; P, progressive. <sup>c</sup>OA, Optic Atrophy. <sup>d</sup>Patients CS291ST, CS040ST, CS309ST, CS276ST, CS260ST correspond to cases 5, 6, 7, 8, 9 respectively in Ref 39 from NC and VL and we include them as new mutations in Fig 1.

Table 2 Mutation and clinical data on CS-B patients

Code	Country (origin)	Sex	Age at onset, yrs	Age at biopsy (latest report or death*), yrs	Clinical classification	Mutation genomic DNA	Mutation cDNA	Protein Alteration	Growth failure	Low birth weight	Cachexia / bird-like faces	Mental retardation <sup>a</sup>	Microcephaly <sup>b</sup>	Cataracts <sup>b</sup>	Microphthalmia	Retinal de-generation <sup>c</sup>	Deafness	Clinical photo-sensitivity	Fitzpatrick skin type scale	Dental anomalies	Arthrogryposis	Reference <sup>d</sup>
<b>HOMOZYGOTES</b>																						
CS18BR	Germany	M	3		I	c.212delC	r.212delc	p.Leu72Cysfs*12	+	-	-			-	-	-	-	+				
CS3SH	UK	F		9		c.466C>T	r.466c>u,423_543del (exon 3)	p.Gln156*,Ser142Asnfs*4			+								+			
CS14LO	UK	F	6	23	I	c.466C>T	r.466c>u,423_543del (exon 3)	p.Gln156*,Ser142Asnfs*4	+		+	M	+				+	+		-		
CS27PV	Italy	M	1.2	6.5	I	c.526C>T	r.526c>u,423_543del (exon 3)	p.Arg176*,Ser142Asnfs*4	-	-	+	+	+		-	-	-	+		-	-	
CS010ST	Lebanon (Iraq)	F		5	I	c.640G>T	r.(640g>u)	p.(Glu214*)	+	-	+	S	P	-	-			+	IV	+	-	
CS13PV	Italy	F	0.4	2.5 (6)	I	c.1070C>G	r.1070c>g	p.Ser357*	+	-	+	+	+		+	+		+		+		
CS201ST	Iran	M	0	3	II	c.1128del	r.(1128del)	p.(Thr377Glnfs*28)	+	+	+	S	P	+	-		+	+	III	+	-	
CS10LO	UK (Africa)	F	0.5	4	I	c.1280dupT	r.(1280dupu)	p.(Ser429Lysfs*7)	+	-	+	+	+	-		-	-	+				
						<b>PiggyBac insertion at c.1397+6912 (in intron 5)</b>																
CS19PV	Italy	F	0	1.5 (2.4*)	II	c.1431_1432delGA	r.1431_1432delga	p.Lys478Thrfs*9	+	-	+	+	+	C	+	-	-	+		-	-	
CS4TAN	Turkey	M			II	c.1551G>A	r.1551g>a	p.Trp517*	+		+	+	+						+			
CS8TAN	Turkey	F			II	c.1551G>A	r.1551g>a	p.Trp517*	+			+	+						+			
CS35NG						c.1627C>T	r.(1627c>u)	p.(Ile543Phe)														
CS107ST	Spain (India)	F	0	2.5	II	c.1690G>T	r.(1690g>u)	p.(Glu564*)	+	+	+	S	P	-	-	+	+		IV	+	-	
CS253ST	India	M	0	3	II	c.1936G>A	r.(1936g>a)	p.(Asp646Asn)	+	+	+	S	+	-		+		-	IV	-	-	
CS10X	UK	F				c.1954C>T	r.1954c>u	p.Arg652*														
CS13MA	Pakistan	F	0		II	c.1954C>T	r.1954c>u	p.Arg652*	+		+		+	+			+					
CS232ST	France (Algeria)	F	0	3	II	c.1971_1974dup	r.(1971_1974dup)	p.(Thr659Cysfs*24)	+	+	+	S	+	C		-	-		III	+		
CS1PL	UK	M		34	III	c.1993-5A>G	r.1992_1993insauag	p.Phe665Tyrfs*18	+		+	-							-		-	
CS9TAN	Turkey					c.2038A>G	r.(2038a>g)	p.(Asn680Asp)														
CS1NE	UK	M				c.2047C>T	r.2047c>u	p.Arg683*					+	+					+			
CS23PV	Italy	M	0.3	4 (6)	I	c.2143G>T	r.2143g>u	p.Gly715*	+	-	+	+	+	+	-	+	+	-			+	
CS3BI	UK				III	c.2167C>T	r.1993_2169del (exon 10)	p.Phe665_Gln723del														[48]
CS11MA	UK	F	0	1	II	c.2167C>T	r.1993_2169del (exon 10)	p.Phe665_Gln723del	+	+		+		+								
CS22BR	Brazil	M				c.2203C>T	r.2203c>u	p.Arg735*														
CS12RO	(Italy)	M		11		c.2203C>T	r.2203c>u	p.Arg735*														
CS17LO	UK (Turkey)	M		13	I		r.2203c>u	p.Arg735*	+	-	+		+									
CS128ST	France (Bosnia)	F	0.75	15	I	c.2203C>T	r.2203c>u	p.Arg735*	+	-	+	M	P	-	-	-	-	+	III	+	-	
CS7MA	UK	M	0	0.5	II	c.2222_2230del	r.2222_2230del	p.Tyr741_Arg744delinsTrp	+	+	+	+	+	+	+		+					
CS8PV	Sri Lanka	M	0.1	1 (4.5*)	II	c.2279_2280insA	r.2279_2280insa	p.Asn760Lysfs*2	+		+	+	+	+	+			+				

CS144ST	India		0	3.5	II	c.2560C>T	r.(2560c>u)	p.(Gln854*)	+		+	S	C	-	-	+		+	IV		+		
CS31PV	Pakistan	M	0	2	II	c.2599-26A>G	r.2598_2599ins2599-25_2599-1 (partial insertion of intron 13)	p.Met867Thrfs*14	+	+	+	+	+	+	+	-	+	-		+	+		
CS20PV	Senegal	F	0	0.5 (1.7*)	II	c.2624T>C	r.2624u>c	p.Leu875Pro	+	+	+	+	+	+	+						+		
CS11LO	UK	M	0.5	0.5	II	c.2830-2A>G	r.2830_2924del (exon 16)	p.Ala944Thrfs*10	+	+		+	+	+	+								
CS23BR	UK (?)	M	0	0.5	II	c.2925-93_3778+527delinsTT	r.2925_3778del (exon 17, 18)	p.Gln976Trpfs*70						+						+			
CS210ST (CS4BL)	UK	M	0	1.5	II	c.3052dupA	r.(3052dupa)	p.(Thr1018Asnfs*32)	+	+	+	S	C	C	-				+	-	+	-	
CS241ST	UK	M				c.3536delA	r.(3536dela)	p.(Tyr1179Leufs*22)	+		+										+		
CS25LO	UK (Pakistan?)			4		c.3627_3628insT	r.3627_3628insu	p.Lys1210*	+	-	+	+	+								+		
CS270ST	Iran	F		2		c.3862C>T	r.(3862c>u)	p.(Arg1288*)	+	-	+	S	+			+	+	+					
CS071ST	India	M	0.3	10	I	c.4063-1G>C	r.(4062_4063ins4063-139_4063-1;4063-1g>c (partial insertion of intron 20))	p.(Asp1355Valfs*32)	+	-	+	M	P	-	-	+	-	+	V		-		
CS204ST	India	M		16	III				+	-	+	M									V	+	-
CS221ST	India	M	4	13	III				+	+	+	M	P	-	-	+	-	+	V	+	-		
CS222ST	India	F		8	I				+		+	S	P			+			V				
CS27LO	UK (Pakistan?)	F	0	0.5		c.4180delA	r.4180dela	p.Arg1394Glufs*6	+			S	+	+									
COMPOUND HETEROZYGOTES																							
CS288ST	France (Reunion)	F	0	10	II	c.[del exon1];[2047C>T]	r.[?];[(2047c>u)]	p.[?];[(Arg683*)]	+	+	+	S	C	P					+	IV		- [44]	
CS195ST	France	F	0	4	II	c.[(?_-176).(422+1_?)del]; [1834C>T]	r.[0];[1834c>u]	p.[0];[Arg612*]	+	-	+	M	+	C	-	-	+	+	II				
CS1PR	UK	F	1	2		c.[466C>T];[2839C>T]	r.[466c>u,423_543del (exon 3)];[2839c>u]	p.[Gln156*,Ser142Asnfs*4]; [Arg947*]	+												-		
CS225ST (CS16LO)	UK	F	2	17	I/III	c.[466C>T];[2599-26A>G]	r.[466c>u,423_543del (exon 3)];[(2598_2599ins2599-25_2599-1 (partial insertion of intron 13))]	p.[(Gln156*,Ser142Asnfs*4)]; [(Met867Thrfs*14)]	+	-	+	M	P	P	-				+	+	II	+	-
CS5MA	UK	M	2			c.[466C>T];[2839C>T]	r.[466c>u,23_543del (exon 3)];[2839c>u]	p.[Gln156*,Ser142Asnfs*4]; [Arg947*]	+	-	+	+		+							+		
CS2LE	UK	F	33		III?	c.[466C>T];[2203C>T]	r.[466c>u,423_543del (exon 3)];[2203c>u]	p.[Gln156*,Ser142Asnfs*4]; [Arg735*]															
CS1SH	UK	F	4	14	I	c.[466C>T]; [1412_1413delTG]	r.[466c>u,423_543del (exon 3)];[1412_1413delug]	p.[Gln156*,Ser142Asnfs*4]; [Leu471Glnfs*16]	+	-		-	-	-							-	+	
CS1SO	UK	F	1.5	5 (14*)	I	c.[526C>T];[1954C>T]	r.[526c>u,423_543del (exon 3)];[1954c>u]	p.[Arg176*,Ser142Asnfs*4]; [Arg652*]	+	-	+	+	+	-	-	+	+	+					
CS1GO	Sweden	M	0.5	9	I	c.[526C>T];[1765_1767delTGG]	r.[526c>u,423_543del (exon 3)];[1765_1767delugg]	p.[Arg176*,Ser142Asnfs*4]; [Trp589del]	+		+	+		-	-	+	+	+					
CCS8	Japan		1		II	c.[536_537insTA];[1834C>T]	r.[(536_537insua)];[(1834c>u)]	p.[(Asn180Ilefs*7)]; [(Arg612*)]															
CS289ST	France	M	2	13	I	c.[543G>T];[543+4delA]	r.[423_543del(exon 3)]; [423_543del(exon 3)]	p.[Ser142Asnfs*4]; [Ser142Asnfs*4]	+	-	+	M	P	-	-	-	-	+	II	+	-	[44]	
CS26PV	Italy	F	1	36	III	c.[543+4delA];[2203C>T]	r.[423_543del (exon 3)];[2203>u]	p.[Ser142Asnfs*4];[Arg735*]	+		+	+	+		+						+	+	-
CS250ST	France	F	0	2	II	c.[818del];[1397+2T>A]	r.[(818del)];[?]	p.[(Lys273Serfs*56)];[?]	+	+	+	S	+	C		+	+	-	II			-	
CS148ST	South Africa	M			I/II	c.[935_936delinsG];[2167C>T]	r.[?];[(1993_2169del(exon 10))].	p.[(?)];[(Phe665_Gln723del)]	+			S								III			

CS1WR	UK	M		1 (8*)	II	c.[1357C>T];[2286+1G>A]	r.[1357c>u];[2286_2287ins2286+1_2286+96;2286+1g>a (partial insertion of intron 11)]	p.[Arg453*];[Val763Ilefs*7]	+		+	+		+									
PiggyBac insertion at c.1397+6912 (in intron 5)																							
CS233ST	France	M	0	2*	II	c.[1518delG];[2923C>T]	r.([1518delg]);[2923c>u]	p.([Lys506Asnfs*37]); [Arg975*]	+	+	+	S	C	C	+	-		+	II	+	-		
CS058ST (CS2LI)	UK	F		10	I	c.[1526+1G>T];[3952_3953delAG]	r.[1398_1684del (exons 6-7)]; [3952_3953delag]	p.[Arg467_Arg562del]; [Arg1318Glyfs*12]	+			M		-				+	+	I		-	
CS18NG	Japan				II	c.[1583G>A];[1834C>T]	r.([1583g>a]);([1834c>u])	p.([Gly528Glu]);[Arg612*]															
CS18PV	Italy	M	0	1.7 (2.6*)	II	c.[1685+6T>G];deletion including exons 9 and 10	r.[1527_1685del (exon 7)]; [1822_2169del (exons 9-10)]	p.[Tyr510_Arg562del]; [Glu608_Gln723del]	+	+	+	+	+	C	+							-	
CS17PV	Italy	F	0.6	1.1 (2.9*)	I/II	c.[1686-1G>A];[c.2827C>T]	r.[1686_1821del (exon 8)];[2827c>u,2826_2829del,2710_2829del (exon 15)]	p.[Phe563Argfs*3];[Gln943* Gln943Profs*8,Asp904_Gln943del]	+	-	+	+	+	C	+	+	+	+	-			-	+
CS25PV	Italy	M				c.[? (deletion including exon 8)];[2203C>T]	r.[1686_1821del (exon 8)];[2203c>u]	p.[Phe563Argfs*3];[Arg735*]															
CS10MA	UK	F	0.4	1.5		c.[1834C>T];[2167C>T]	r.[0];[1993_2169del (exon 10)]	p.[0];[Phe665_Gln723del]	+	+		+	+	-	+	-	-		+				
CS1LI	UK	M	0	1.5	II	c.[1939G>A];[2167C>T]	r.[0];[1993_2169del (exon 10)]	p.[0];[Phe665_Gln723del]	+	-	+		+	+				+	+				
CS18LO	UK	F	0	0.1	II	c.[2008C>T];[2287-2A>G]	r.[2008c>u];[2287_2382del (exon 12)]	p.[Arg670Trp];[Val763_Gln794del]	+				+	+								+	
CS32LO	UK	F				c.[2047C>T];[3862C>T]	r.[2047c>u];[3862c>u]	p.[Arg683*];[Arg1288*]				+											
CS12MA	UK	M	0		II	c.[2047C>T];[2167C>T]	r.[2047c>u];[1993_2169del (exon 10)]	p.[Arg683*];[Phe665_Gln723del]					+	+					+				
CS1GGO	Germany	M			II	c.[2060C>T];[2203C>T]	r.[2060c>u];[2203c>u]	p.[Ser687Leu];[Arg735*]	+													+	
CS278ST	France	M	0	3	II	c.[2060C>T];[3862C>T]	r.([2060c>u]);([3862c>u])	p.([Ser687Leu]);[Arg1288*]	+	+	+	S	+	C	+	+	-	-	II	+	-	[44]	
CS3BL	UK	M		11		c.[2092_2093insG]; [2857C>A]	r.[1993_2169del (exon 10)]; [2857c>a]	p.[Phe665_Gln723del]; [Gln953Lys]															
CS22PV	Italy	M	0.5	3 (5)	I	c.[2096_2097insC];[2203C>T]	r.[2096_2097insc];[2203c>u]	p.[Leu700Valfs*60];[Arg735*]	+	+	+	+	+	-	-	OA	-	-				+	-
CS28PV	Italy	M	3	4.5	I	c.[2096_2097insC];[2203C>T]	r.[2096_2097insc];[2203c>u]	p.[Leu700Valfs*60];[Arg735*]	+	+	+	+	+	-				+					-
CS2BL	UK	F	0			c.[2143G>T];[2167C>T]	r.[0]; [1993_2169del (exon 10)]	p.[0]; [Phe665_Gln723del]						+	+								
CS26LO	UK	F				c.[2167C>T];[2092_2093insG]	r.[1993_2169del (exon 10)]; [1993_2169del (exon 10)]	p.[Phe665_Gln723del]; [Phe665_Gln723del]															
CS8MA	UK	M	0	1	II	c.[2167C>T];[3661C>T]	r.[1993_2169del (exon 10)];[3661c>u]	p.[Phe665_Gln723del];[Arg1221*]	+	+	+	+	+	+								+	
CS19LO	UK	M	0		II	c.[2167C>T];[3259C>T]	r.[1993_2169del (exon 10)];[3259c>u]	p.[Phe665_Gln723del];[Arg1087*]	+	-				+	+								
CS19BR	UK	F	1		II	c.[2167C>T];[2776G>C]	r.([1993_2169del(exon 10)]);([2776g>c])	p.[Phe665_Gln723del];[Ala926Pro]	+	+	+	+	+	+									
CS215ST	UK	M	0	0.6*	II	c.[2170-1G>A];[2599-26A>G]	r.([2170_2286del (exon 11)]); [(2598_2599ins2599-25_2599-1 (partial insertion of intron 13))]	p.([?]);[Met867Thrfs*14]	+	+	+	S	C	C	-	+	+	+				+	
CS2GR	Austria	F	0	15	I	c.[2203C>T];[3607_3608ins26]	r.[2203c>u];[3607_3608ins26]	p.[Arg735*];[Lys1203fs]	+	+	+	+	+				+	+					
CS14PV	Italy	M	1	3.5 (5)	I	c.[2203C>T];[? (deletion of about 5.5Mb in 10q11.2)]	r.[2203c>u];[0]	p.[Arg735*];[0]	+	-	+	+	+					-	+			-	-
CS21PV	Italy	F	1	21 (27)	I	c.[2551T>C];[3952_3953delAG]	r.[2551u>c];[3952_3953delag]	p.[Trp851Arg]; [Arg1318Glyfs*12]	+	+	+	+	+						+	-		+	-

CS286ST	France (Algeria)	M	0	4	I/II	c.[2599-26A>G];[4115delG]	r.[(2598_2599ins2599-25_2599-1 (partial insertion of intron 13)); [(4115delg)]	p.[(Met 867Thrfs*14)]; [(Gly1372Glufs*22)]	+	-	+	M	P	C	-	-	-	-	II	-	-	[44]
CS1BEL	UK	F		7 (18*)		c.[2830-2A>G];[3536delA]	r.[2830_2924del (exon 16)];[3536delA]	p.[Ala944Thrfs*10]; [Tyr1179Leufs*22]	+								+	+				

Details of 85 CS-B patients are summarised. Nucleotide numbering starts with the A of the ATG translation initiation site as nucleotide 1. Mis-sense and small in-frame deletion mutations are indicated in italics on shaded background. The position of the PiggyBac insertion is indicated. Mutation nomenclature follows the format indicated at <http://varnomen.hgvs.org/>.

<sup>a</sup>M, moderate; S, severe. <sup>b</sup>C, congenital; P, progressive. <sup>c</sup>Patients CS288ST ,CS286ST, CS278ST, CS289ST correspond to cases 1, 2, 3 and 4, respectively in Ref 39 from NC and VL and we include them as new mutations in Fig 3.



The distribution of *CSB* truncation mutations is presented in Figure 3B (lower panel). Some general conclusions may be drawn from the present in-depth investigation together with previous studies (data up to 2010 reviewed by Laugel et al [39], and since 2011 listed in Supplementary Table 2). With two exceptions, all of the *CSB* mis-sense mutations are located either in or very close to the seven helicase domains, in particular in domains I and III (4 mutations each) and domains IV-VI (9 mutations within 110 amino acids), emphasising the crucial role of these domains in *CSB* function. These helicase domains are involved in the DNA-dependent ATPase activity of the protein and confirm that this activity is vital for preventing the features of CS. The N- and C-terminal extensions are likely to be much more amenable to genetic variants that do not affect function, despite the demonstration that the C-terminal part of the protein is essential for a normal cellular response to UV-irradiation [13,16]. This may imply that the structure of the C-terminal ubiquitin - binding domain needs to be intact, but the precise amino acid sequence is less crucial. Again, as might be anticipated, the truncation mutations are spread rather evenly across the protein (Fig 3B).

Interestingly, the c.1834C>T and c.2143G>T result in p.Arg612\* and p.Gly715\*, respectively, and their transcript was detected only in homozygous or hemizygous patients. This transcript must be poorly expressed, presumably because of nonsense-mediated decay, because in compound heterozygotes only the transcript resulting from the second allele was detected.

Furthermore, several genetic variants affect *ERCC6/CSB* splicing giving rise to either truncations or in-frame deletions. In particular, 10 mutations map at the canonical splice sites of different exons, 4 are located inside introns (c.1993-5A>G; c.2599-26A>G, c.1685+6T>G, c.543 + 4delA) and 3 inside exons (c.466C>T, c.526C>T and c.2092\_2093insG). All the exonic-changes are likely to alter the splicing by creating novel exon-splicing enhancer (ESE

in the case of c.466C>T) or exon-splicing silencer (ESS for c.526C>T and c.2092\_2093insG) sites according to HSF prediction.

### **Recurring pathogenic genetic variants**

Mutations found in several patients are indicative of either founder effects or mutation hotspots. Although haplotype analysis would be required to distinguish definitively between these two alternatives, as a first approximation, we assume that if the pathogenic genetic variant is only found in a relatively limited geographical location, it is more likely to be a founder effect. In *ERCC8/CSA*, we found a complex rearrangement involving exon 4 in 8 Japanese patients, also previously reported in four Japanese patients by Ren et al [41], strongly indicating a founder mutation. Moreover, the c.966C>A mutation found in two patients of our cohort (CS9IAF, CS5IAF) was also previously described in 3 cases (CS2IAF, CS886VI/CS887VI) [39]. Since all the patients are of Arabic origin, albeit from different countries (Israel or Lebanon), this is also most likely a founder mutation.

Nine *CSB/ERCC6* pathogenic genetic variants occur in three or more patients (Table 3). The most common of these multiple occurrences are c.2203C>T, c.2167C>T and c.466C>T respectively found in 12, 11 and 7 patients. Whereas c.2167C>T and c.466C>T are found almost exclusively in UK patients and may result from founder effects, c.2203C>T is found in individuals from several different countries and likely results from independent mutations. Interestingly, the C>T mutations in Table 3 that are more likely to result from a founder mutation are at CpA sites, whereas those more likely to result from independent mutations are at CpG sites. CpG sites are known to be mutational hotspots in the human genome [49].

**Table 3 ERCC6/CSB mutations identified in three or more patients**

<b>c.466C&gt;T</b> <b>aaCag</b>	<b>c.526C&gt;T</b> <b>aaCga</b>	<b>c.1834C&gt;T</b> <b>tgCag</b>	<b>c.1954C&gt;T</b> <b>ttCga</b>	<b>c.2047C&gt;T</b> <b>tcCga</b>	<b>c.2167C&gt;T</b> <b>taCag</b>	<b>c.2203C&gt;T</b> <b>taCga</b>	<b>c.2599-26A&gt;G</b> <b>caAac</b>	<b>c.3862C&gt;T</b> <b>acCga</b>	<b>c.4063-1G&gt;C</b> <b>taGga</b>
CS3SH UK	CS1SO UK	CS10MA UK	CS1OX UK	CS1NE UK	CS26LO UK	CS22BR Brazil	CS31PV Pakistan	CS32LO UK	CS071ST India
CS1PR UK	CS1GO Sweden	CCS8 Japan	CS13MA Pakistan	CS32LO UK	CS1LI UK	CS12RO Italy	CS215ST UK	CS270ST Iran	CS204ST India
CS225ST UK	CS27PV Italy	CS18NG Japan	CS1SO UK	CS12MA UK	CS8MA UK	CS2LE UK-Turkey	CS225ST UK	CS278ST France	CS221ST India
CS5MA UK		CS195ST France		CS288ST Reunion	CS3BI UK	CS1GGO Germany	CS286ST France-Algeria		CS222ST India
CS14LO UK					CS11MA UK	CS2GR Austria			
CS2LE UK					CS19LO UK	CS14PV Italy			
CS1SH UK					CS10MA UK	CS22PV Italy			
					CS12MA UK	CS25PV Italy			
					CS148ST South Africa	CS26PV Italy			
					CS19BR UK	CS17LO UK-Turkey			
					CS2BL UK	CS128ST France-Bosnia			
						CS28PV Italy			

For each mutation (top row), the sequence around the mutated base (CAPS) is indicated (second row), followed by the cell strain designations and country of origin of the patients' families. No indication implies origin is unknown.

## Relationship to clinical features

No obvious genotype-phenotype correlation was identified in the CS-A patients reported in previous investigations (45 cases from 33 families). With the present study we have expanded the cohort of CS-A patients by describing 39 new cases, the majority of which are homozygotes. Focusing on the homozygous CS-A patients (33 from 24 families in the literature and 30 from 30 families in our cohort, excluding the 9 Japanese cases with a recurrent mutation), mis-sense mutations appear to be more frequently associated with mild phenotypes than protein-truncating mutations. The observation that the mis-sense alteration p.Trp361Cys, which interferes with transcription-coupled NER but not with the oxidative stress response, is associated with UVSS, a rare disorder characterized only by cutaneous photosensitivity [20], strongly supports the notion that the severity of the clinical features is related to the effects of the mutation on the additional roles of CSA outside transcription-coupled NER, which include oxidative damage response, mitochondrial function maintenance and ribosomal DNA transcription.

Previous analysis of mutations in CS-B patients (51 homozygous cases from 29 families/kindreds and 37 compound heterozygotes from 32 families) have not identified any clear correlation between the site or the nature of the mutations with the type and severity of the clinical features [50,51], although some more subtle relationships have been suggested. Several years ago, Tanaka and co-workers suggested that CSB truncations generating no functional protein resulted in the mild phenotype of UVSS, whereas more C-terminal truncations might generate inactive protein that could interfere with other processes, thereby resulting in more severe phenotypes [19]. Weiner and colleagues showed that the human *ERCC6/CSB* gene contains a PiggyBac transposon insertion in intron 5 [52,53] (see Table 2 and Figure 3). They showed that translation of *ERCC6/CSB* resulted in bona fide CSB protein, but also a CSB-PiggyBac fusion protein. Truncation mutations upstream of intron 5

would generate neither protein, whereas those downstream would generate only the CSB-PiggyBac fusion, which was proposed to have deleterious effects [52]. We have analysed the severity of the clinical features in our patient cohort to see if they are in accord with these suggestions. In eight patients homozygous for truncations in the first 5 exons, six could be categorised as Type I, and one as Type II. No information is available for one patient. In contrast, in 28 patients homozygous for truncations downstream of exon 5, the numbers assigned to types I, II and III are 5, 12 and 3 respectively. There thus appears to be a tendency to more severe phenotypes (Type II) associated with downstream truncations, although this does not seem to be an absolute correlation. Patients with truncations upstream of the PiggyBac insertion but severe clinical features have been reported previously (eg [54]). Furthermore, of the four patients homozygous for the mutation Asp1355Valfs\*32, two were classified as type I and two as type III (see Table 2). Altogether these observations indicate that other factors, apart from the site of mutation, contribute to the severity of the pathological phenotype.

In an earlier analysis, Laugel suggested that type II features were more prevalent in CS-B than in CS-A patients [6]. This is supported by our current data. The distributions for those patients for whom we have clinical data for Types I, II and III are 67, 21 and 12.5% for CS-A (21 patients) and 35, 56 and 10% for CS-B (60 patients). The individual clinical features for which we have information are summarised in Table 4, where they are also compared, where possible, with data from a recent analysis of 102 CS individuals by Wilson et al [4]. Within our own cohort, there are few differences between CS-A and CS-B patients, with the possible exceptions of cataracts, low birth weight and microphthalmia, which are more prevalent in CS-B patients. The incidence of several features appears to be higher in our cohort than in that studied by Wilson et al (see Table 4). Two possible explanations for this are: (1) they

could represent genuine differences between the two cohorts; (2) the analytical clinical criteria may differ between the two studies. Of the patients subjected to molecular analysis in [4] the ratio of CS-B to CS-A cases is very similar to that reported here.

In a recent survey of CS patients in Japan, nearly all of them (41/47) were categorised clinically as Type I [3]. Unfortunately this survey did not include molecular analyses.

However our data strongly suggest that there is a *ERCC8/CSA* founder mutation in Japanese CS patients. We may extrapolate this to suggest that many of the patients analysed in the survey by Kubota et al are likely also to have carried this founder mutation. As mentioned above, CS-A patients are more likely to fall into the Type I category. The features of the 41 Japanese Type I patients are also included in Table 4. Deafness, photosensitivity and retinal

**Table 4 Summary of clinical features**

<b>Clinical Feature</b>	<b>CS-A<sup>a</sup></b>	<b>CS-B<sup>a</sup></b>	<b>Wilson et al<sup>b</sup></b>	<b>Kubota et al<sup>d</sup></b>
Growth Failure	28/29 (97)	66/67 (99)	100	36/36 (100)
Low birth weight	7/25 (28)	24/48 (50)	0	
Cachexia	26/27 (96)	55/56 (98)		38/39 (97)
Mental retardation	27/27 (100)	55/57 (96)		41/41 (100)
Microcephaly	26/27 (96)	55/57 (96)	100	
Cataracts	12/22 (54)	34/49 (69)	48	20/31 (65)
Microphthalmia	2/16 (12.5)	13/33 (39)		
Retinal degeneration	10/18 (55)	16/30 (53)	43	25/28 (89)
Deafness	15/21 (71)	26/43 (60)	44	28/31 (90)
Photosensitivity	17/23 (74)	38/50 (76)	47	34/37 (92)
Dental anomalies	11/14 (78)	17/25 (68)	46	18/25 (72)
CSA/CSB mutations	39/39 (100)	85/85 (100)	39/40 (98) <sup>c</sup>	

The data present a summary from Tables 1 and 2. <sup>a</sup>Number of cases with indicated feature/total number for whom we have relevant information. % in parentheses; <sup>b</sup>Data, expressed as %, from [4]; <sup>c</sup>Molecular analysis only available from 40 families – 39 were mutated in *CSA* or *CSB*, 1 was mutated in *XPD*. <sup>d</sup>Data from [3].

degeneration appear to be higher in the Japanese cohort. This may be partially explained by the average age of the Japanese patients (17.5 years), which appears to be significantly higher than in our cohort. Deafness and retinal degeneration are progressive and therefore more likely to occur in older patients.

As also reported in earlier studies, clinical photosensitivity was found in the majority of our patients, even those with skin types IV and V on the Fitzpatrick scale (see Tables 1, 2 and 4). Nevertheless, as in other reports (eg [55]), we found no skin cancers in any of our patients. This may be explained by a recent finding that CS fibroblasts are not hypermutable by UV radiation [56].

In conclusion, our analyses show that the human mutation spectrum of the CS genes is not yet saturated, but mis-sense mutations are largely confined to a few relatively short regions. There are no definitive correlations between genotype and phenotype, but truncation mutations C-terminal to the PiggyBac insertion in *ERCC6/CSB* are more likely to confer a severe clinical phenotype than mutations N-terminal to this insertion or mutations in *ERCC8/CSA*. Our data will be important for diagnosis of and genetic counselling for this disorder.

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**Contributors:** NC, EB, NJ and CO carried out the sequence analysis; HF, TN, ML and CO did the RRS measurements and contributed to the genetic analysis, as did YN and NJ, who also did the lentivirus complementation. SM, KS, MK, MAS, MS, VL and ARL provided patient material and clinical data; MS, VL, DO, TO and ARL conceived and designed the study, and, together with NC and EB, wrote the paper. All authors approved the final manuscript.

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**Patient consent:** Obtained.

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## FIGURE LEGENDS

### Figure 1 Mutation distribution in CSA

(A) Distribution of mis-sense mutations across the CSA protein. Mutations identified in this study are indicated below the protein with new mutations indicated in bold. Other previously identified mis-sense mutations are indicated above the protein. 1-7 indicate the seven WD40 domains of the CSA protein according to the reference sequence NP\_000073.1. (B) Missense mutations associated with different CS phenotypes mapped onto the three-dimensional CSA protein structure (RCSB PDB, DOI: [10.2210/pdb4a11/pdb](https://doi.org/10.2210/pdb4a11/pdb), [11 12]). Yellow, UVSS; Orange, CS type I; Violet, CS type II; dark Grey, CS type III. (C) Distribution of truncation mutations. Mutations have been grouped in intervals of 40 aa and columns represent the number of mutations for each group. The interval 0-40 includes mutations resulting in no transcript (asterisks). Black: new mutations identified in this study; grey: previously reported mutations also present in this study; white: other previously reported mutations.

### Figure 2 Lack of complementation with CSA and CSB mutations

Wild type and various mutant *ERCC8/CSA* (A, B) or *ERCC6/CSB* (C, D) cDNAs were ectopically expressed by recombinant lentivirus infection in fibroblasts derived from a CS-A patient CS9LO or CS-B patient, CS10LO, respectively. (A, C) RRS activities were detected 12 hours after UV irradiation (filled bars, 12 J/m<sup>2</sup> UVC-irradiation; open bars, no-UV irradiation), and the value was normalised to activity measurement in non-irradiated cells. (B, D) Viral infection efficiency was confirmed by immunofluorescent staining of V5-tagged wild type and mutant CSA or CSB proteins, and calculated as the number of Alexa 488-positive cells using a semi-automatic VTI system. w/o, without virus infection; w.t., wild type. Results from at least three independent experiments. Error bars indicate SD.

### Figure 3 Mutation distribution in CSB

(A) Distribution of mis-sense mutations across the CSB protein. Mutations identified in this study are indicated below the protein with new mutations indicated in bold. Other mis-sense mutations reported as pathogenic are indicated above the protein, with those now classified as polymorphic variants in parenthesis. Different domains of the protein are indicated: A, acidic domain; N, nuclear localisation domain; I, IA, II-VI, helicase-like domains; U, ubiquitin-binding domain. (B) Distribution of truncation mutations. Mutations have been grouped in intervals of 100 aa and columns represent the number of mutations number for each group. The interval 0-100 includes mutations resulting in no transcript (asterisks). Black: new mutations identified in this study; grey: previously reported mutations also present in this study; white: other previously reported mutations.

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