Novel missense mutations in a conserved loop between ERCC-6 (CSB) helicase motifs V and

VI: Insights into Cockayne syndrome

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ABSTRACT

Cockayne syndrome is caused by biallelic *ERCC8 (CSA)* or *ERCC6 (CSB)* mutations and is characterized by growth restriction, microcephaly, developmental delay, and premature pathological aging. Typically affected patients also have dermal photosensitivity. Although

Cockayne syndrome is considered a DNA repair disorder, patients with UV-sensitive syndrome, with *ERCC8* (*CSA*) or *ERCC6* (*CSB*) mutations have indistinguishable DNA repair defects, but none of the extradermal features of Cockayne syndrome. We report novel missense mutations affecting a conserved loop in the ERCC-6 (*CSB*) protein, associated with the Cockayne syndrome phenotype. Indeed, the amino acid sequence of this loop is more highly conserved than the adjacent helicase motifs V and VI, suggesting that this is a crucial structural component of the SWI/SNF family of proteins, to which ERCC-6 (*CSB*) belongs. These comprise two RecA-like domains, separated by an interdomain linker, which interact through helicase motif VI. As the observed mutations are likely to act through destabilizing the tertiary protein structure, this prompted us to re-evaluate *ERCC6* (*CSB*) mutation data in relation to the structure of SWI/SNF proteins. Our analysis suggests that antimorphic mutations cause Cockayne syndrome and that biallelic interdomain linker deletions produce more severe phenotypes. Based on our observations, we propose that further investigation of the pathogenic mechanisms underlying Cockayne syndrome should focus on the effect of antimorphic rather than null *ERCC6* (*CSB*) mutations.

Key words: Cockayne syndrome; Cockayne syndrome protein B (*CSB*); ERCC-6

INTRODUCTION

Cockayne syndrome (CS; OMIM 133540/216400) is a rare, autosomal recessive disorder characterized by restricted postnatal growth, disproportionate microcephaly, developmental delay, and premature pathological ageing [Wilson et al, 2015]. It is principally caused by mutations in *ERCC6* (*CSB*; OMIM 609413) and *ERCC8* (*CSA*; OMIM 609412) and is historically

considered a DNA repair disorder [Nardo et al., 2009; Horibata et al., 2004]. ERCC-8 (CSA) and ERCC-6 (CSB) cooperate in the recognition of bulky DNA adducts during transcription. ERCC-6 (CSB) is a nuclear resident protein, which recognizes and binds such lesions in concert with XPG [Harker et al, 2005]. ERCC-8 (CSA) is a cytoplasmic E3-ubiquitin ligase, which is recruited into the nucleus in response to DNA damage in complex with its chaperone, UVSS-A [Saijo et al, 2007]. ERCC-8 (CSA) polyubiquitinates ERCC-6 (CSB), removing this protein from damaged template strand lesions, which are then resolved through the common nucleotide excision repair pathway. The fibroblasts from patients with classical CS exhibit a defect in transcription-coupled nucleotide excision repair (tc-NER) following UVC irradiation. However, defective tc-NER cannot be the cause of the CS phenotype, as an indistinguishable DNA repair defect caused by mutations in *ERCC8* (CSA) or *ERCC6* (CSB) occurs in patients with UV-sensitive syndrome (UVSS; OMIM 600630/614621), who experience photosensitivity, but lack the impaired growth, developmental problems, or signs of pathological ageing seen in patients with CS. A number of alternative roles for the ERCC-6 (CSB) protein have been proposed, including altering the action of base excision repair factors [Wong et al, 2007], in telomere maintenance [Battenburg et al, 2012] and in mitochondrial DNA repair [Scheibye-Knudsen et al, 2012]. However, ERCC-8 (CSA) has not been found to participate in any of these processes, suggesting that while defects in these pathways may act as modifiers, they are not the major influence on the CS phenotype. Further clues are needed to establish the pathology underlying CS. Here, we describe two patients from different ethnic backgrounds with novel homozygous *ERCC6* (CSB) mutations in an evolutionarily conserved motif common to the SWI/SNF family of proteins, associated with the CS phenotype.

CLINICAL REPORTS

Patient 1 (Figure 1A) is the first child of consanguineous parents. He was born at term, weighing 3.3 kg (-0.5 SD). He presented at 2.5 years with postnatal growth failure, disproportionate microcephaly and developmental delay. He developed intention tremor at 5 years and had a broad-based ataxic gait. He also had a mild, predominantly motor demyelinating polyneuropathy and proximal weakness of the legs. He had bilateral sensorineural hearing loss from 15 years. He was hypermetropic and underwent regular ophthalmology evaluations, but did not have cataracts or retinal abnormalities. He had subcutaneous fat loss, although the age at onset was unclear. He also had a photosensitive rash, managed with the use of sunscreen. At 22 years, his height was 125 cm (-7.7 SD), weight was 25 kg (-12.6 SD) and OFC was 49 cm (-4.8 SD for 18y). He was initially diagnosed with CS following skin biopsy, as his fibroblasts demonstrated impaired recovery of RNA synthesis after UVC irradiation (Figure 1B).

Patient 2 (Figure 1C) is the second child of South African parents who are not known to be consanguineous, but belong to the same tribe. She was born at term, weighing 2.1 kg (-3.1 SD) with an OFC of 33 cm (-1.3 SD). She had postnatal growth failure with disproportionate microcephaly. Following normal early development, she regressed from 8 months of age. She had a single febrile seizure at 10 months of age. She had bilateral divergent strabismus and progressive eye abnormalities, comprising bilateral corneal clouding and nystagmus. She also had bilateral conductive hearing loss. Dental anomalies included conical teeth and multiple dental caries, likely representing enamel hypoplasia. She also had subcutaneous fat loss with

loose skin, and developed progressive contractures, principally affecting her large joints. Additionally, she had a severe thoracic kyphosis (Figure 1D). She died at 4 years of age.

METHODS

Both patients were recruited to the Cockayne Syndrome Natural History study, which was granted ethics approval by the NRES Committee North East – Newcastle and North Tyneside 2. Written consent was obtained from both families for participation and publication of clinical information. For Patient 1, recovery of RNA synthesis in fibroblasts following UVC irradiation was assessed as described [Colella et al., 2009], before recruitment to our study. Genomic DNA extracted from blood was used for genetic investigations. Bidirectional fluorescent Sanger sequencing was performed on amplicons encompassing all exons of *ERCC6*, including non-coding exon 1, and flanking intronic sequences; data were analyzed using the current version of Mutation SurveyorTM software. Details of primers are available on request.

RESULTS

Sanger sequencing of the *ERCC8* (*CSA*) and *ERCC6* (*CSB*) genes showed patient 1 to be homozygous for *ERCC6* (*CSB*) c.2800C>A, p.Pro934Thr (Supplemental Online Figure 1A); both parents were confirmed to be carriers. Patient 2 was apparently homozygous for *ERCC6* (*CSB*) c.2808G>C, p.Trp936Cys (Supplemental Online Figure 1B). In this case, testing of both parents was not possible to rule out the presence of a deletion in *trans* with the missense mutation. Further samples were not available from the proband for quantitative PCR or MLPA. However, as Patient 2 was heterozygous for other SNPs in *ERCC6* (*CSB*), including in the same fragment as the reported missense mutation, and both parents belong to the same tribe, it is probable that this change is homozygous.

DISCUSSION

Pro934 and Trp936 form part of a loop between SWI/SNF helicase motifs V and VI. While much attention has been paid to the adjacent motifs, the role of this loop has not been evaluated previously. Throughout this family of proteins, this loop has retained an identical amino acid sequence from soil living amoeba (slime mould), through to man (Figure 2A). Such remarkable conservation suggests that this structure is essential for the function of SWI/SNF proteins generally. Crystal structures show that SWI/SNF family members, such as *Saccharomyces cerevisiae* Rad54, adopt a bilobar structure, with two RecA-like domains [Thomä et al, 2005]. Helicase motifs I, IA, II, and III contribute to the N-terminal RecA-like domain, while motifs IV, V, and VI form the C-terminal domain. A linker region between motifs III and IV allows the two RecA-like domains to interact, forming an interdomain cleft. Motif VI is essential for this interdomain association. As this conserved loop lies buried close to this cleft and is immediately upstream of motif VI (Figure 2B), it may be that mutations here affect the ability of motif VI to associate with the first RecA-like domain, disrupting the tertiary protein structure.

In an attempt to glean further mechanistic insights from available *ERCC6* (*CSB*) mutation data, we reanalyzed a recent review of published mutations in CS and cerebro-oculofacioskeletal syndrome [Laugel et al, 2010], which is essentially a prenatal onset form of the disorder. What becomes clear from this analysis is that homozygous or compound heterozygous missense mutations are uncommon molecular causes of CS (5 of 44 allele combinations, including the patients reported here; Figure 2C). Most patients have at least one truncating mutation (nonsense/frameshift). The next commonest change in CSB is deletion of residues from the interdomain linker between motifs III and IV, which tends to be seen in

patients with more severe phenotypes. Interestingly, most amino acid substitutions caused by missense mutations also lie within this region. There is no association between the length of truncated ERCC-6 (CSB) and phenotype, except that if both alleles predict proteins <200 amino acids, the patient displays minimal features of CS, or a UVSS phenotype. Mutations that result in major disruption of the tertiary structure of ERCC-6 (CSB), e.g., truncations and changes preventing physical interaction of the two RecA-like domains, can be termed antimorphic. These data suggest that in order to develop CS, a patient generally requires two antimorphic mutations, or one antimorphic mutation in tandem with a hypomorphic allele. The mutations we report here in the conserved motif V-VI loop are in keeping with this, as putative structural determinants of ERCC-6 (CSB) interdomain stability. The principal mechanism underlying CS pathogenesis is currently unknown. We hypothesize that in patients with *ERCC6 (CSB)* mutations, this mechanism will be dependent on the presence of structurally abnormal ERCC-6 (CSB) proteins, rather than the impaired tc-NER that results from antimorphic, hypomorphic and null mutations alike, and does not correlate with phenotype.

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CONFLICT OF INTEREST

None of the authors has any conflict of interest to disclose.

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FIGURE LEGENDS

Figure 1.

Study patients. A, patient 1 at 24 years of age. B, response of fibroblasts to UVC irradiation. Open squares, Patient 1. Open triangles and filled circles, normal controls. Filled triangles, CS control. C, patient 2 at 3.5 years. D, lateral spine X-ray showing severe thoracic kyphosis.

Figure 2.

Conservation of the SWI/SNF family motif V-VI loop and mutation analysis in CS. A, the motif V-VI loop (grey) shows 100% sequence identity from slime mold to man. *H sapiens* (human), *M musculus* (mouse), *G gallus* (chicken), *D rerio* (zebrafish), *P bivattus* (Burmese python), *L chalumnae* (coelacanth), *S cerevisae* (brewer's yeast), *A thaliana* (thale cress), *D discoideum* (slime mould). Arrows show the position of P934 and W936. B, structure of the SWI2/SNF2 chromatin remodeling domain of *Saccharomyces cerevisiae* Rad54 (PDB accession code 1Z3I) [Thomä et al, 2005]. The interdomain cleft (IDC), helicase motifs V and VI are shown; the

conserved loop between these motifs appears in black with amino acids visible. C, *ERCC6* (CSB) mutations in patients with CS or UVSS. Reported patients appear on the x-axis, grouped by phenotype and numbered arbitrarily. Mutant alleles in each patient are represented by bars, one black and one gray. Amino acid position is shown on the y-axis; the corresponding domain structure of ERCC-6 (CSB) protein appears at the right. The degree of truncation represents the residue at which the sequence departs from the published reference sequence, +(n) non-reference residues for frameshift mutations. Triangles show the location of missense mutations. The area between the broken lines represents the interdomain linker region. Clinical correlates appear below. Biallelic microdeletion of the 5' region of *ERCC6* (CSB) is reported in two patients with CS from a highly inbred island population; as sequencing of *ERCC8* (CSA) was not undertaken, we cannot be sure that there is no additional contribution to their phenotype. In addition to helicase domains I-VI, CSB has acidic (A), glycine-rich (G), and nucleotide-binding (N) domains. S, serine phosphorylation site.

Supplemental Online Figure 1.

Molecular genetic analysis of *ERCC6* (CSB). A, Sanger sequence showing homozygous change c.2800C>A (Patient 1). B, Sanger sequence showing apparently homozygous change c.2808G>C (Patient 2). Normal control traces appear above.

Wilson et al (Figure 2) .

Wilson et al (supplemental online figure 1) .

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Wilson_et_al_(Figure_1-500 rev) .

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