Special Issue: Rare Diseases: Advances in Diagnosis, Prevention, Treatment and Management

Journal of INTERNATIONAL MEDICAL RESEARCH

Journal of International Medical Research
48(2) 1–9

DOI: 10.1177/0300060519877997
journals.sagepub.com/home/imr
SAGE

Two heterozygous mutations in the *ERCC6* gene associated with Cockayne syndrome in a Chinese patient

Qin Zhang[1,2,*], Minjuan Liu[1,2,*], Yinghua Liu[1,2,*], Hui Tang[1,2], Ting Wang[1,2], Hong Li[1,2] and Jingjing Xiang[1,2]

Abstract

Objective: To confirm diagnosis and explore the genetic aetiology in a Chinese patient suspected to have Cockayne syndrome (CS).

Methods: The patient was clinically examined, and the patient and her biological parents underwent genetic analysis using whole exome sequencing (WES) and Sanger sequencing. The foetus of the patient's mother underwent prenatal diagnostic Sanger sequencing using amniotic fluid obtained at 19 weeks' gestation.

Results: Clinical examination of the patient showed developmental delay, progressive neurologic dysfunction and premature aging. Two compound, heterozygous ERCC excision repair 6, chromatin remodelling factor (*ERCC6*) gene mutations were detected in the proband by WES and confirmed by Sanger sequencing, comprising a known paternal nonsense mutation (c.643G > T, p.E215X) and a novel maternal short insertion and deletion mutation (c.1614_c.1616delGACinsAAACGTCTT, p.K538_T539delinsKNVF). The patient was consequently diagnosed with CS type I. The foetus of the patient's mother was found to carry only the maternally-derived c.1614_c.1616delGACinsAAACGTCTT variant.

Conclusion: This study emphasized the value of WES in clinical diagnosis, and enriched the known spectrum of *ERCC6* gene mutations.

[1]Centre for Reproduction and Genetics, the Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, Jiangsu, China
[2]Centre for Reproduction and Genetics, Suzhou Municipal Hospital, Suzhou, Jiangsu, China

*These authors contributed equally to this work.

Corresponding author:
Jingjing Xiang, Centre for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, 26 Daoqian Street, Suzhou, Jiangsu, 215002, China.
Email: xiangjingjing2013@163.com

Keywords
Cockayne syndrome, *ERCC6*, whole exome sequencing, prenatal diagnosis

Date received: 15 July 2019; accepted: 2 September 2019

Introduction

Cockayne syndrome (CS; Mendelian Inheritance in Man® [MIM] phenotype numbers 133540 and 216400) is a rare autosomal recessive genetic disorder, first described by Cockayne in 1936.[1] CS is characterized by growth failure, progressive neurologic impairment and premature aging.[2] Other features of CS include cachectic dwarfism, cutaneous photosensitivity, impaired vision and hearing, and dental caries.[3,4] Depending on the time of onset and severity of manifestations, CS can be divided into three types: type I, a moderate form of CS with normal prenatal growth and onset of growth and developmental delay within the first 2 years of age; type II, a severe or early-onset form of CS, with growth failure at birth and little or no postnatal neurologic development; and type III, a mild or late-onset form of CS, with essentially normal growth and development or late onset of abnormalities in growth and development.[5,6]

Mutations in two genes are known to cause CS: ERCC excision repair 6, chromatin remodelling factor (*ERCC6*, also known as *CSB*, MIM#609413) that encodes excision repair cross-complementation group 6 protein; and ERCC excision repair 8, CSA ubiquitin ligase complex subunit (*ERCC8*, also known as *CSA*, MIM#609412) that encodes excision repair cross-complementation group 8 protein.[7,8] Mutations in the *ERCC6* gene account for 65% of CS cases.[5] The *ERCC6* gene, comprising 21 exons and 20 introns, is located at chromosome 10q11.23 and encodes a protein of 1493 amino acid residues belonging to the switch (SWI)2/ sucrose non-fermentable (SNF)2 family.[9] The *ERCC8* gene is located at chromosome 5q12.1, and encodes a WD repeat protein of 396 amino acids.[8] The ERCC6 and ERCC8 proteins have been implicated in the nucleotide excision repair pathway that eliminates a wide variety of DNA lesions.[10–12] Early diagnosis of CS can be difficult due to its broad phenotype spectrum, however, molecular genetic testing could be used to confirm the clinical diagnosis and identify underlying genetic causes.

In the present study, clinical data from a Chinese female patient suspected to have CS is reported. Further molecular analysis using whole exome sequencing (WES) and Sanger sequencing validated the CS diagnosis and revealed the presence of two compound heterozygous variants in the *ERCC6* gene that may be responsible for this disorder.

Patient and methods

Study population

This study was conducted at the Centre for Reproduction and Genetics, The Affiliated Suzhou Hospital of Nanjing Medical University, Suzhou, China between December 2018 and June 2019, and included a 13-year-old female patient (the proband), with clinical features suggestive of CS, and her biological parents. The study was approved by the institutional ethics

committee of the Affiliated Suzhou Hospital of Nanjing Medical University, and written informed consent was obtained from the patient's parents.

Whole exome sequencing and data analysis

Peripheral blood from the patient and her parents was collected into 6 ml EDTA tubes (BD Bio-sciences, San Jose, CA, USA) and stored at 4°C prior to use. Genomic DNA was then extracted for sequencing using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Whole exome sequencing (WES) of the patient/parent trio was performed by Chigene Beijing Zhiyin Oriental Translational Medicine Research Centre Co., Ltd (Beijing, China) using the xGen® Exome Research Panel v1.0 (Integrated DNA Technologies, Inc., Coralville, IA, USA) and the Illumina NovaSeq 6000 platform (Illumina, San Diego, CA, USA), according to the manufacturer's instructions. Subsequent data analysis was conducted using an online Genetic Diagnostic Platform provided by Chigene. The identified variants were classified according to the Standards and Guidelines for the Interpretation of Sequence Variants released by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.[13]

Sanger sequencing

To confirm the WES results, exons 4 and 7 of the *ERCC6* gene were amplified by polymerase chain reaction (PCR) using DNA samples from the patient and her parents. Foetal DNA from amniotic fluid obtained from the mother during prenatal amniocentesis at 19 weeks' gestation also underwent amplification of exons 4 and 7 of the *ERCC6* gene. Each 20 µl PCR reaction mix contained the following: 25 ng sample DNA, 0.2 mM each dNTP, 0.5 µM each primer, 1 U Faststart™ Taq DNA polymerase (Roche, Basel, Switzerland) and 1 × Faststart™ Taq PCR reaction buffer with 2 mM $MgCl_2$. The primer sequences were: forward, 5′-TCACGGCCCCTTT ACTCCTA-3′, reverse, 5′-AGGGATTT GTTCTGCAGGT-3' for exon 4, and forward, 5′-TCCCGCATGTTTCTCTGACT-3′, reverse, 5′-TGCCCTACAGCTCCAT TGTC-3′ for exon 7 (Shanghai Generay Biotech Company, Shanghai, China). PCR was performed using a GeneAmp 9700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA), with the following cycling conditions: an initial denaturation at 94°C for 5 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 58°C for 30 s, extension at 72°C for 30s, a final extension at 72°C for 5 min and holding at 4°C. The PCR products were purified and sequenced in two orientations using an Applied Biosystems™ 3130 Genetic Analyser and associated reagents (ThermoFisher Scientific, Waltham, MA, USA) according to the manufacturer's instructions. The mutation sites were determined by comparison with the National Institutes of Health (NIH) GenBank reference sequence for *ERCC6* (NM_000124.4; https://www.ncbi.nlm.nih.gov/genbank/).

In silico analysis

The identified variants were searched in the NIH dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/), the 1000 Genomes Project database (http://www.1000genomes.org/), the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org/), and the Genome Aggregation Database (gnomAD; http://gnomad.broadinstitute.org/). The pathogenicity of mutations was predicted using the Protein Variation Effect Analyzer (PROVEAN; http://provean.jcvi.org) software tool, version 1.1.3.[14]

The ERCC6 protein and its orthologs were aligned using Clustal Omega software, version 1.2.2 (http://www.clustal.org/omega/).[15] Iterative Threading ASSEmbly Refinement (I-TASSER) software, version 5.1 (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) was used to predict the protein structure.[16]

Results

Clinical data

At the time of the study, the female patient was 13 years of age, with healthy and non-consanguineous parents. She was born at 40 weeks of gestation by caesarean delivery without abnormalities, weighing 3.25 kg and measuring 50 cm in length. She presented with skin craze at 4 months of age, which was treated as eczema, and she was able to walk at 16 months of age. Her development was then delayed, and behavioural and intellectual deterioration was observed. At 5 years of age, her face showed signs of premature aging. Magnetic resonance imaging (MRI) of the brain at 10 years of age revealed thinning corpus callosum, enlargement of the ventricles and cerebral atrophy. Chromosomal microarray analysis of the patient showed a normal female profile. At the time of the study, the patient showed growth failure (height 100 cm, weight 12 kg), intellectual disability and language regression. She had thin hair, wizened face, sunken eyes, dental caries and anomalies of tooth size and shape (Figure 1a–c). The patient also exhibited progressive impairment of vision and hearing, and subcutaneous fat loss. Based on these clinical phenotypes, CS type I was suspected in the patient.

Genetic analysis

Whole exome sequencing in the family trio was performed with DNA from the proband and her parents. For the proband, approximately 99.73% of the targeted bases were sufficiently covered, and the coverage for 99.21% of the targeted bases were over 20×. A total of 61 732 variants were detected in the proband by WES, and 948 variants were obtained after filtering synonymous variants, and variants with low sequencing depth (depth < 10×)

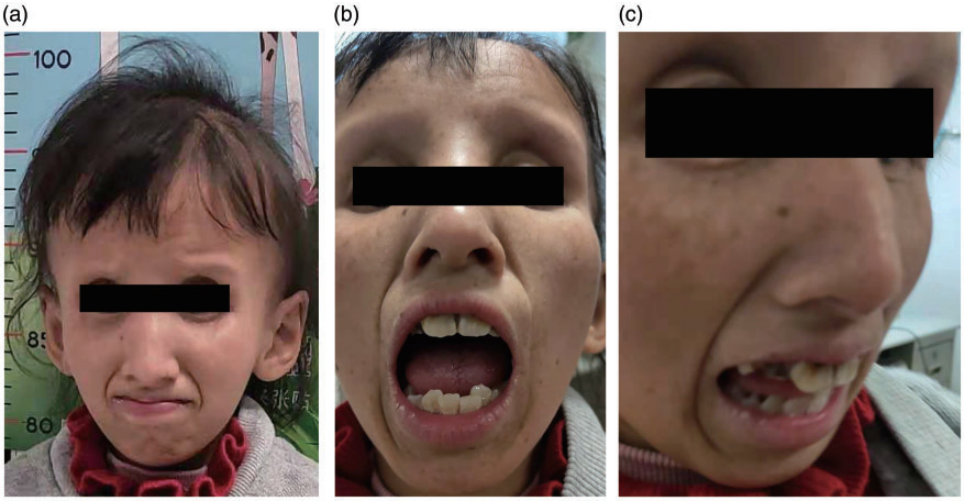

Figure 1. Representative images showing clinical features of a 13-year-old female Chinese patient with Cockayne syndrome: (a) facial features; and (b and c) close up view of the teeth.

or minor allele frequencies >0.01 in the 1000 Genome Project and ExAC databases. Ultimately, two compound heterozygous mutations in the *ERCC6* gene (c.643G>T and c.1614_c.1616delGACins AAACGTCTT) were detected. Sanger sequencing validated the two compound heterozygous variants in the proband, the c.643G>T variant in exon 4 of the *ERCC6* gene was inherited from the father, and the c.1614_c.1616delGACinsA AACGTCTT variant in exon 7 of the *ERCC6* gene was inherited from the mother. The family pedigree and Sanger sequencing results are shown in Figure 2a and 2b, respectively.

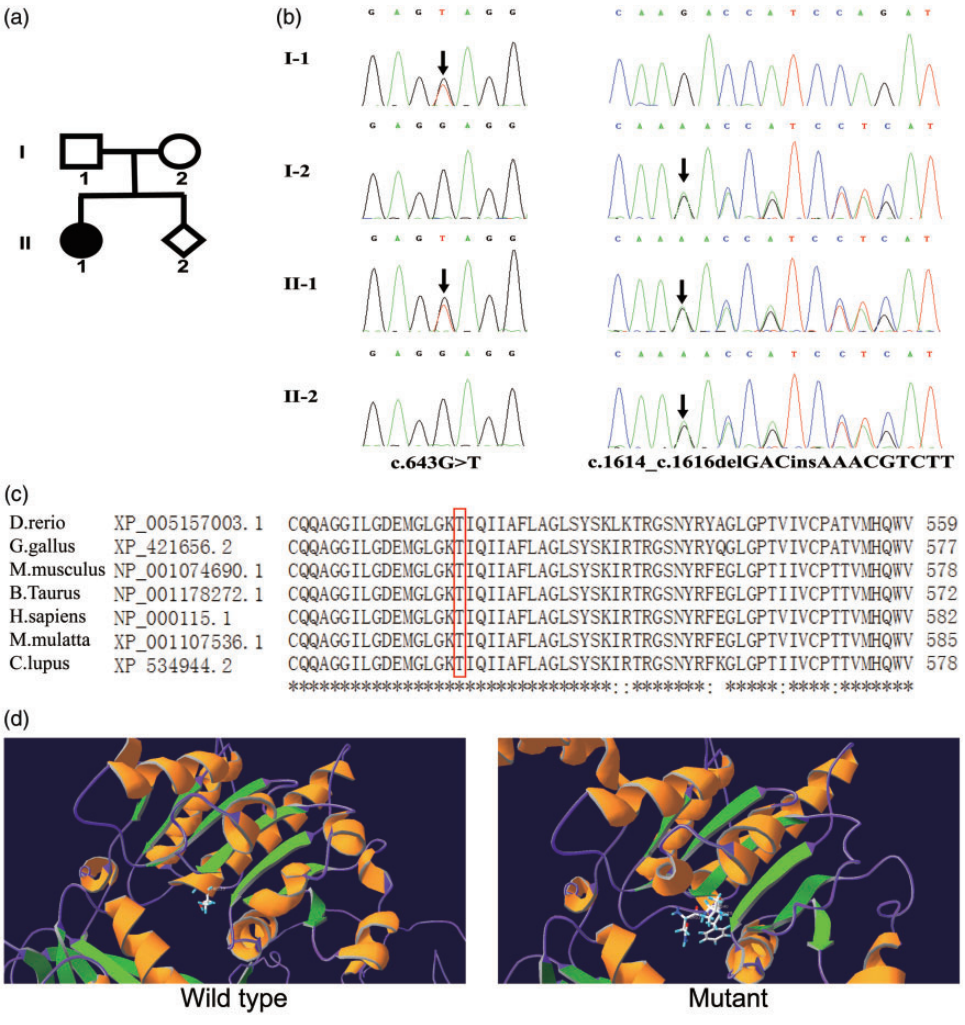

Figure 2. Genetic analysis of a proband/parent trio showing: (a) the family pedigree; (b) results of Sanger sequencing of ERCC excision repair 6, chromatin remodelling factor (*ERCC6*) in family members, demonstrating mutations in the proband, her parents and the foetus of the mother (black arrows indicate mutations); (c) multiple sequence alignment of the ERCC-6 protein and its orthologs in other species (red box indicates the threonine residue in position 539); and (d) predicted structures of wild type and mutated ERCC-6 proteins. The α-helices were shown in orange, β-sheets in green, and coils in purple.

The c.643G>T variant was found to be a nonsense mutation causing a premature stop codon and a truncated protein (p.E215X), which has been reported previously in a patient with CS[17] and classified as likely pathogenic in the NIH ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/225905/). The c.1614_c.1616delGACinsAAACGTCTT variant is a novel in-frame short insertion and deletion mutation, which was not recorded in the NIH dbSNP database, the 1000 Genomes Project database, or the ExAC or gnomAD databases. The c.1614_c.1616delGACinsAAACGTCTT variant leads to the deletion of threonine at position 539 of the ERCC6 protein and insertion of three residues, asparagine, valine and phenylalanine (p.K538_T539delinsKNVF), which are located in a highly conserved region of the ERCC6 protein (Figure 2c) and predicted by I-TASSER to form coil instead of α-helix (Figure 2d). Furthermore, the c.1614_c.1616delGACinsAAACGTCTT variant is predicted by PROVEAN to be deleterious, with a score of –13.52. According to the American College of Medical Genetics and Genomics variant classification guideline,[13] the c.1614_c.1616delGACinsAAACGTCTT variant could be classified as likely pathogenic (iv) with three moderate evidences (PM2, PM3 and PM4). Furthermore, prenatal amniocentesis and Sanger sequencing for the identified variants in the foetus of the proband's mother, at 19 weeks of gestation, indicated that the foetus was a carrier who only inherited the maternal c.1614_c.1616delGACinsAAACGTCTT mutation (Figure 2b).

Discussion

Historically, most patients with CS have been diagnosed late after birth due to its wide spectrum of clinical symptoms, and skin-fibroblast DNA repair assay or direct sequencing of the *ERCC6* and *ERCC8* genes were performed to validate the diagnosis.[18–20] The recent advent of next-generation sequencing has enabled the detection of *ERCC6* or *ERCC8* variants and aids the diagnosis of CS.[21–24] In the present study, the patient received a confirmed diagnosis of CS type I following clinical evaluation and identification of two compound heterozygous mutations in the *ERCC6* gene using whole exome sequencing.

As a member of the SNF2/SW12 family of ATPases, the human ERCC6 protein consists of an acidic domain, a glycine-rich region, two putative nuclear localization sequences, and seven conserved helicase-like ATPase motifs.[25] ERCC6 possesses DNA-dependent ATPase activity, and participates in various DNA repair and transcription processes.[10] To date, 84 different *ERCC6* mutations have been reported in patients with CS, including short insertion and deletion mutations (indels), nonsense mutations, missense mutations, splicing mutations, and promoter mutations, with the two major mutation types being short insertion and deletion mutations and nonsense mutations.[20] In the present study, two compound heterozygous mutations of the *ERCC6* gene were identified in the proband, c.643G > T and c.1614_c.1616delGACinsAAACGTCTT, each of which was inherited from the father and mother, respectively. The nonsense c.643G > T variant has been reported previously in a patient with CS[17] and classified as likely pathogenic in the NIH ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/225905/). The novel short insertion and deletion mutation, c.1614_c.1616delGACinsAAACGTCTT variant, results in p.K538_T539delinsKNVF of the ERCC6 protein, located in the highly conserved helicase-like ATPase motif I. According to I-TASSER software, this mutation will result in the alteration of protein secondary structure from α-helix to

Table 1. Summary of phenotypes and genotypes of reported Chinese patients with Cockayne syndrome and ERCC excision repair 6, chromatin remodelling factor (*ERCC6*) mutations.

Reference/ Case	Mutations on cDNA	Protein (predicted)	Growth failure	Low birth weight	Intellectual disability	Microcephaly	Vision decrease	Hearing decrease	Photosensitivity	Dental anomalies	Bird-like features	Age at onset	Age at latest report	Age at death
[27]Two male siblings	c.1357C>T, c.1607T>G	p.Arg453X p.Leu536Trp	+ +	– –	+ +	+ +	+ +	+ +	+ +	+ +	+ +	1y 6m 1y 6m	7y 5m 4y 8m	
[22]Three female siblings	c.1595A>G, c.1607T>G	p.Asp532Gly p.Leu536Trp	+ + +	– – –	+ + +	+ + +	– – –	+	+ + +	+ + +	+ + +	1y 1y 1y	4y 8m 3y 5m 1y 9m	
[28]Two male siblings	c.1834C>T, c.2923C>T	p.Arg612X p.Arg975Trp	+ +	– –	+ +	+ +	+ +	– –	– +		+ +	2y 2y		8y 5m 9y 4m
[29]Female	c.1387C>T, 2.82Mb deletion including entire *ERCC6*	p.Gln463X	+	–	+	+			+	+	+	1y 8m	1y 8m	

Yes, +; No, –; blank box, missing data; y, year; m, months.

coil. An *in vitro* functional study revealed that a point mutation in ATPase motif I of the ERCC6 protein (K538R) abolished its ATPase activity, but the mutated protein could partially rescue the defect in RNA synthesis recovery after UV exposure when microinjected into CS-B fibroblasts.[26] The phenotypes and genotypes of reported Chinese patients with CS involving the *ERCC6* mutations are summarized in Table 1.[22,27–29] Consistent with previous reports,[5,18,30] no obvious genotype-phenotype correlation was observed in patients with CS involving pathogenic variants in the *ERCC6* gene.

In summary, the present study describes the diagnosis of a patient with CS by a combination of clinical examination and whole exome sequencing, and the identification of two compound heterozygous mutations including a known nonsense variant and a novel short insertion and deletion mutation in the *ERCC6* gene of the patient. The present findings not only broaden the known spectrum of *ERCC6* mutation in CS, but also demonstrate the potential of whole exome sequencing in clinical diagnosis and discovery of disease-causing mutations.

Acknowledgements

We thank the family for participating in this research project.

Declaration of conflicting interest

The authors declare that there is no conflict of interest.

Funding

This research was supported by Jiangsu Province Association of Maternal and Child Health Project (FYX201603), Jiangsu Provincial commission of health and family planning (H2017073), Jiangsu Maternal and Children health care key discipline (FXK201748), Jiangsu Maternal and Children health care research project (F201603), Jiangsu Provincial

Medical Innovation Team (CXTDB2017013), Suzhou Key Medical Centre (SZZX201505), Suzhou Introduced Project of Clinical Medical Expert Team (SZYJTD201708) and Suzhou Industry Technology Innovation Project (SYS201770, SS201873 and SYSD2018119).

ORCID iD

Ting Wang https://orcid.org/0000-0002-9477-1951
Jingjing Xiang https://orcid.org/0000-0001-7191-7156

References

1. Cockayne EA. Dwarfism with retinal atrophy and deafness. *Arch Dis Child* 1936; 11: 1–8.
2. Nance MA and Berry SA. Cockayne syndrome: review of 140 cases. *Am J Med Genet* 1992; 42: 68–84.
3. Laugel V. Cockayne syndrome: the expanding clinical and mutational spectrum. *Mech Ageing Dev* 2013; 134: 161–170.
4. Wilson BT, Stark Z, Sutton RE, et al. The Cockayne Syndrome Natural History (CoSyNH) study: clinical findings in 102 individuals and recommendations for care. *Genet Med* 2016; 18: 483–493.
5. Laugel V. Cockayne syndrome. In: Adam MP, Ardinger HH, Pagon RA, et al. (eds) *GeneReviews® [Internet]*. Seattle: University of Washington, Seattle https://www.ncbi.nlm.nih.gov/books/NBK1342/ (2000 [Updated 29 August 2019], accessed 21 August 2019).
6. Hafsi W and Badri T. Cockayne syndrome. *StatPearls [Internet]*. Treasure Island (FL), StatPearls Publishing, 2018, http://knowledge.statpearls.com/chapter/internal%20medicine-med%20student/19676/ (Updated 2 May 2019, accessed 21 August 2019).
7. Troelstra C, van Gool A, de Wit J, et al. ERCC6, a member of a subfamily of putative helicases, is involved in Cockayne's syndrome and preferential repair of active genes. *Cell* 1992; 71: 939–953.
8. Henning KA, Li L, Iyer N, et al. The Cockayne syndrome group A gene encodes a WD repeat protein that interacts with CSB protein and a subunit of RNA polymerase II TFIIH. *Cell* 1995; 82: 555–564.
9. Troelstra C, Hesen W, Bootsma D, et al. Structure and expression of the excision repair gene ERCC6, involved in the human disorder Cockayne's syndrome group B. *Nucleic Acids Res* 1993; 21: 419–426.
10. Lake RJ and Fan HY. Structure, function and regulation of CSB: a multi-talented gymnast. *Mech Ageing Dev* 2013; 134: 202–211.
11. Velez-Cruz R and Egly JM. Cockayne syndrome group B (CSB) protein: at the crossroads of transcriptional networks. *Mech Ageing Dev* 2013; 134: 234–242.
12. Fousteri M, Vermeulen W, van Zeeland AA, et al. Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. *Mol Cell* 2006; 23: 471–482.
13. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 2015; 17: 405–424.
14. Choi Y, Sims GE, Murphy S, et al. Predicting the functional effect of amino acid substitutions and indels. *PLoS One* 2012; 7: e46688.
15. Sievers F, Wilm A, Dineen D, et al. Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 2011; 7: 539.
16. Yang J, Yan R, Roy A, et al. The I-TASSER Suite: protein structure and function prediction. *Nat Methods* 2015; 12: 7–8.
17. Wu Y, Zheng Y, Yan X, et al. Ocular findings in a patient with Cockayne syndrome with two mutations in the ERCC6 gene. *Ophthalmic Genet* 2017; 38: 175–177.
18. Calmels N, Botta E, Jia N, et al. Functional and clinical relevance of novel mutations in a large cohort of patients with Cockayne syndrome. *J Med Genet* 2018; 55: 329–343.
19. Cui YP, Chen YY, Wang XM, et al. Two novel heterozygous mutations in ERCC8 cause Cockayne Syndrome in a Chinese patient. *Pediatr Neurol* 2015; 53: 262–265.

20. Kou Y, Shboul M, Wang Z, et al. Novel frame shift mutation in ERCC6 leads to a severe form of Cockayne syndrome with postnatal growth failure and early death: a case report and brief literature review. *Medicine* 2018; 97: e11636.
21. Shehata L, Simeonov DR, Raams A, et al. ERCC6 dysfunction presenting as progressive neurological decline with brain hypomyelination. *Am J Med Genet A* 2014; 164A: 2892–2900.
22. Chen L, Yu S, Wu W, et al. Genetic analysis for a family with Cockayne syndrome. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 2014; 31: 285–288 [In Chinese, English abstract].
23. Swartz JM, Akinci A, Andrew SF, et al. A novel ERCC6 splicing variant associated with a mild Cockayne syndrome phenotype. *Horm Res Paediatr* 2014; 82: 344–352.
24. Xie H, Li X, Peng J, et al. A complex intragenic rearrangement of ERCC8 in Chinese siblings with Cockayne syndrome. *Sci Rep* 2017; 7: 44271.
25. Durr H, Korner C, Muller M, et al. X-ray structures of the Sulfolobus solfataricus SWI2/SNF2 ATPase core and its complex with DNA. *Cell* 2005; 121: 363–373.
26. Citterio E, Rademakers S, van der Horst GT, et al. Biochemical and biological characterization of wild-type and ATPase-deficient Cockayne syndrome B repair protein. *J Biol Chem* 1998; 273: 11844–11851.
27. Zhou Z, Liu L, Wu M, et al. Clinical and molecular analysis of two Chinese siblings with Cockayne syndrome. *Zhonghua Er Ke Za Zhi* 2016; 54: 56–60 [In Chinese, English abstract].
28. He C, Sun M, Wang G, et al. Two novel mutations in ERCC6 cause Cockayne syndrome B in a Chinese family. *Mol Med Rep* 2017; 15: 3957–3962.
29. Zhang H, Gao J, Ye J, et al. Maternal origin of a de novo microdeletion spanning the ERCC6 gene in a classic form of the Cockayne syndrome. *Eur J Med Genet* 2011; 54: e389–e393.
30. Laugel V, Dalloz C, Durand M, et al. Mutation update for the CSB/ERCC6 and CSA/ERCC8 genes involved in Cockayne syndrome. *Hum Mutat* 2010; 31: 113–126.