Two heterozygous mutations in the \textit{ERCC6} gene associated with Cockayne syndrome in a Chinese patient

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Abstract

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

\textbf{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.

\textbf{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 (\textit{ERCC6}) gene mutations were detected in the proband by WES and confirmed by Sanger sequencing, comprising a known paternal nonsense mutation (c.643G \textgreater 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.

\textbf{Conclusion:} This study emphasized the value of WES in clinical diagnosis, and enriched the known spectrum of \textit{ERCC6} gene mutations.

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**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. CS is characterized by growth failure, progressive neurologic impairment and premature aging. Other features of CS include cachectic dwarfism, cutaneous photosensitivity, impaired vision and hearing, and dental caries. 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.

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. Mutations in the ERCC6 gene account for 65% of CS cases. 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. The ERCC8 gene is located at chromosome 5q12.1, and encodes a WD repeat protein of 396 amino acids. The ERCC6 and ERCC8 proteins have been implicated in the nucleotide excision repair pathway that eliminates a wide variety of DNA lesions. 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 MgCl2. The primer sequences were: forward, 5'-TCACGGCCCCCTTT ACTCTTA-3', reverse, 5'-AGGGATTT GTTCTGCAAGGT-3' for exon 4, and forward, 5'-TCCCCGATGTTTCTCTGACT-3', reverse, 5'-TGCCCCTACAGCTCCAT 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 30 s, 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/). 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.

**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](image-url). 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 \textit{ERCC6} gene (c.643G>T and c.1614_c.1616delGACinsAAACGTCTT) were detected. Sanger sequencing validated the two compound heterozygous variants in the proband, the c.643G>T variant in exon 4 of the \textit{ERCC6} gene was inherited from the father, and the c.1614_c.1616delGACinsAAACGTCTT variant in exon 7 of the \textit{ERCC6} gene was inherited from the mother. The family pedigree and Sanger sequencing results are shown in Figure 2a and 2b, respectively.

\textbf{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 (\textit{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 \(\alpha\)-helices were shown in orange, \(\beta\)-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\textsuperscript{17} and classified as likely pathogenic in the NIH ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/variation/225905/). The c.1614_c.1616delGACinsAAACGTCTTT 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.1616delGACinsAAACGTCTTT 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 \(\alpha\)-helix (Figure 2d). Furthermore, the c.1614_c.1616delGACinsAAACGTCTTT 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,\textsuperscript{13} the c.1614_c.1616delGACinsAAACGTCTTT 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.1616delGACinsAAACGTCTTT 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 \(\text{ERCC6}\) and \(\text{ERCC8}\) genes were performed to validate the diagnosis.\textsuperscript{18–20} The recent advent of next-generation sequencing has enabled the detection of \(\text{ERCC6}\) or \(\text{ERCC8}\) variants and aids the diagnosis of CS.\textsuperscript{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 \(\text{ERCC6}\) 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.\textsuperscript{25} ERCC6 possesses DNA-dependent ATPase activity, and participates in various DNA repair and transcription processes.\textsuperscript{10} To date, 84 different \(\text{ERCC6}\) 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.\textsuperscript{20} In the present study, two compound heterozygous mutations of the \(\text{ERCC6}\) gene were identified in the proband, c.643G>T and c.1614_c.1616delGACinsAAACGTCTTT, 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\textsuperscript{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.1616delGACinsAAACGTCTTT 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 \(\alpha\)-helix to

\(\alpha\)-helix
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. The phenotypes and genotypes of reported Chinese patients with CS involving the ERCC6 mutations are summarized in Table 1. Consistent with previous reports, 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.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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