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ORIGINAL ARTICLE

Identification of a novel mutation of the *COL2A1* gene in a Chinese family with spondyloepiphyseal dysplasia congenita

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Abstract
Purpose To identify potential disease-causing mutation in the *COL2A1* gene in a Chinese family with autosomal dominant spondyloepiphyseal dysplasia congenita (SEDC) and to analyze the phenotype–genotype correlation.
Methods Complete physical and radiographic examinations of four affected individuals from SEDC family were conducted. Genomic DNA were isolated from peripheral blood leukocytes. All 54 exons and exon–intron boundaries of the *COL2A1* gene were amplified by polymerase chain reaction (PCR) and bidirectionally sequenced.
Results All four affected individuals were found carried a novel missense mutation of c.2224G>A (p.Gly687Ser) in *COL2A1*, while normal members of the family and 50 healthy controls did not have this mutation. Protein prediction of missense mutation by polyphen-2 and SIFT software and mutation taster indicated severe damage to the function.
Conclusions c.2224G>A (p.Gly687Ser) is a novel mutation of *COL2A1* associated with spondyloepiphyseal dysplasia congenital. There are heterozygous of phenotype for the mutation in members of the pedigree analyzed. Onset becomes more earlier and severe with each successive generation.

Keywords Spondyloepiphyseal dysplasia congenita · *COL2A1* · Mutation · Phenotype

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Introduction

Spondyloepiphyseal dysplasia congenita (SEDC; OMIM 183900) is an autosomal dominant inherited chondrodysplasia that mainly affects vertebral bodies and proximal epiphyses of the long bones [1, 2]. Patients diagnosed as SEDC can present with various clinical features involving short stature and bone malformation. Spinal deformities like scoliosis and flattened vertebral bodies are common. Hip deformities including coxavara and avascular necrosis-like changes in bilateral femoral epiphyses may be present, sometimes accompanied by hip pain or decreased walking tolerance. In addition, the patients can have hearing loss, cleft palate and ocular anomalies including myopia with retinal detachment [3–5].

According to the earlier genetic investigations, most cases of SEDC are resulting from mutations sparsely distributed in the 54 exons of *COL2A1* gene [6]. *COL2A1* gene, localized to 12q13.11–q13.2, provides key elements for making the α1 chain of procollagen type II, which is the main structural protein of cartilage and bone. Exons 6–48 encoded the core area of type II collagen, which is composed of triple-helical domain. Until now, the 27 different mutations [7–11] related to SEDC had been identified in

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world. There are eight noval mutations (G810S, G1176V, G504S, G447A, G456A, G1152D [11–13], G546S [14], G1086V [15]) associated to the SEDC in Chinese patients.

In the present study, we report our findings of phenotype and mutation of *COL2A1* from a three-generation family affected with SEDC.

Methods

Subjects

The three-generation family enrolled in this study, whose nationality is Han, was found in Peace Hospital Attached to Changzhi Medical College, Changzhi, China. The detailed clinical records on medical history, physical examinations and laboratory studies were obtained.

We mapped the pedigree of the family (Fig. 1). The ethical approval was obtained from the Changzhi Medical College Ethics Committee and the study conformed to the Declaration of Helsinki. Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study.

Genomic DNA preparation

Genomic DNA was extracted and purified from whole blood by using the Maxwell® 16 System (Pomega Corporation, USA). High concentration (>100 ng/μL) genomic DNA was directly used for PCR after a short centrifugation.

Mutation analysis of *COL2A1*

Genetic analysis was performed by sequencing *COL2A1* gene as the candidate gene which linked inheritable epiphyseal dysplasia. The 54 exons primers of *COL2A1* (Gene registration number in NCBI was MIM#120140) were designed by Primer 5.0 software (PREMIER Biosoft International Inc., Canada), primers listed in "Appendix". All 54 exons and exon–intron boundaries of *COL2A1* gene were amplified by PCR. PCR was conducted in a 20 μl tube containing 100 ng DNA template, 2.0 μl of 10 × PCR buffer, 1.5 mmol $MgCl_2$, 1 U Taq DNA-polymerase (TaKaRa, Dalian, China), 200 μmol dNTPs and 200 nmol each primer. PCR cycling conditions were 5 min at 95 °C followed by 35 cycles at 95 °C for 1 min, 57 °C for 45 s, 72 °C for 1 min and 72 °C for 10 min. A 5 μL PCR product was obtained and subjected to separating on a 2 % agarose gel stained with ethidium bromide. After electrophoresis, the products were sequenced by ABI (Applied Biosystems)3730XL in BGI (Beijing Genomics Institute, China). BioEdit Software (Borland Inc, Scotts Valley, USA) was used to compare the sequencing and the reference sequence of *COL2A1* gene. Query the UCSC dbSNPs database and the international human genome mutation database to know if there exists single nucleotide variants (SNVs) or known pathogenic mutations. Deleterious SNVs were predicted by SIFT, Polyphen-2 Software and MutationTaster programs.

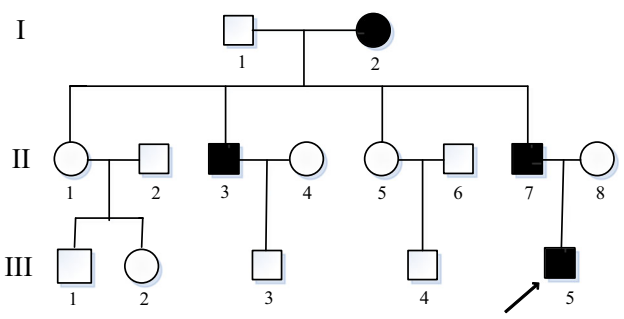

Fig. 1 Pedigrees of the three-generation SEDC family

Results

Case presentation

The investigated pedigree of the family was shown in Fig. 1. The proband was 16-year-old boy with shortened trunk stature, flat face, short neck, and moderate thoracic hyperkyphosis (160 cm height). Besides the patient presented with waddling gait, his hearing vision and intellectual development were normal, and he did not have a cleft palate. The laboratory examination results showed normal growth hormone and normal serum 1,25-dihydroxyvitamin D3. The magnetic resonance imaging (MRI) of spine manifested epiphyseal dysplasia, ovoid vertebral bodies, mild scoliosis. X-rays of his pelvis revealed abnormalities of the capital femoral epiphyses, flattening of the acetabular roof (Fig. 2). With regard to proband's father, the curve severity of scoliosis was moderate and os odontoideum in the cervical spine was found by X-ray (Fig. 3). As for the uncle of the proband, no scoliosis was found from his anterior–posterior standing X-ray film except flattened vertebral bodies. Overall, the proband's father and uncle have similar pelvis radiograph showed femoral head epiphyses, flattening of the acetabular roof, and shortening of the femoral neck. The abnormalities noted in grandmother's pelvis included flattening of the acetabular roofs, collapsed femoral heads. Spine disorders involved obvious thoracic hyperkyphosis as well as platyspondyly (Fig. 4). There was no abnormality in the epiphyses of both knees of the proband and his family members. Physical findings and radiographic manifestations of affected family members are summarized in Table 1.

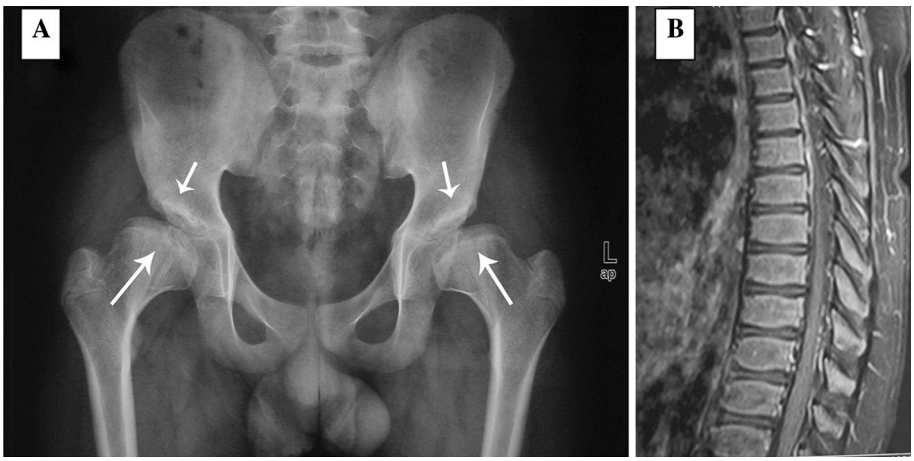

Fig. 2 **a** The pelvis radiograph shows dysplasia of both femoral head epiphyses, flattening of the acetabular roof. **b** Radiograph of the proband's spine showing mild scoliosis, and ovoid vertebral bodies

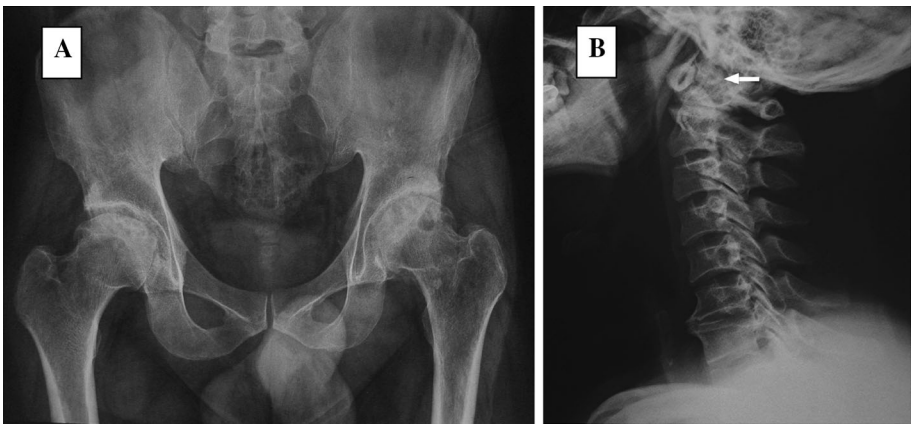

Fig. 3 **a** Radiograph of the pelvis from the father reveals flattening of the acetabular roof, subchondral bone sclerosis and cystic. **b** Os odontoideum of the cervical spine indicated by the *arrow*

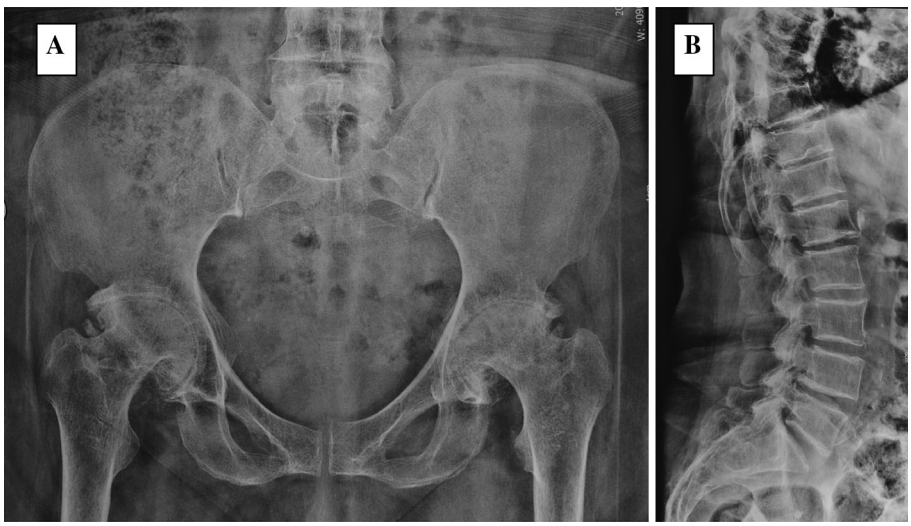

Fig. 4 **a** Radiograph of proband's grandmother's pelvis showing degenerative changes in the hip joints which had flattened acetabular roofs, dysplasia of the femoral heads epiphyses,marginal osteophytes, cystic and joint space narrowed. **b** The lateral view of the spine shows thoracic hyperkyphosis as well as platyspondyly

Mutation analysis

Sequencing of *COL2A1* showed a heterozygous G>A change in the affected individuals in exon 32 at nucleotide position c.2224. This nucleotide change results in a serine substitution for a highly conserved glycine at codon 687 (p.Gly687Ser), but no same nucleotide change was detected in the other unaffected members and the 100 healthy control subjects (Fig. 5).

Table 1 Clinical manifestation of affected family members

Pedigree	Age at onset (years)	Age (years)	Gender	Height (cm), SD	Retinal detachment	Hearing loss	Cleft palate	Complaint	Scoliosis	Hyperkyphosis	Platyspondyly	Os odontoideum	Flat acetabular roofs	Dysplasia of the femoral heads
I-1	30	74	F	155 <−2SD	–	–	–	Groin pain	+	+	+	–	+	+
II-3	19	48	M	162 <−2SD	–	–	–	Groin pain, waddling gait	–	–	+	–	+	+
II-7	12	42	M	161 <−2SD	–	–	–	Groin pain, waddling gait	+	–	+	+	+	+
III-5	3	17	M	160 <−2SD	–	–	–	Groin pain, waddling gait	+	–	+	–	+	+

This mutation resulting a deleterious missense mutation was predicted by 3 different programs; SIFT (affecting protein function with score of 0.01), polyphen-2 (probably damaging with score of 0.998), and mutation taster (disease causing). This mutation was not found in ESP6500, 1000 Genome, and in-house exome database, suggesting it is a rare variant co-segregated with the phenotype in the family.

Discussion

Mutation in the type II collagen gene was associated with human disorders termed type II collagenopathies. Type II collagen mutations are found not only in phenotypes with premature-onset arthritis but also in conditions with abnormal spondylar, epiphyseal and metaphyseal development, which included achondrogenesis type II (ACG2, MIM 200610), Czech dysplasia (MIM 609162), Kniest dysplasia (MIM 156550), spondyloepiphyseal dysplasia congenita (SEDC, MIM 183900), spondyloepimetaphyseal dysplasia (SEMD, MIM 184250), spondyloperipheral dysplasia (SED,MIM 271700), Stickler syndrome type II (STD-1, MIM 108300) and Torrance type platyspondylic dysplasia (MIM 151210). Uncommon phenotypes, such as avascular necrosis of the femoral head (MIM 608805) and Legg–Calve–Perthes disease (LCPD, OMIN 150600) have also been reported [16, 17]. Over 200 different *COL2A1* human mutations are described and a wide spectrum of disease exists. The most common type of mutation is a substitution in the triple-helical glycine residue of the α chains, however, there was no mutation hotspots [18]. Till now, 27 different mutations related to SEDC have been reported since SEDC was firstly reported by Spranger and Wiedemann in 1966 [1], most of which are located within the triple-helical domain of the protein.

In the current study, four SEDC-affected individuals from a three-generation family were investigated. The affected individuals had many features of SDEC, such as short stature (−2SD), flat face, wide set eyes, short neck, barrel chest and waddling gait, no patient had cleft palate, hearing loss or retinal detachment. The proband was 2.5 kg weight at birth and walked after age of 18 months. He firstly reported his groin pain at age 3 years. Skeletal radiographs findings were consistent with SEDC including ovoid shaped vertebrate, scoliosis and thoracic hyperkyphosis, flattening of the acetabular roof and hypoplastic ossification of femoral head. Compared to proband's early-onset, I-2, II-3 and II-7 delayed, respectively. Grandmother felt groin pain and decreased walking tolerance at age of 30 years. Her radiographic examinations showed a sever platyspondyly and avascular necrosis-like changes in bilateral femoral epiphyses. His father and uncle's hip pain

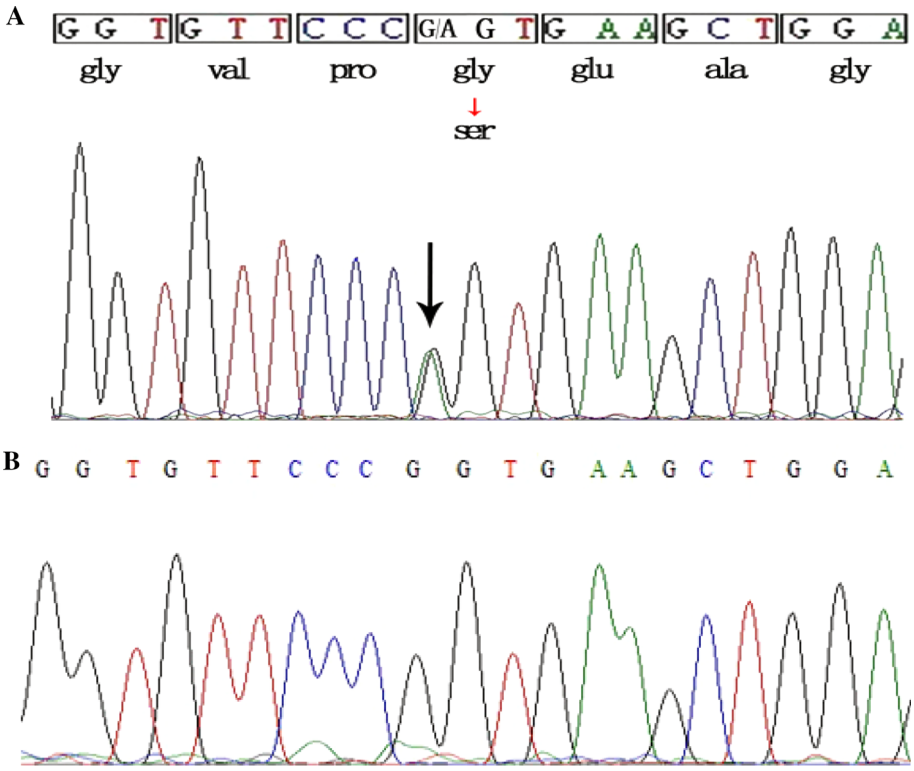

Fig. 5 **a** Partial sequence diagram of *COL2A1* exon 32 of the proband. A heterozygous G>A transition, which results in substitution of Gly to Ser, is indicated by the *arrow*. **b** No mutation was detected in the same coding region of the mother or in a normal control

occurred lately and progressed more quickly recently. Their X-rays indicated mild spinal deformities but a severity hip deformities, such as cystic, osteophytes and joint space narrowed. Specifically, X-rays of the proband's father revealed os odontoideum in the cervical spine. His phenotype was not consistent with Jung's study [19] that os odontoideum is rare in SEDC and patients with os odontoideum may have atlantoaxial instability and more seriously phenotypes. In this study, although affected individual had similar shorter height (−2SD), they had different spinal deformity. Overall, onset becomes more earlier with each successive generation, the phenotypic expression of different individuals become more severe with age.

We identified a novel glycine substitution mutation (c.2224G>A; p.Gly687Ser) of *COL2A1*. Until now, 27 different mutations related to SEDC had been identified, most of which are substitution of the glycine by serine in position one of the triple helix of the α1 chains. The triple helix domain is characterized by about 330 repeating Gly–X–Y triplets that are highly conserved. The serine-to-glycine substitution adds a large hydroxymethyl group to the center of the superhelix that is predicted to disrupt the local structure and loosen the superhelix. The abnormal α1 chain composed of variant triple helix mainly expressed in cartilage instead of in cells. It is reasonable that abnormal α1 chains can assemble into fibrils and form abnormal cross-linked, could further lead to the degradation of premature collagen molecules, or product over modified type II collagen [20], or serve as important binding sites of telopeptides in the early stages of fibril formation [21, 22] or interrupts intracellular transport and secretion of collagen [23]. It is very possible there are several common mechanisms leading to spondyloepiphyseal dysplasia. In future studies, we will examine removed cartilage tissue from patients, and to assess the effects of these mutations on chondrogenesis, should be helpful to better delineate the consequence of p.Gly687Ser mutations in *COL2A1* on collagen structure and cartilage matrix integrity.

Taken together, our results provide compelling evidence that c.2224G>A (p.Gly687Ser) is a novel mutation of *COL2A1* associated with spondyloepiphyseal dysplasia congenital, based on the following reasons: (1) this mutation located at Gly–X–Y repetitive sequence;(2) this mutation in the *COL2A1* gene cosegrated with the patients; (3) the mutation was not detected in the normal members of same family and 100 healthy controls; (4) protein prediction of missense mutation by Polyphen-2, SIFT software and MutationTaster indicated severe damage to the function. However, variation in the phenotypic expression of different individuals suggests that some other genetic and environmental effect may play a role in the final clinical

expression. Ultimately, this study on this pedigree can be extended by including the distal relatives of the family that may assist in establishing a relationship between the nature of *COL2A1* mutations and the clinical features of SEDC.

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Conflict of interest The authors declare that they have no conflicts of interest concerning this article.

Appendix

See Table 2.

Table 2 Sequences of primers for *COL2A1* exons

Exon	Sense primer	Anti-sense primer
Exon 1	5′AGTTCGCCAGCCTCGAAAG3′	5′ATTGGCAGAACTCTTCTTGGTGA3′
Exon 2	5′TAAGTTCTATATGTCCAGGTGG3′	5′TCTATGGGAGCGTGTTTG3′
Exon 3	5′TGTGTCCTTTTACTTGTTTGAT3′	5′GCCGAGTAAGCCCACTAA3′
Exon 4–5	5′GCTTCAAAAGCAATGCTATGT3′	5′TGACAGCAAGGCCAGGAGC3′
Exon 6–7	5′CTCGCTGCGCGCAAGTTA3′	5′GCCAGCCAGGTAAGTGCAAG3′
Exon 8	5′TAGTCTCCAACAATCAGAGTTTATC3′	5′GCCACCTCCTGCCATTTT3′
Exon 9-10	5′TGCCCACAGAGTAACTTCTTGT3′	5′CCGACTGTGGGAAAGAGC3′
Exon 11	5′CTATGATCTCTGCACCTTTGCT3′	5′GATGGCGTCAGGGTTTGG3′
Exon 12	5′GGGAGATGAGTGAGCCGGTAG3′	5′GCACTGTGTTTAAGGCCACAGG 3′
Exon 13–14	5′GTGTGGGGATTCGAGACAACG3′	5′TGTAGGGCTGGTGTTCCCAGC3′
Exon 15	5′CAGCCCTACATCCTGATGG3′	5′GCAGCCATCTGATAGTCTGAA3′
Exon 16	5′AGTCAAGGAGCCAGCACCA3′	5′CCTGGGAAGTTTTGATGGG3′
Exon 17	5′ACACCTCGCCATCCTCGT3′	5′TCAGAGTGCTGCTGTGGTT3′
Exon 18	5′TGGATATGGAGTGAAATCAGTA3′	5′GCAGGTGGTTGTTGAGGG3′
Exon 19	5′TGTGTGTGAACGCACATGTT3′	5′AAGGTTTGGTGGTTGGAG3′
Exon 20–21	5′CTATCCCATCTTCCCCTTG3′	5′AGATGCCCTCGGATGGAG3′
Exon 22	5′GTAAGAGCCCAAAGTGACC3′	5′TGAAAGGACCCAGATTGG3′
Exon 23	5′CCAGCCCTGAAACAGTTGC3′	5′GAGGATGACATGCGGAAA3′
Exon 24–25	5′TGCAGGCCACAGCTACTGCTC3′	5′GGGGTCAGGAGCCGGCC3′
Exon 26	5′GTAAGTAGCAGAGCTGCTGTTG3′	5′CCCTAACCCAAACTCCATCT3′
Exon 27	5′GTGGTCAATCCTAGATGCTGA3′	5′CCCACTCATCACTGTCCCT3′
Exon 28	5′GGCTGATACTTTGCTTTATCTTGG3′	5′GCCACAGAGATCAACACTCAATACT 3′
Exon 29	5′GCTGGGGTACCGTGGAG3′	5′GCTCAGCCCACATTCACA3′
Exon 30	5′TACTAGCTGTGGCTCTCAGGGTCTC3′	5′GCCCTGGGTATGGCAAAGGACTG3′
Exon 31	5′TGCTAACGCTTGTCACTTCGGCTTCT3′	5′ATGCCCTCTTGCCCTTGCCTCCT3′
Exon 32	5′GAGGCAAGGGCAAGAGG3′	5′CACCAAGAAGTGATCAACCAAC3′
Exon 33	5′GTCCTATGCTCCTGCTCCTTTCCC3′	5′CCTTCCTCCCATGTAGACCTCCTTT3′
Exon 34	5′GTAAGTGAGGCTGCATCCTGTAGGG3′	5′ACCAGGTGCCATAAGGGAACGGA3′
Exon 35	5′CCCTGAGACCACAGCAAATT3′	5′CCGACAGAGACAGGACCAG3′
Exon 36	5′TCTGTTAAAATGGGGCCAGA3′	5′AAGACAGAACCGCCTTTGG3′
Exon 37	5′CTGTGCCAGGGGGACCTG3′	5′CATGAGGGCCTGCACTGACT3′
Exon 38	5′GTTAACTAAGGCTGCTTTCAGA3′	5′AGTATGGAGGCGGGAAAG3′
Exon 39	5′GCCTCCATACTAATAGAACCATCAT3′	5′CTGCGAACCATCCTCTGC3′
Exon 40	5′AGTGCCAAGAAAGCTGCATCTT3′	5′AACGCAGGGCTGGGAAAA3′
Exon 41	5′CTTGCTGCTTTGCAGTCCCT3′	5′AACGGACTCAGAGGAGTGAAGG3′
Exon 42	5′TTAAGCTGCCTGCCCTTAG3′	5′CCCAGCCTCTTCTGTCCT3′
Exon 43	5′GTAAGTCCCTCACCAGGCCCAT3′	5′GAGGGCAGACAAGGGACAGTCC3′
Exon 44	5′TGCCAAGGCTTCTACCTCC3′	5′TGACTGGGACTTGTCCTTGCT3′
Exon 45	5′GAGGAGAGGCCTGGGCT3′	5′TAGGCTGAGATGAGACTTGTTC3′

Table 2 continued

Exon	Sense primer	Anti-sense primer
Exon 46	5′GTCAGCTGGGGGTGGCAG3′	5′GACAGGTGGGGGCCTCCT3′
Exon 47–48	5′GGTGGGGCTGAGGCAGTCC3′	5′GGAAATCCTAGAAACTGCTTAGGGT 3′
Exon 49	5′ACAGCTTGGGATCACCCTA3′	5′AGGCACATGAGCCAGTCC3′
Exon 50	5′TGCTATCAGGACAGCCACCT3′	5′TAAAAGAGGCCCTGAGCAAA3′
Exon 51	5′TAGACATGGTGCTGTGGTTTC3′	5′AAACTTCCAGGCCCAGCT3′
Exon 52	5′CATGTGAACCTCATCCCTTGTC3′	5′GGAAAATATGGGGAAGGTGCTA3′
Exon 53	5′TTGAGGTCCTTGAACCATGAA3′	5′GACCCTCAAACTCATGCCTCT3′
Exon 54-1	5′CAGTGTGGTTCAACCTTGTGG3′	5′ATAGAACACCGAGATTTTATTTTGC 3′
Exon 54-2	5′CCCGAGCAGGAATTCGGT3′	5′ACGGAGGATTAATGGAAAACAAACT 3′

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