Identification of novel compound heterozygous RECQL4 mutations and prenatal diagnosis of Baller-Gerold syndrome: a case report

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ABSTRACT. Birth defects are structural and/or functional malformations present at birth that cause physical or mental disability and are important public health problems. Our study was aimed at genetic analysis and prenatal diagnosis of congenital anomalies to understand the cause of certain birth defects. Karyotypes and array-comparative genomic hybridization (aCGH) were performed on a pregnant woman, surrounding amniotic fluid, and her husband. A short-stature panel genetic test was conducted in accordance with the phenotype of the fetus. Following examination, it was determined that the karyotype and aCGH results were normal. The *RECQL4* gene in the fetus showed compound heterozygous mutations, and each parent was found to be a carrier of one of the mutations. The two heterozygous mutations (c.2059-1G>C and c.2141_2142delAG) were detected in the *RECQL4* (NM_004260) gene

in the fetus; therefore, the fetus was predicted to have Baller-Gerold syndrome. These two mutations have not previously been reported. In addition, these results identified a 25% risk of the parents having a second conceptus with this congenital disease. Therefore, prenatal genetic diagnosis was highly recommended for future pregnancies.

Key words: Genotyping; Prenatal Diagnosis; Baller-Gerold syndrome; *RECQL4*

INTRODUCTION

Birth defects (BDs) are structural and/or functional malformations present at birth that cause physical or mental disability; some BDs may be fatal. BDs are an important public health problem due to their overall incidence, with major congenital anomalies occurring in approximately 2-3% of live births in the European Union (EU) (Taruscio et al., 2011). The 2006 March of Dimes report estimated that, on average, 7.9 million children (approximately 6% of total births worldwide) are born with BDs (Shannon et al., 2014). However, the incidence is 5.6% in China, and there are 0.9 million new cases every year according to The Prevention Report of China's Birth Defects (2012), which was published by China's Ministry of Health. (The Central People's Government of the People's Republic of China Web Services). To control China's BD incidence, prenatal screening and diagnosis are integral to antenatal care throughout the country. Prospective parents are offered screening for common fetal chromosomal and structural congenital malformations. Prenatal screening is routinely offered in a package that includes an ultrasound scan of the fetus and an assessment of maternal blood examining biochemical markers of aneuploidy. We describe the case of a fetus with signs of Baller-Gerold syndrome (BGS) and the performance of molecular studies to assess the diagnosis of a *RECQL4* mutation.

MATERIAL AND METHODS

Clinical case

The case involved a 27-year-old pregnant woman and her 31-year-old husband; there was no history of consanguinity. The patient's routine blood, urine, liver, and renal function analyses were normal. Electrocardiography and chest X-rays showed no abnormalities in the heart and lungs. The woman was 24 weeks pregnant, and the ultrasonic description was as follows: we detected bilateral arms and humerus; bilateral forearms and ulnas; bilateral thighs and femurs; bilateral legs, tibias, and fibulas; and bilateral feet. Furthermore, the left ulna was 25 mm long, with an "arc" shape, and no radius echo was detected; the right ulna was 24 mm long, and no radius echo was detected; and bilateral hands were detected in an indistinctly "hook" shape. A referral to the prenatal diagnosis clinic and chromosomal analysis were recommended. A previous patient had been pregnant with a fetus that exhibited the same ultrasonic detection patterns. Therefore, we suggested a single recessive gene disorder, and the fetus and the couple received related genetic examinations. Informed consent was obtained from the patients and the study was approved by the No. 202 Hospital of PLA Ethics Committee. All the patients consented to use their information in this study, and were aware that their

information would be used in future research. The patient information was not anonymized. All the authors had access to any identifying information. We documented the verbal consent in their files.

Karyotyping

Karyotyping was performed on G-banded metaphases obtained from peripheral blood cultures using standard procedures. Karyotypes were obtained from the pregnant woman and her husband. Karyotyping of amniotic fluid was performed at 18 weeks of gestation according to standard techniques (Chandler and Yunis, 1978). Results are described in accordance with the International System for Chromosome Nomenclature (ISCN) 2009.

Array-comparative genomic hybridization (aCGH) analysis

Genomic DNA was extracted from peripheral blood samples (the pregnant woman and her husband) and amniocytes (fetus) using a DNA extraction kit (Tiangen Biotech; Beijing, China) according to manufacturer instructions. aCGH was performed with oligonucleotide-based custom arrays (Agilent Technologies; Santa Clara, CA, USA) using the standard protocol. Briefly, equal amounts of test DNA and normal sex-matched DNA were digested with *Alu*I and *Rsa*I, and differentially labeled with cyanine-5 (cy5) and cyanine-3 (Cy3) fluorescent dyes, respectively, using a SureTag Complete DNA Labeling Kit (Agilent). Hybridizations were carried out at 65°C for 24 h. After washing, slides were scanned using an Agilent SureScan Microarray Scanner and the images were extracted and analyzed using the Feature Extraction v11.5 (Agilent) and the Cytogenomics v2.5 (Agilent) software, respectively.

Short-stature panel genetic testing

Targeted sequence capture and next-generation sequencing

Targeted sequence capture was performed with a BGI (Beijing Genomics Institute)-developed protocol. In brief, 1 μg genomic DNA was fragmented into 200-bp lengths, followed in sequence by end-repair, polyA-tailing and adaptor ligation, a 4-cycle pre-capture polymerase chain reaction (PCR) amplification, targeted sequence capture, and 15-cycle post-capture PCR. Targeted sequence capture was performed using custom oligonucleotide probe sets (NimbleGen, Roche, Basel, Switzerland). Sequencing was performed on a HiSeq2000 platform (Illumina, San Diego, California) as paired-end 90 bp (PE90) according to manufacturer instructions.

Read mapping, variant detection, annotation, and interpretation

Image analyses, error estimation, and base calling were performed using the Illumina Pipeline (version 1.3.4) to generate the primary sequence data. Low-quality reads and potential adaptor contamination were removed from the primary data using a local algorithm. The remaining short reads were mapped to the human genome (UCSC, hg18) using the BurrowsWheelerAligner (BWA) software. Single nucleotide variants (SNVs) were detected using the SOAP SNP software and small insertions and deletions (InDels) were detected using the

SAMtools Pileup software. Variants were annotated using a BGI in-house-developed annotation pipeline and were interpreted according to the American College of Medical Genetics and Genomics (ACMG)-recommended standards (Richards et al., 2008).

RESULTS

Karyotyping

Karyotyping of G-banded metaphases showed that the karyotypes of both the couple and the fetus were normal.

aCGH analysis

We identified a copy number gain of chromosome bands 14q32.33 spanning approximately 0.34 Mb; 16p11.2 spanning approximately 1.52 Mb; and 22q11.22 spanning approximately 0.36 Mb in non-disease associated regions. These copy number gain was determined to be polymorphism by retrieving the public database (DGV; OMIM; DECIPHER; PubMed).

Targeted gene sequence capture and next-generation sequencing analysis

Targeted gene sequence capture and next-generation sequencing

The exons and immediately adjacent intronic regions of 37 genes associated with disorders including clinical features of short limb in OMIM database (Table 1) were captured and sequenced in the proband and his healthy parents with custom oligonucleotide probe sets (NimbleGen, Roche). The targeted regions of the 37short-limbed related genes were covered by a mean depth of over 190-fold, with 99.45% of the targeted region covered by at least one read and 97.71% by at least 20 reads.

Table 1. Genes associated with short limbs Panel.

Disease name	Gene	Disease name	Gene
Spondyloepimetaphyseal dysplasia, aggrecan type	*ACAN*	Acromesomelic dysplasia, Hunter-Thompson type	*GDF5*
Osteogenesi simperfecta, type V	*IFITM5*	Osteogenesis imperfecta, type XIII	*BMP1*
Chrondrodysplasia, acromesomelic, with genital anomalies	*BMPR1B*	Cranioectodermal dysplasia 3	*IFT43*
Osteogenesis imperfecta, type VIII	*LEPRE1*	Desbuquois dysplasia	*CANT1*
Spondyloepiphyseal dysplasia with congenital joint dislocations	*CHST3*	Epiphyseal dysplasia, multiple, 5	*MATN3*
Short rib-polydactyly syndrome, type II/II digenic	*NEK1*	Osteogenesis imperfecta, types I-IV	*COL1A1*
Spondyloepimetaphyseal dysplasia, Pakistani type	*PAPSS2*	Osteogenesis imperfecta, types II-IV	*COL1A2*
Spondyloepimetaphyseal dysplasia, Strudwick type	*COL2A1*	Osteogenesis imperfecta, type IX	*PPIB*
Epiphyseal dysplasia, multiple, 6	*COL9A1*	Rothmund-Thomson syndrome	*RECQL4*
Pseudoachondroplasia	*COMP*	Cartilage-hair hypoplasia	*RMRP*
Osteogenesis imperfecta, type VII	*CRTAP*	Osteogenesis imperfecta, type VI	*SERPINF1*
Spondylometaphyseal dysplasia, short limb-hand type	*DDR2*	Osteogenesis imperfecta, type X	*SERPINH1*
Short rib-polydactyly syndrome, type III/II digenic	*DYNC2H1*	Epiphyseal dysplasia, multiple, 4	*SLC26A2*
Ellis-van Creveld syndrome	*EVC*	Campomelic dysplasia	*SOX9*
Ellis-van Creveld syndrome	*EVC2*	Osteogenesis imperfecta, type XII	*SP7*
Spondyloepiphyseal dysplasia tarda	*TRAPPC2*	Hypochondroplasia	*FGFR3*
Short rib-polydactyly syndrome, type V	*WDR35*	Osteogenesis imperfecta, type XI	*FKBP10*
Spondylocarpotarsal synostosis syndrome	*FLNB*		

Identification of a potential disease-causing mutation

More than 800 SNVs and small InDels were identified in the 37 genes analyzed in each of the three samples (Table 2). After filtering the variants with frequency more than 5% in the 1000 Genomes Database (May 2011 release; http://www.1000genomes.org/node/506) and 2% in a BGI (Beijing Genomic Institute) in-house Database (composed of 1125 healthy control subjects) and non-functional relevance, there were approximately 86-114 non-synonymous variants (NS), splicing site (SS), or coding InDels remaining. Considering the disease inheritance pattern of genes in which these variants occur, the phenotypic overlap between the expected diseases and the proband, and the transmission pattern of the variants in the trio, the two heterozygous variants in the *RECQL4* gene (c.2059-1G>C and c.2141_2142 delAG) were left as the only potential disease-causing mutations (Table 3). Homozygous or compound heterozygous mutations in the *RECQL4* gene induce two short-limb-related syndromic autosomal recessive disorders, including Rothmund-Thomson Syndrome (RTS; OMIM: 268400) and BGS (OMIM: 218600). The c.2141_2142delAG mutation (Figure 1) transmitted from the mother induced a frameshift in exon 13 of the *RECQL4* gene (the gene includes 22 exons). In addition, the c.2059-1G>C mutation (Figure 2) transmitted from the father disrupted the acceptor splice site in intron 12 of the *RECQL4* gene. Neither of these mutations has been previously reported in the literature, and they are expected to be the cause of the disease in the proband.

Table 2. Summary statistics on target gene coverage.

| Sample	13S0122887 (proband)	13B0122885 (mother)	13B0122886 (father)
Sequence depth	271.07	190.89	282.96
>1X coverage rate	99.64%	99.52%	99.45%
>4X coverage rate	99.49%	99.37%	99.18%
>10X coverage rate	98.94%	99.04%	98.75%
>20X coverage rate	98.05%	98.5%	97.71%

Table 3. Summary statistics on variants by filtering.

| Filter	Proband 13S0122887	Mother 13B0122885	Father 13B0122886
All SNVs/InDels	5400	6152	6715
NS/SS/InDel	441	428	436
<5% variation in the 1000 Human Genomes Database, and <2% in the BGI in-house database	56	74	59
Related to short upper limbs disease	2	-	-

SNV = single nucleotide variants; InDel = insertion or deletion mutation; NS = non-synonymous variant; SS = splice site; BGI = Beijing Genomics Institute.

DISCUSSION

The *RECQL4* gene is a member of the human RecQ helicase family and plays a role in the initiation of replication. In addition, *RECQL4* is implicated in several DNA repair pathways (Fan and Luo, 2008; Singh et al., 2010; Thangavel et al., 2010; Croteau et al., 2012).
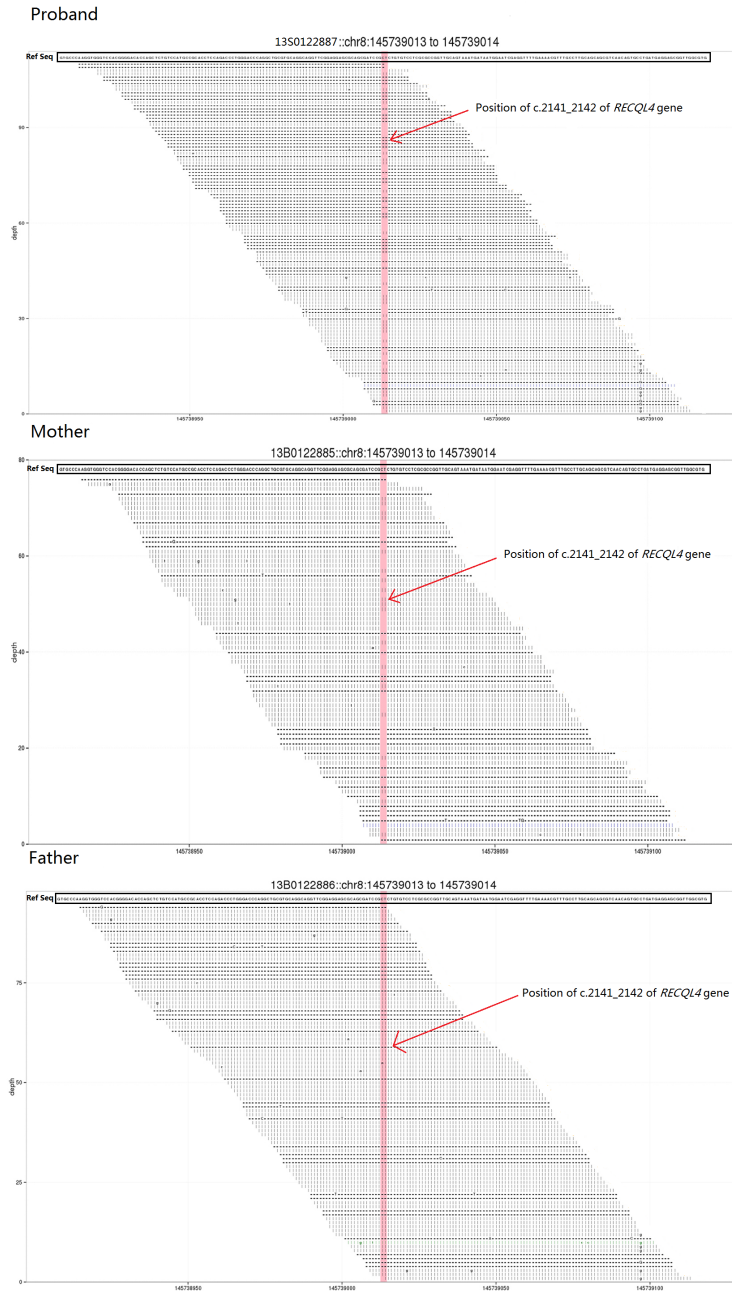
Figure 1. Sequencing reads in support of the c.2141_2142delAG mutation. The DNA sequence inside the top black box indicates reference sequence. The pink shaded box highlights the position of c.2141_2142 of *RECQL4* gene. For 13B0122886 (father), no read supports c.2141_2142delAG mutation. For 13S0122887 (proband) and 13B0122885 (mother), the support reads for c.2141_2142delAG is 45 and 44 respectively, which means this mutation is inherited from mother.
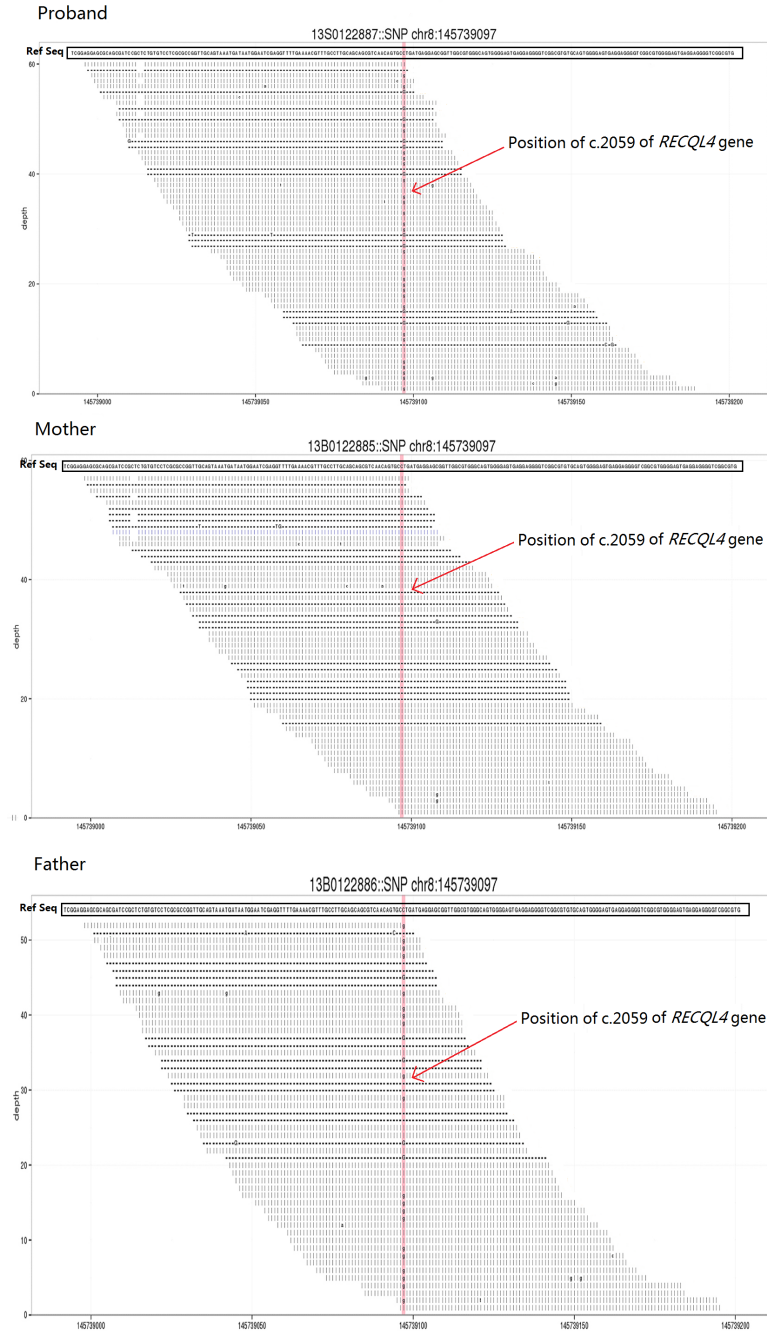
Figure 2. Sequencing reads in support of the 2059-1G>C mutation. The DNA sequence inside the top black box indicates reference sequence. The pink shaded box highlights the position of c.2059-1 of *RECQL4* gene. For 13B0122885 (mother), no read supports c.2059-1 G>C. For 13S0122887 (proband) and 13B0122886 (father), the support reads for c.2059-1 G>C is 37 and 27 respectively, which means this mutation is inherited from father.

It was originally identified in 1998 in a human genome search for RecQ helicases (Kitao et al., 1998) and some years later was found to be associated with the rare type II RTS (Kitao et al., 1999), RAPADILINO Syndrome (Siitonen et al., 2003), and BGS (Van Maldergem et al., 2006). RTS, RAPADILINO, and BGS exhibit a significant overlap of symptoms (Capp et al., 2010), all occurring in tissues with a high proliferation rate. RTS is characterized by poikiloderma; sparse hair, eyelashes, and/or eyebrows; small stature; skeletal and dental abnormalities; cataracts; and an increased risk for cancer, especially osteosarcoma. The skin is typically normal at birth; the typical rash of RTS develops between ages three and six months as erythema, swelling, and blistering on the face and subsequently spreads to the buttocks and extremities. The rash evolves over months to years into the chronic pattern of reticulated hypo- and hyperpigmentation, punctate atrophy, and telangiectases, collectively known as poikiloderma. Hyperkeratotic lesions occur in approximately one third of individuals. Skeletal abnormalities include dysplasia, absent or malformed bones (such as absent radii), osteopenia, and delayed bone formation. The population prevalence of RTS and the carrier frequency are unknown (Larizza et al., 2012). BGS is characterized by coronal craniosynostosis manifested as abnormal shape of the skull (brachycephaly) with ocular proptosis and a bulging forehead, radial ray defect manifested as oligodactyly (reduction in number of digits), aplasia or hypoplasia of the thumb, and/or aplasia or hypoplasia of the radius, growth retardation, and poikiloderma.

In the current study, we found that the 24-week fetus exhibited a bilateral radial agenesis by ultrasound examination. In addition, bilateral thumbs were not clearly shown in the ultrasound imaging and seemed to be absent. Because this woman was pregnant with a fetus presenting the same symptoms as another fetus encountered a year and half previously, we speculated that the defects were genetic in origin. Given that the karyotype analysis and aCGH levels were normal in the blood samples of this couple and the amniotic fluid sample from the fetus, there was a high possibility that that genetic defect observed in the fetus was a single-gene disorder. Taken together, we chose to perform further genetic testing using the Short-stature Panel, and performed gene sequencing in 448 genes associated with limb development. We identified two heterozygous mutations (c.2059-1G>C and c.2141_2142delAG) in the *RECQL4* (NM_004260) gene. These mutations have not been previously reported, and both were predicted to be severe loss-of-function mutations. The c.2059-1G>C mutation is located in the acceptor splice site of intron 12 and is assumed to disrupt the splice site. The c.2141_2142delAG mutation is a frameshift mutation in exon 13 that leads to a premature stop at codon 807. Several nonsense mutations [p.Gln810*, p.Gln821*, p.Arg826*, p.Gln888*, and p.Gln1175* (Wang et al., 2003; Jin et al., 2008; Siitonen et al., 2009)], which occurred downstream of codon 807, have been previously reported to be the cause of RTS. The c.2059-1G>C and c.2141_2142delAG mutations have not been observed in at least 6000 controls (1000 Genomes Project and BGI in-house database). RTS and BGS are two short-limb-related syndromes and autosomal recessive disorders. We detected the c.2059-1G>C mutation in the blood sample of the husband, and the c.2141_2142delAG mutation was detected in that of the mother; therefore, these two mutations were found to be compound heterozygous in the proband. On this basis, these mutations (c.2059-1G>C and c.2141_2142delAG) in the *RECQL4* gene are assumed to be the disease-causing mutations in this patient.

The pregnancy was terminated after obtaining written informed consent from the patient. The fetus aborted was found to have bilateral short upper forearms and bilateral absence of thumbs. The fetus had an identical presentation to the BGS patient previously reported (Savarirayan et al., 1998; Markose et al., 1999; Lype et al., 2008). Therefore, we believe this

fetus had BGS. The use of the short-stature panel for genetic testing covered all the mutations, including point mutations and insertions in the exons and ± 10-bp neighboring introns in short-stature-associated genes. This new method of testing genes associated with similar symptoms and/or presentations in genetic disorders greatly improves the efficiency of genetic diagnosis. The identification of abnormal genes in genetic diseases can greatly aid prenatal genetic diagnosis and intervention, thus allowing the possibility of ensuring that patients are delivered of a healthy child.

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