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Case reports

A Cleidocranial Dysplasia Case with a Novel Mutation and Growth Velocity Gain with Growth Hormone Treatment

Emine Çamtosun,MD[1], Ayşehan Akıncı,MD[1], Emine Demiral, MD[2], İbrahim Tekedereli,MD[2], Ahmet Sığırcı,MD[3]
[1] İnonu University Faculty of Medicine, Department of Pediatric Endocrinology, Malatya, Turkey
[2] İnonu University Faculty of Medicine, Department of Medical Biology and Genetics, Malatya, Turkey
[3] İnonu University Faculty of Medicine, Department of Radiology, Malatya, Turkey

Short Title: Cleidocranial Dysplasia Case, Novel Mutation

ABSTRACT

Cleidocranial dysplasia (CCD) is a rare congenital autosomal dominant skeletal disorder that is characterized by hypoplasia or aplasia of clavicles, failure of cranial suture closure, dental anomalies, short stature and other changes in skeletal patterning and growth. The gene responsible for pathogenesis has been mapped on the short arm of chromosome 6p21, Core Binding Factor Alpha-1 (*CBFA1*) or Runt Related Transcription Factor 2 (*RUNX2).* Here we describe a CCD patient with a novel mutation in the *RUNX2* gene. An eight-and-a-half years old girl presented with severe short stature, dysmorphic facial appearance (hypertelorism, prominent forehead, high palate, midfacial hypoplasia), macrocephaly, large anterior fontanel, increased anteroposterior chest diameter when she was five-and-a-half years old. Her shoulders were close to each other and her bilateral clavicles seemed too short on physical examination. Bilateral hypoplastic clavicles, coxa valga, hypoplasia of iliac bones wide symphysis pubis and phalangeal dysplastic features were detected on her skeletal x-ray examination. She was diagnosed as having CCD. Molecular analysis detected a novel heterozygous mutation 'NM_001024630.3 p. T155P(c.463A>C)' in the *RUNX2* gene. Because of her severe short stature growth hormone (GH) treatment was started and she responded well to this one-year therapy with no adverse effects. In conclusion hypoplasia or aplasia of the clavicles, failure of cranial suture closure, dental anomalies, and short stature should bring CCD to mind. We present a novel mutation in the *RUNX2* gene for CCD. We obtained growth velocity gain with GH treatment in our patient.

Key Words: Cleidocranial dysplasia, *RUNX2*, severe short stature

Corresponding Author: Emine Çamtosun, Assistant Professor, MD, Department of Pediatric Endocrinology, İnonu University Faculty of Medicine, Malatya, Turkey.
Tel: +90 422 3410660 (5377)
E-mail: epurcuklu@gmail.com
0000-0002-8144-4409

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What is already known about this topic?
Classical Cleidocranial dysplasia is characterised by hypoplasia or aplasia of clavicles, failure of cranial suture closure and dental anomalies. Short stature is a frequent feature of the syndrome. Nearly two hundred mutations reported associated with the CCD.

What this study adds?
We present a likely novel mutation for CCD in a patient. Although data about Growth hormone therapy for CCD with severe short stature is very limited, we obtained growth velocity gain with growth hormone treatment.

INTRODUCTION:
Cleidocranial dysplasia (OMIM:119600) is a skeletal dysplasia characterized by hypoplasia or aplasia of the clavicles permitting abnormal facility in apposing the shoulders, persistently open skull sutures with bulging calvaria and dental anomalies (delayed exfoliation of primary teeth, delayed eruption of permanent teeth, multiple impacted supernumerary teeth). Short stature, generalized bone dysplasia, vertebral malformations, a depressed nasal bridge,and a wide pubic symphysis can also be seen (1). The estimated prevalence of CCD is one per million births (most likely underdiagnosed), and there is no sex predilection (2)
CCD is caused by heterozygous loss-of-function mutation in the *RUNX2* gene, encoding transcription factor CBFA1 on chromosome 6p21 (1,3). Human *RUNX2* (*CBFA1*) gen, consists of eight exons and it controls differentiation of precursor cells into osteoblasts and is essential for membranous and endochondral bone formation (3,4). It is a master regulatory gene for skeletal development and morphogenesis. The majority of *RUNX2* mutations in classic CCD patients are missense or nonsense mutations. Frame shift and exon skipping mutations (4), insertions, deletions were also described (3). The disease is commonly autosomal dominantly inherited but can be sporadic.
Here we present a CCD patient with significant short stature with typical characteristics of CCD and a novel *RUNX2* mutation. She had GH therapy with growth velocity gain .

CASE:
A five-and-a-half years old girl was admitted to our hospital due to her short stature and dysmorphic features. Her anthropometric measures and SD scores according to Turkish curves (5) were as follows: height: 94.3 cm (-3.69SD), weight: 13.7 kg (-2.45 SD), body mass index: 15.4 (-0.05 SD), head circumference: 52 cm (0.77 SD); upper/lower segment ratio: 1.25 (> +2 SD); and mid parenteral target height: 161.15 (-0.31 SD). Parents had no history of CDPG. She had a dysmorphic face with hypertelorism, a prominent forehead, high palate, midfacial hypoplasia, down-slanting palpebral fissures as well as macrocephaly, large anterior fontanel, increased anteroposterior chest diameter, and laxity in her distal joints and pes planus. Her shoulders were close to each other and her bilateral clavicles seemed too short on physical examination (Figure 1). Exfoliation of her primary teeth was delayed. She had normal developmental milestones and intelligence, except for a mild speech delay. Her neurological examination was normal.
Her bone age was 3-3.5 years according to the Greulich and Pyle atlas. Bilateral hypoplastic clavicles, wide and open anterior fontanel, coxa valga, hypoplasia of iliac bones, wide symphysis pubis were detected on her skeletal x-ray examination (Figure 2,3). Her hand x-ray examination revealed cone shaped epiphysis, a pseudo-epiphysis of second metacarpal, tapering of distal phalanges, severe dysplasia of middle phalanx of fifth finger and a wide phalangeal epiphysis. These findings were compatible with the diagnosis of CCD. She had no scoliosis.

In laboratory studies her blood count, biochemical tests, thyroid function tests, urine examinations were normal. Tissue transglutaminase immunoglobulins were negative. Her IGF1 and IGFBP3 levels were 74 ng/ml (-1.15 SD), 2860 ng/ml (-0.12SD) respectively. Her peak growth hormone level was 13.4 ng/ml (non-deficient) with L-DOPA stimulation test. Karyotype analysis revealed 46, XX. After genetic consultation, a next generation sequencing detected a novel heterozygous mutation 'NM_001024630.3 p. T155P (c.463A>C)' in the *RUNX2* gene (Figure 4). *RUNX2* gene sequence analysis was performed by using MiSeq next generation sequencing (NGS) platform, a FDA approved diagnostic system (Illumina, San Diego, CA, USA). Genomic DNA was extracted according to the manufacturer's standard procedure using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany). All coding exonsof the *RUNX2* gene and their flanking splice site junctions were amplified using PCR primers, designed with PRİMER©-Primer Designer v.2.0 (Scientific and Educational Software programme) software. PCRs were validated by using agarose gel electrophoresis. After PCR amplification, the libraries were prepared with the NexteraXT kit (Illumina Inc.), according to the manufacturer's instructions. Next-gene sequencing was carried on MiSeq (Illumina Inc.). Sequences were aligned to the hg19 genome within MiSeq Reporter software (Illumina Inc.) Visualisation of the data was performed with IGV 2.3 (Broad Institute) software."

This mutation was not reported before and it is highly likely to be the cause of the disease according to PolyPhen-2 (score: 1.00, sensitivity: 0.00, specificity: 1.00) (http://genetics.bwh.harvard.edu/pph2), SIFT (score:0,0001 converted rank score:0,912), Provean (score:-5,46 -5,53 converted rankscore:0,86) and Mutation Taster (score: 0,99) software records. Her mother's genotype was normal for this mutation. We couldn't perform her father's genetic analysis.

At age seven years and two months old, her anthropometric measures were as follows, height: 104.1 cm, height SDS: -3.8 SD, body weight: 17.1 kg (-2.3 SD); and upper/lower segment ratio: 1.28. Her bone age was five years. Because of her severe short stature, an IGF generation test was performed with 0.1 mg/kg/day growth hormone for four days, it revealed a % 200 increase in IGF1. We started 30 mcg /kg/ day subcutaneous growth hormone (GH) therapy. After one year of treatment (at eight years and three months old) her growth velocity was 8.2 cm/year while it is 5.28 cm/year before treatment. Height SDS was increased to -3.15 SD. She was still prepubertal and her bone age was 6.5-7 years old. Her IGF1 level was 123 ng/ml (-0.03 SD), IGFBP3 level was 5460 ng/ml (1.08 SD) after GH treatment. We followed her every three months and observed no side effects associated with GH treatment. She has also been followed by orthopedist for pes planus and she has been followed by a pediatric dentist for delayed exfoliation of primary teeth. She continues to receive growth hormone therapy. After 21 months of GH therapy she was nine years old. She was prepubertal and her anthropometric measures were as follows height: 119.2 cm (-2,28 SD), weight: 22,6 kg (-1,48), VKI: 15,9 (-0,26 SD), upper/lower segment ratio: 1.16 (> +2SD) (+2SD=1.08), and her arm span: 115 cm. Her body proportions were not worsened.

A written informed consent was obtained from the patient's family regarding the scientific publication of the patient's photographs, medical informations and imagines.

DISCUSSION:

Cleidocranial dysplasia is generally diagnosed with clinical features and the diagnosis is supported by radiography. Genetic analysis reveals a *RUNX2* mutation in almost 70 % of patients. Our patient was diagnosed with classical phenotypic and radiological features.

Short stature can be a feature of CCD because of generalized bone dysplasia. Reports about gender difference and severity of short stature are controversial (2, 6, 7). Short stature is usually mildly disproportionate but it can be proportionate (2,7). Studies which include

younger CCD patients indicate birth lengths are normal, but heights drop below -2 SD at the age of 4-8 years (6).

Jensen BL. investigated somatic development in 17 CCD patients from Denmark aged 5-46 years. Stature was documented for 6 males and 8 females, and compared with Danish curves. The report noted growth retardation, especially in females. Heights of CCD males were clustered between the 5th and 50th percentiles, but all CCD females' heights were below 5th percentile (between -1.81SD and -3.56 SD). Because of smaller number of patients, having no data about parental heights and noting that four females belonged to same family they concluded that the observed severity of short stature in females may have occurred by chance. Also, the females had smaller head circumference than the boys (respectively, -0.77 SD, +0.27 SD) (6). Our female patient had a significant short stature and her height SDS was significantly lower than her mid-parental height SDS. Her head circumference was relatively macrocephalic (head circumference SD: +0.77 SD).

In the study of Cooper SC et al., (2) 21 female and 21 male CCD patients aged >18 years were evaluated for height. The authors observed that their patients had shorter statures than their healthy relatives. The mean height percentiles of girls and boys were 10 p (38 % were < 5p) and < 5p (62% were < 5p), respectively. Unlike Jensen BL's report, short stature in this study was more prominent in males, and severe short stature was not observed among the cases. Most prevalent skeletal deformities of the cases were genu valga and pes planus.

Dinçsoy Bir F et al., reported 15 CCD cases in 11 independent families. Short stature was seen in three males (height SDS were -4.2, -2.9, -2.24 SD) and a female (height SDS was -2.55), and proportionated for all of them (7). Three of these short patients had low IGF1 levels (<-2 SD). The female patient showed partial GH deficiency in her GH stimulation tests, and had normal hypophyseal MRG. She had not received GH therapy yet. The male patient had -4.2 height SDS, no low IGF1 and no GH deficiency. He didn't respond to one-year GH therapy at the age of 15 years, but his bone age had not been reported in the study.

Different frequency and severity of short stature in CCD patients can be explained with various effects of the known mutations. There are studies investigating genotype-phenotype correlations for *RUNX2* mutations (4,8). Yoshida T et al., studied genotype-phenotype correlations in 17 Japanese CCD patients, and they reported that *RUNX2* mutations which affect the Runt domain (responsible for binding to DNA) are correlated with short stature and its severity. They showed that patients had normal stature when they had mutations with an intact Runt domain (8).

The mutation detected in our patient ('NM_001024630.3 p. T155P (c.463A>C)') was a missense mutation leading to a change in 155th amino acid in Exon 4 and located within the Runt domain. It was likely pathogenic in *in silico* analysis. This situation can explain our patient's severe short stature.

Yoshida T et al., found that short stature and number of supernumerary teeth were correlated significantly. Different studies showed, mutations which affect the Runt domain of the *RUNX2* gene, which causes classical CCD phenotype and severe dental anomalies (4). Genotype-phenotype correlation studies also showed mutations of the *RUNX2* gene could lead to various phenotypic features even in the same family (4).

Data on GH treatment for CCD patients is very limited. One patient with CCD who was treated with GH for one year didn't benefit in the study of Dinçsoy Bir F et al. But the patient was 15 years old and his bone age data was absent. Our patient's height was increased 8.2 cm/year (prepubertal), which was 3 cm/years more than before GH treatment. Her height SDS increased 0.65 SD/year. Starting GH at an early age could have resulted in a better outcome for this patient. However, in terms of efficacy and safety of GH therapy, there is a need for randomized controlled trials involving more patients.

It is suggested that there can be increased bone fragility in CCD patients. Cooper SC et al., reported two patients with multiple bone fractures, but they found similar fracture and osteoporosis rates between 90 CCD patients and control group (2). Dinçsoy Bir F et al., reported more than 50% of their patients have osteoporosis; they also reported no relationship between osteoporosis in their patients with vitamin D deficiency (7). Our patient had no bone fracture, and her radiography of her vertebrates showed neither vertebral fractures nor scoliosis.

In conclusion a patient who presented with severe short stature, failure of cranial suture closure and hypoplasia of clavicles was diagnosed as having CCD. A novel mutation in the *RUNX2* gene for CCD was detected. We obtained growth velocity gain with GH treatment for severe short stature, with no side effects. Randomized controlled trials are necessary, however for evaluating effectiveness and safety of GH therapy for this population.

REFERENCES

1) Cleidocranial dysplasia, CCD. https://www.omim.org/entry/119600

2) Cooper SC, Flaitz CM, Johnston DA, Lee B, Hecht JT. A natural history of cleidocranial dysplasia. Am J Med Genet. 2001; 104(1):1-6.

3) Mundlos S. Cleidocranial dysplasia: clinical and molecular genetics. J Med Gen.1999; 36: 177-182.

4) Jaruga A, Hordyjewska E, Kandzierski G, Tylzanowski P. Cleidocranial dysplasia and RUNX2-clinical phenotype-genotype correlation. Clin Genet. 2016; 90(5):393-402.

5) Neyzi O, Bundak R, Gökçay G, Günöz H, Furman A, Darendeliler F, Baş F. Reference values for weight, heihgt, head circumference and body mass index in Turkish children. J Clin Res Pediatr Endocrinol. 2015; 7(4):280-293.

6) Jensen BL. Somatic Development in Cleidocranial Dysplasia. Am J Med Genet. 1990; 35: 69-74.

7) Dinçsoy Bir F, Dinçkan N, Güven Y, Baş F, Altunoğlu U, Kuvvetli SS, Poyrazoğlu Ş, Toksoy G, Kayserili H, Uyguner ZO. Cleidocranial dysplasia: Clinical, endocrinologic and molecular findings in 15 patients from 11 families. Eur J Med Genet. 2017; 60(3):163-168.

8) Yoshida T, Kanegane H, Osato M, Yanagida M, Miyawaki T, Ito Y, Shigesada K. Functional analysis of RUNX2 mutations in Japanese patients with cleidocranial dysplasia demonstrates novel genotype-phenotype correlations. Am J Hum Genet. 2002; 71(4):724-738.

Figure 1: Hypoplasia of the clavicles permitting abnormal facility in apposing the shoulders

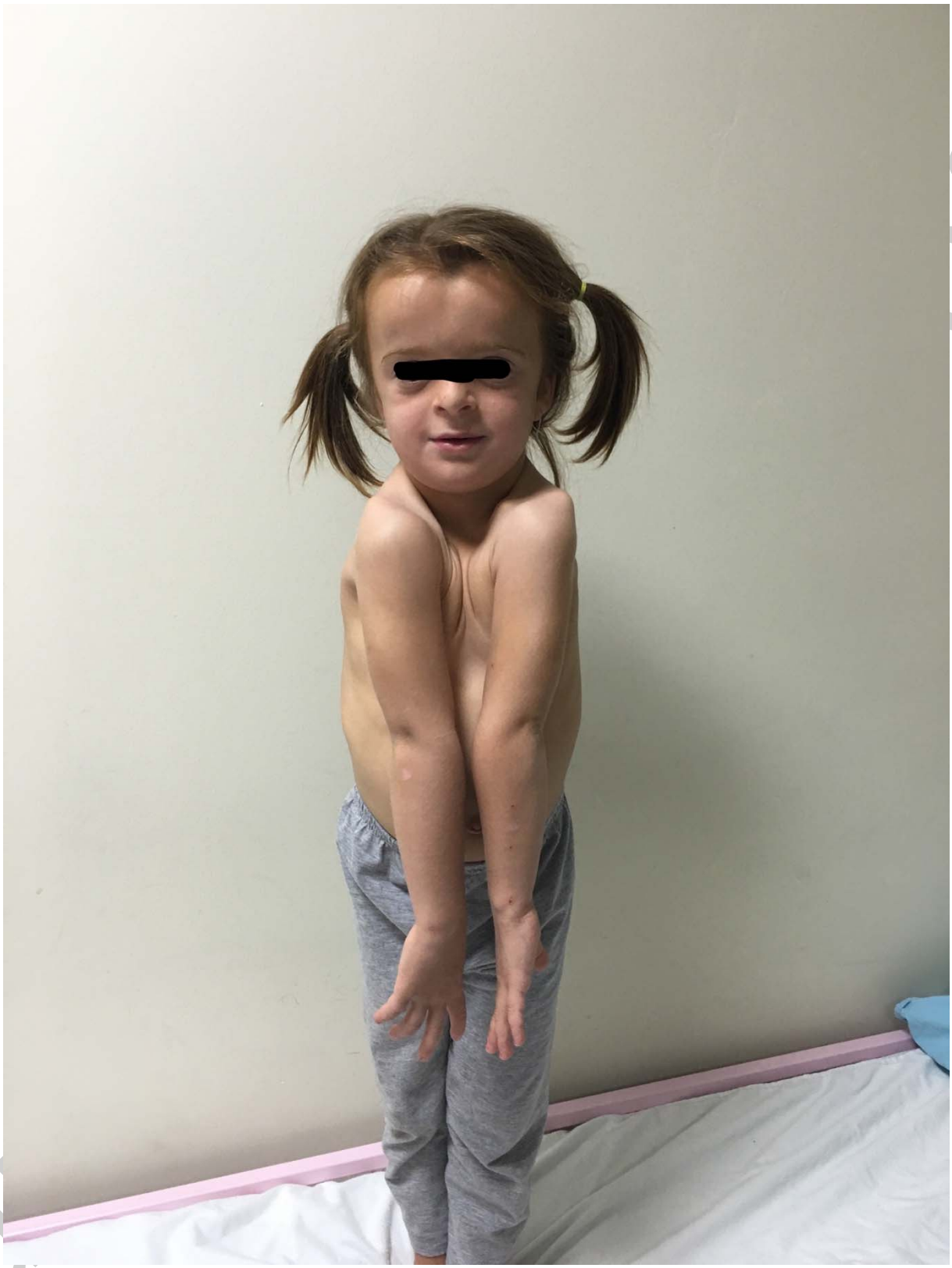

Figure 2: Skull x-ray of patient shows wide and open anterior fontanel.

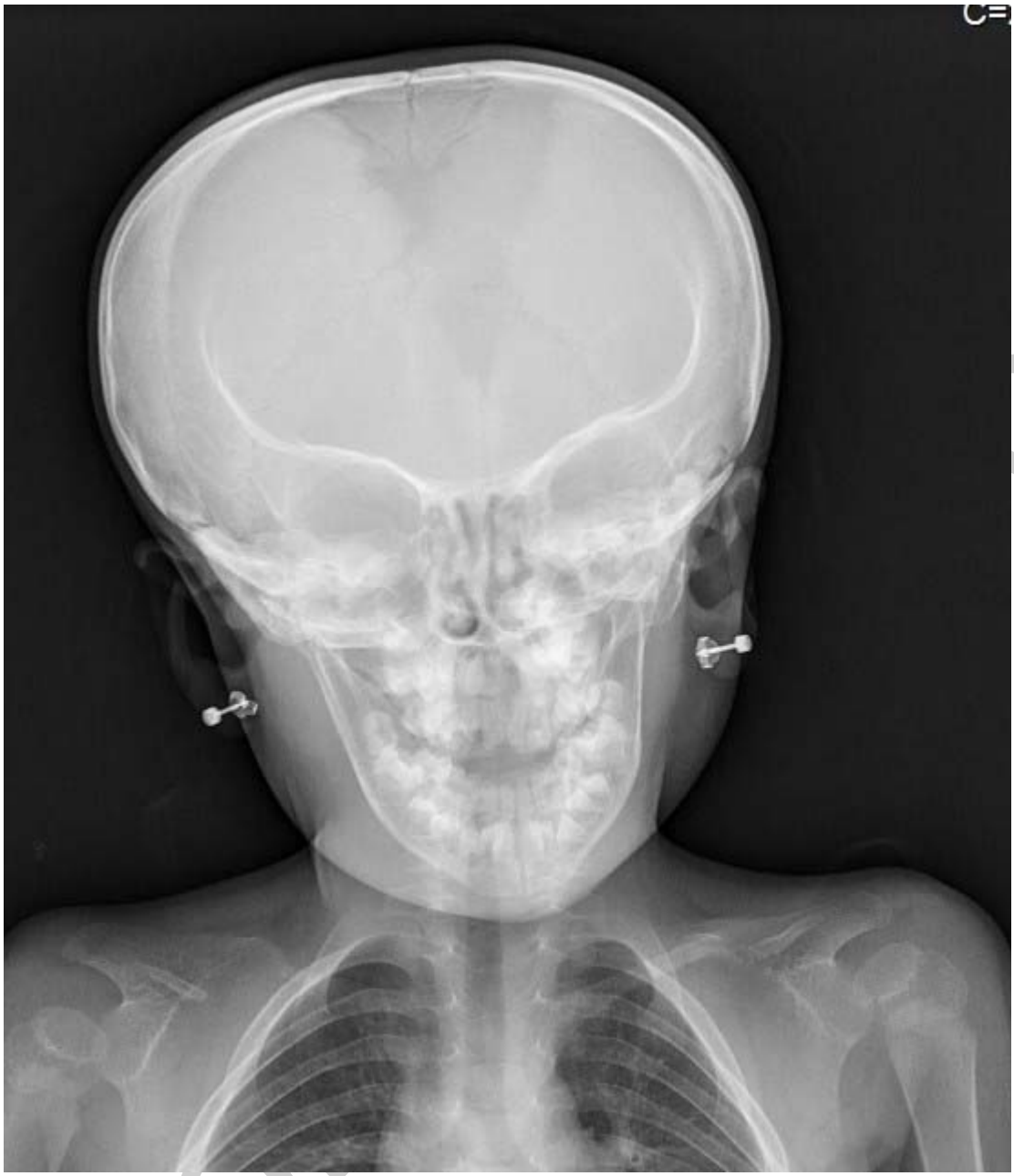

Figure 3: X-ray of her trunk shows bilateral hypoplastic clavicles, hypoplasia of iliac bones, wide symphysis pubis.

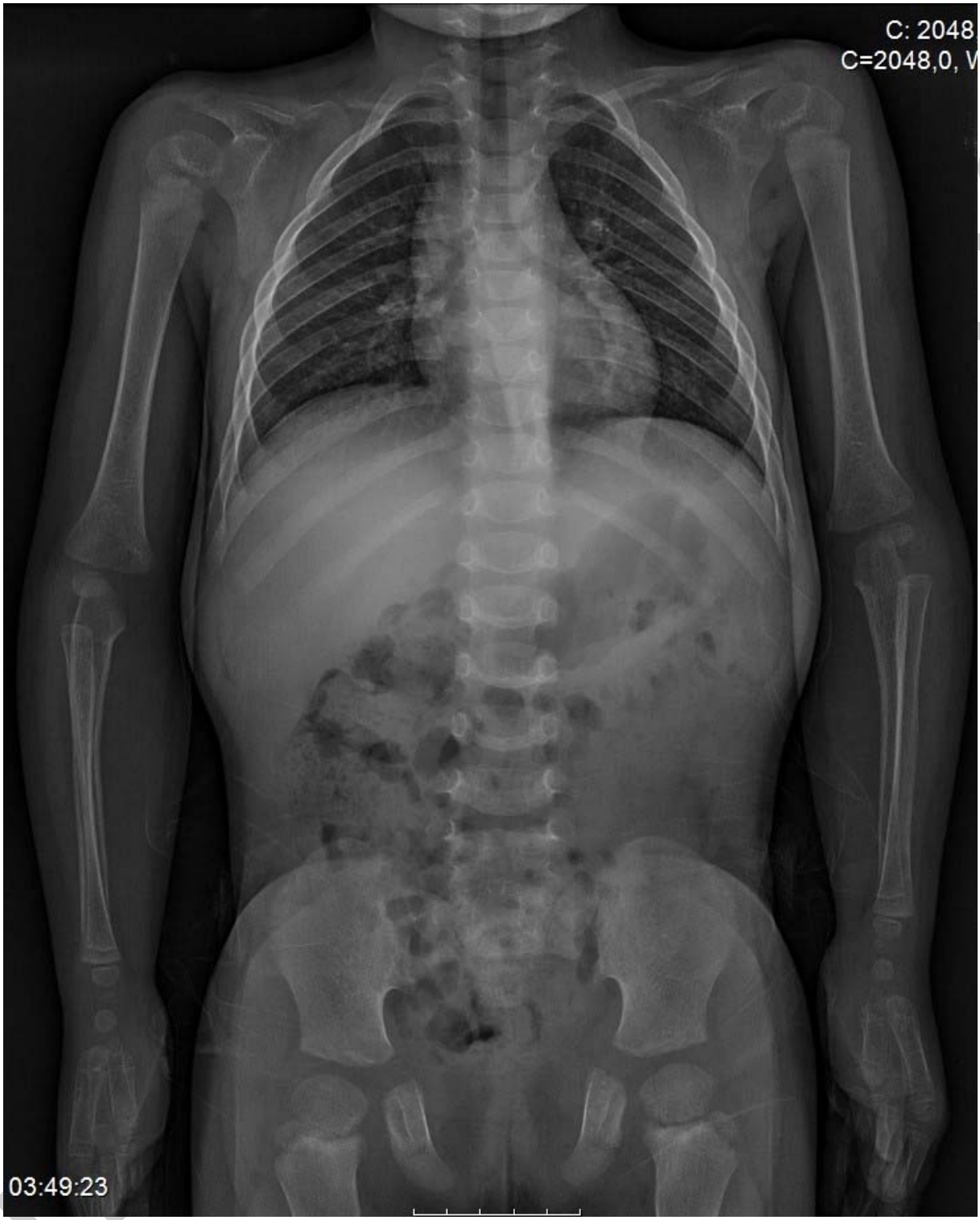

Figure 4: MISEQ sequence image of the mutation in *RUNX2* gene.