Gene 577 (2016) 289–292

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Short communication

Clinical, genetics and bioinformatics characterization of a campomelic dysplasia case report

Nerea Carvajal [a,b,1], Mónica Martínez-García [a,c,1], Monica Chagoyen [d], Nieves Morcillo [e], Ana Pino [b], I. Lorda [a], María José Trujillo-Tiebas [a,c,*]

[a] Genetics Department, Health Research Institute, Fundación Jiménez Díaz, UAM (IIS-FJD), UAM, Madrid, Spain
[b] Department of Pathological Anatomy, Fundación Jiménez Díaz, Madrid, Spain
[c] Center of Biomedical Researches of Rare Diseases (CIBER), ISCIII, Madrid, Spain
[d] Bioinformatics Department, National Center of Biotechnology (CNB-CSIC), Madrid, Spain
[e] Gynecological Department, Santa Cristina Hospital, Albacete, Spain

ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form 20 September 2015
Accepted 25 November 2015
Available online 27 November 2015

Keywords:
Campomelic dysplasia syndrome
SOX9 gene
Clinico-pathological approach
Frameshift

ABSTRACT

Campomelic dysplasia is a rare disorder characterized by skeletal and extraskeletal defects. Up to two-thirds of affected XY individuals have a gradation of genital defects or may develop as phenotypic females. This syndrome is caused by alterations in SRY-related HMG-Box Gene 9 (SOX9), a transcription factor essential in both chondrocyte differentiation and sex determination.

We report a 27-week fetus with ambiguous genitalia and upper and lower extremities bone malformations. Gross photographs, radiologic and pathological studies led the clinical diagnosis to campomelic dysplasia. A new frameshift mutation (p.Pro415Serfs*163) was identified in the *SOX9* gene by genetic analysis. This mutation not only alters almost the entire sequence of the C-terminal transactivation (TA) domain of *SOX9*, but also enlarges it. This altered sequence does not resemble any other existing sequence. Since TA domain is entirely affected, SOX9 could not establish its normal function. The comparison between p.Pro415Serfs*163 and other frameshift mutations that enlarges SOX9 showed the same nucleotides added. This new sequence is not conserved either. We speculate that the fact of adding a sequence downstream of the C-terminal domain alters SOX9 and leads to campomelic dysplasia. The clinical information is essential not only to achieve a correct diagnosis in fetuses with pathologic ultrasound findings, but also to offer a proper genetic counseling.

1. Introduction

Campomelic dysplasia (CD) (OMIM: 608160) is a very rare autosomal dominant disorder characterized by a variable association of skeletal and extraeskeletal abnormalities: bowed and fragile long bones, pelvis and chest abnormalities, eleven rib pairs instead of the usual twelve, facial dysmorphology, cleft palate, sexual ambiguity and heart, brain and kidney malformations.

Prevalence at birth ranges from 1/40,000 to 1/80,000 and the majority of CD patients die neonatally due to respiratory distress (Unger et al., 2013). The diagnosis of CD is usually based on clinical and radiographic findings. Although no single clinical feature is obligatory, the radiographic features are consistent and are the most reliable diagnostic clues (Unger et al., 2013). Radiological findings such as bowing of the femora and tibiae, hypoplasia of the scapulae, widely spaced vertical ischia and hypoplastic pubes, and cervical vertebrae abnormalities, definitely diagnosis CD in a majority of affected individuals.

However, genetic testing of CD is essential in order to establish a genotype–phenotype correlation, determine the prognosis of the disease and offer a proper genetic counseling. In addition, not all the patients show the full skeletal manifestation, and might present a few skeletal changes only (f.i. brachydactyly or Pierre Robin sequence).

The only gene in which mutations are known to cause CD is *SOX9* (Sex-Determining Region Y-Box 9, localized to 17q24.3). Anomalies in this gene can occur either as a result of chromosomal recombination involving this locus or due to heterozygous *de novo* mutation with recurrence risk of a few percent (estimate ~5%) due to germ line mosaicism in one of the parents (Scherer et al., 2013).

Molecular testing is also important as affected individuals with aberration of the regulatory domain of SOX9 and chromosome rearrangement tend to have better clinical outcomes. Mutation screening consists in sequencing of the three *SOX9* exons, which allows for the detection of ~90% of mutations in CD cases. The remaining cases need to be further screened for large deletions by quantitative PCR, MLPA or

Abbreviations: AMH, anti-Müllerian hormone; CD, campomelic dysplasia; DIM, dimerization domain; HMG, the DNA-binding/bending high mobility group domain; HGMD, Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff; K2, central transactivation domain; PQA, glutamine and alanine-rich domain; SOX9, HMG-Box Gene 9; TA, C-terminal transactivation domain.

* Corresponding author at: Avenida Reyes Católicos 2, CP: 28040 Madrid, Spain.
E-mail address: mjtrujillo@fjd.es (M.J. Trujillo-Tiebas).

[1] These two authors contribute equally to this work.

http://dx.doi.org/10.1016/j.gene.2015.11.039
0378-1119/

array CGH, and by cytogenetic analyses to detect translocations or larger inversions, present in 5% of these cases (Scherer et al., 2013).

SOX9 presents at least five conserved domains including the dimerization domain (DIM); the DNA-binding/bending high mobility group domain (HMG); central transactivation domain (K2); the proline, glutamine and alanine-rich domain (PQA); and C-terminal transactivation domain (TA).

To date, 107 mutations within the *SOX9* gene have been published in the Human Gene Mutation Database at the Institute of Medical Genetics in Cardiff (HGMD). All known missense point mutations in *SOX9* occur in the HMG domain. With one exception, all *SOX9* nonsense and frame-shift mutations described so far in CD/sex reversal patients lead to truncation of the trans-activation domain. The majority of CD patients present heterozygous de novo mutations mainly located in HMG domain in *SOX9* (Meyer et al., 1997; McDowall et al., 1999).

CD has been shown to be caused by haploinsufficiency of SOX9. This gene is a transcription factor essential in both chondrocyte differentiation and sex determination.

Transient expression of the Y chromosome gene SRY initiates a cascade of gene interactions orchestrated by SOX9, leading to the formation of testes from bipotential gonads (Cox et al., 2011). It acts during chondrocyte differentiation and, with steroidogenic factor 1, regulates transcription of the anti-Müllerian hormone (AMH). Altered SOX9 protein inhibits the activation of COL2A1 leading to premature bone mineralization (Bi et al., 2001).

We report a 27-week fetus with ambiguous genitalia and upper and lower extremities bone malformations. The final diagnosis of campomelic dysplasia was suggested by the radiologic findings, and was confirmed by sequencing analysis of *SOX9* gene.

2. Case report

A female fetus of 27 weeks of gestation was obtained by means of therapeutic abortion due to multiples anomalies from a 32 year-old primigravida. Routine ultrasound scan showed polyhydramnios, protrusion of the parietal bones, general and predominantly mesoacromelic shortness of long bones, dorsal hemivertebrae, hyperextended feet, bilateral hydronephrosis with pielic dilatation and flattened nose.

No known toxic, medical exposures or unusual events were reported during the gestation. There was no history of birth defects, or increased miscarriage rate in relatives.

Photographs and radiographs were taken immediately after delivery. Fetal tissue was collected fresh in order to perform cytogenetic and molecular assays. Since the tissue culture failed, the karyotype could not be done. After dissection and chemical digestion, genomic DNA was isolated from fetal tissue for molecular techniques. As ambiguous genitalia were present in the fetus, MLPA P185-C1 intersex was also performed. Neither deletions nor duplications were detected in any of the sex-determining genes analyzed. Since SRY-probe was not amplified, the results were compatible with female fetal sex. All the clinical information obtained from photographs, radiographs and anatomic pathology reports was gathered in order to get a clinical suspicion of a concise pathology.

Gross examination revealed midface hypoplasia, broad nasal root, retro/micrognathia, hypertelorism, upper and lower limbs malformations with mesomelic predominance affecting radius/ulna and tibia/fibula ratios.

Bone parameters were consistent with a gestational age of 27 weeks. The radiographs showed: lumbar hiperlordosis, vertebral size decrease, small and flattened ribcage, vascular congestion, hyperextension of tibia-tarsus joints, short and blunt metacarpals, viscera generalized immaturity, and acral malformations in both feet and hands. (See Fig. 1).

Taking into account all these features, the final clinical suspicious was campomelic dysplasia. Therefore, the 3 coding exons of *SOX9* were amplified and sequenced using primers published elsewhere (Kwok et al., 1995) and were electrophoresed by ABI PRISM® 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA).

A non-previously described mutation c.1242_1243insT (RefSeq NM_000346.3) was identified in the fetal sample. It consists of a Timine insertion affecting the 415 codon, located in the C-terminal transactivation domain. This frameshift mutation (p. Pro415Ser*fs**163) displaces the wild type stop codon 163 amino acids downstream. Mutant DNA sequences were translated to protein sequences using EMBOSS SixPack (Rice et al., 2000), and aligned with JalView (Waterhouse et al., 2009). Disordered regions were assessed with IUPred (Dosztányi et al., 2005). Search of similar protein sequences was performed with BLAST (Altschul et al., 1990).

3. Discussion

Skeletal dysplasias encompass a large number of disorders and its clinical features overlap among them. Therefore, establishing a specific diagnosis only based on prenatal ultrasound examination is considered a challenge. Minor anomalies are not always detected by ultrasound scans. Given that a specific diagnosis is crucial to provide an appropriate genetic counseling, a detailed necropsy and a radiologic study might lead to a more concise clinical suspicion and can direct molecular testing. Mutation detection rate is higher when molecular assays are performed after pathological and radiological confirmation (Gentilin et al., 2010).

The skeletal features are the most prominent macroscopic characteristics of CD and can be associated with extraeskeletal anomalies. Thus, the cardinal signs of CD are: macrocephaly, flat small face, high forehead, hypertelorism, short palpebral fissures, low-set ears, depressed nasal root, cleft palate, retromicrognathia, congenital heart defects, tracheobronchomalacia, respiratory distress, small thoracic cage, hypoplastic scapulae, slender ribs, sex reversal, hydronephrosis, hypoplastic vertebrae, bowed femur and tibia, polyhydramnios and short phalanges both hand and feet.

XY sex reversal or CD severity are not predicted by the type or position of mutation within *SOX9*. Missense and nonsense mutations in *SOX9* are found in patients with and without XY sex reversal, indicating that sex reversal in CD is subject to variable penetrance (Meyer et al., 1997).

SOX9 protein is highly conserved through-out the vertebrates and differences in this sequence are poorly tolerated (Marshall and Harley, 2000). The frameshift mutation identified in our patient generates a

Fig. 1. Radiograph showing shortening and mild bowing of femora and humeri, poorly developed iliac bones and polydactyly.