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Original Article

Skeletal dysplasia with bowing long bones: Proposed flowchart for prenatal diagnosis with case demonstration

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ABSTRACT

Objective: Skeletal dysplasia with bowing long bones is a rare group of multiple characterized congenital anomalies.
Materials and Methods: We introduce a simple, practical diagnostic flowchart that may be helpful in identifying the appropriate pathway of obstetrical management.
Results: Herein, we describe four fetal cases of bent bony dysplasia that focus on ultrasound findings, phenotype, molecular tests, distinctive X-ray features, and chondral growth plate histology. The first case was a typical campomelic dysplasia resulting from a de novo mutation in the SOX9 gene. The second fetus was affected by osteogenesis imperfecta Type II carrying a mutation in the COLA1 gene. The third case was a rare presentation of campomelic dysplasia, Cumming type, in which SOX9 examination was normal. Subsequently, a femoral hypoplasia unusual facies syndrome is also discussed.
Conclusion: Targeted molecular tests and genetic counseling are required for supplementing ultrasound imaging in order to diagnose the correct skeletal disorders.

Introduction

Bowing long bones represents a rare, heterogeneous finding of potentially lethal skeletal dysplasia that mainly involves the lower limbs. Early ultrasound (US) detection is a clinical challenge for effecting differential diagnosis, genetic counseling and appropriate molecular tests. Four pathologic fetuses with bowing of long bones are described. All underwent termination of pregnancy and post mortem examination.

* Corresponding author. Prenatal Diagnostic Service, Department of Obstetrics and Gynecology, AUSL (Azienda Unità Sanitaria Locale) Reggio Emilia, Via Amendola 1, Reggio Emilia, Italy.
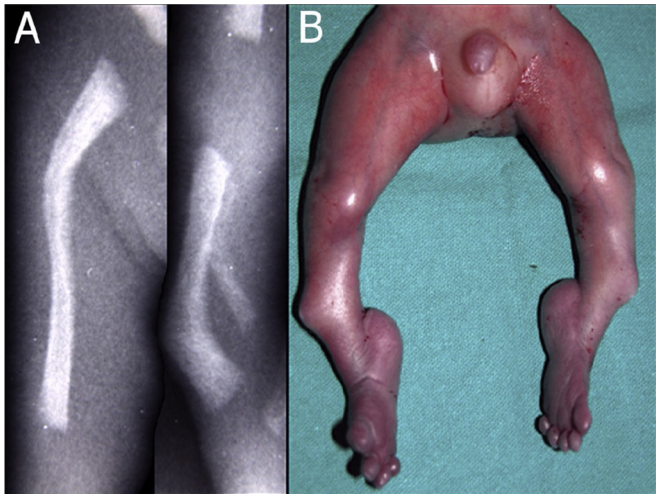E-mail address: tonnig@ausl.re.it (G. Tonni).

Case reports

Case 1. Campomelic dysplasia

A 34-year-old G1P0 was referred at 21^{+1} weeks for small thorax. The femur and the tibia measured 28.9 mm (3.9^{th} centile for gestational age) and 18.8 mm ($< 2.5^{th}$ centile for gestational age) in length, and were bowed with maximum convexity at the level of the medial portion of the diaphysis (Figure 1). The fibula was severely shortened (11 mm). A diagnosis of campomelic dysplasia was presumed. Genomic DNA on SOX9 exons 1–3 documented a de novo c.1320G>A on one allele. Post mortem radiography showed 11 pairs of ribs, a narrow chest, severe bowing of the long, tubular bones, and osseous spikes on both tibiae.

Figure 1. Case 1. (A) Radiograph and (B) *post mortem* details of the lower limb. Note confirmation of the severe bowing of the long tubular bones as identified by prenatal ultrasound. The *post mortem* confirmed clubfoot and spurs.

Figure 3. Case 2. (A) *Post mortem* computed tomography *plus* three-dimensional reformatting and (B) radiography showed arched shape of the long tubular bones, with morphology handset. The thorax was characterized by dysmorphic ribs, with asymmetry of the rib cage. In addition, minimum attitude in subluxation of the fifth ray of the right hand was also observed.

Histological examination revealed well-represented resting cartilage, although the proliferative and maturation zone showed fewer clustered columns, with piled up chondrocytes, as compared with a normal control group. By contrast, the hypertrophic, degenerative, and osteogenic zones were fairly regular. In the tibial spikes, woven bone formation was disorganized and presented areas of abnormal remodeling (Figure 2).

Case 2. Osteogenesis imperfecta Type II

A 34-year-old G1P0 was referred at 20^{+6} weeks for narrow fetal thorax and horizontal ribs. The femur and tibia were bent and measured 19.7 mm (3.9^{th} centile for gestational age) and 14 mm (2.9^{th} centile for gestational age), respectively. The femur presented features consistent with microfracture. The fibula was particularly short (10.2 mm). US findings were consistent with a lethal type of
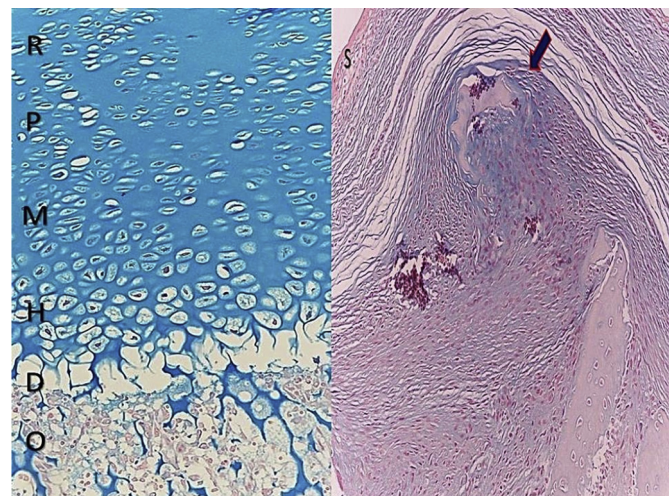
osteogenesis imperfecta (OI), and mutation in the *COLA1* gene on chromosome 17q21.33 was documented.

Post mortem radiography confirmed reduced calcification with bilateral bowing of femurs. Computed tomography *plus* three-dimensional (3D) reformatting of the skeleton showed handset morphology of the tubular bones and minimum attitude in subluxation of the fifth ray of the right hand (Figure 3).

Histological examination of the growth plate was generally normal. However, in the metaphysis, the bony trabeculae were irregularly organized and narrow; in the diaphysis, they were severely reduced in dimension and population. The bony trabeculae were small, slender, and haphazardly arranged.

Bone formation was reduced, with abundant osteoid matrix; however, the medulla was fibrotic. Osteocyte hypercellularity was prominent along the spiculae and in the periosteum, and microfractures and bone remodeling were frequently observed (Figure 4).

Figure 2. Case 1. Epiphyseal growth plate (femur): in campomelic dysplasia the cartilage growth plate is almost normal. Resting cartilage (R) is usually well represented, proliferative (P) and maturation zone (M) show less clustered columns, but chondrocytes remain piled up. Hypertrophic (H), degenerative (D) and osteogenic (O) zones are fairly regular. (A) Hematoxylin and eosin (10×). (B) Tibial spike in campomelic dysplasia. Underneath the skin (S) the woven bone is less organized with islets pointing towards the apex of the spike (arrow).
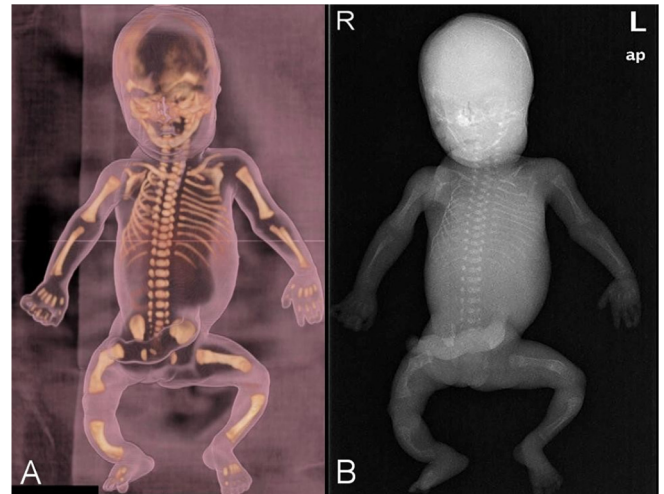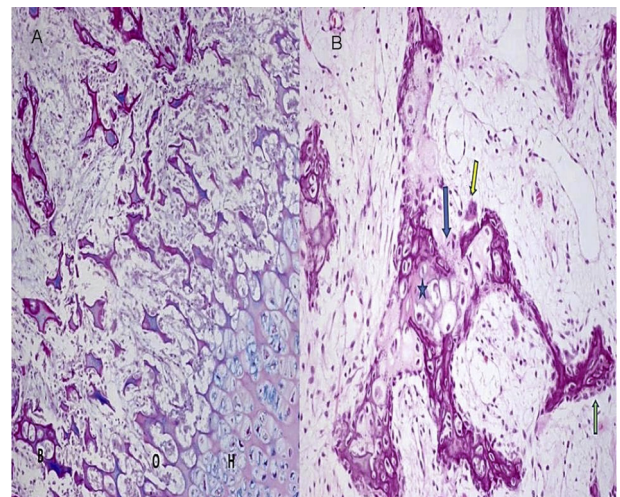

Figure 4. Case 2. (A) Epiphysial growth plate (femur), particular, Alcian Blu PAS (10×). The osteogenic zone (O) has reduced and thin spicules of calcified cartilage (B = basal zone; H = hypertrophy zone). (B) Hematoxylin 2 and Eosin (20×). A microfracture (blue arrow) in a bony trabecula with metaplastic cartilage (star). Of note the osteoclasts (yellow arrow) and the typical hypercellular osteoblast layer (green arrow) are present.

Case 3. Cumming syndrome

A 31-year-old G1P0 was referred at 20^+3 weeks with second trimester scan consistent with dolichocephalic, and with a cephalic index of 72 (compared with abnormal mean value for age of 79.1) and poor mineralization of the calvarium. Lower limbs were bowed and short, with the femur measuring 18.2 mm (< 2.3^rd centile for gestational age) and the tibia measuring 16 mm (< 2.3^rd centile for gestational age). Bilateral microcystic dysplastic kidney disease with severe oligohydramnios was an associated finding.

The *COLA1*, *SOX9*, *FGFR1*, *FGFR2*, *FGFR3*, *Twist*, and *RECQL4* genes were negative for mutations. Radiography confirmed reduced ossification of cranial bones and bilateral bowing of the femurs. *Post mortem* examination showed hypertelorism and low-set ears; the skull bones were soft, and the thorax was enlarged (Figure 5). Histological examination of the epiphyseal growth plate showed enlargement of the maturation and hypertrophy zones. Cartilage degeneration was sparse, with reduced osteoid deposition (Figure 6). The osteogenic zone presented increased bone remodeling, with osteoblast and osteoclast activity (Figure 7). Diagnosis of
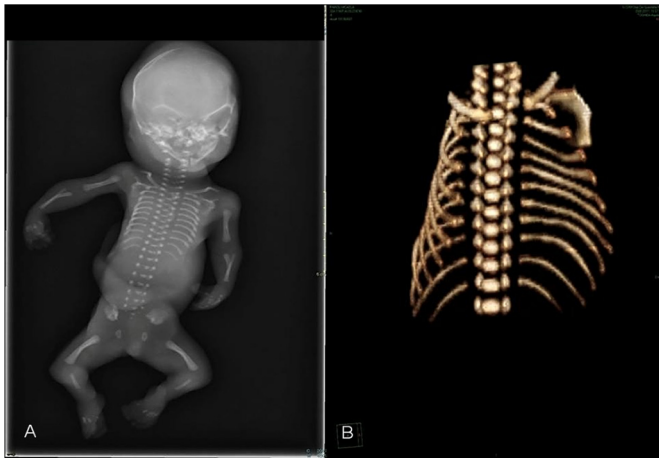

Figure 5. Case 3. (A) Radiography confirmed reduced ossification of cranial bones and bilateral bowing of the femurs. (B) Detail of the thoracic cage by three-dimensional computed tomography.
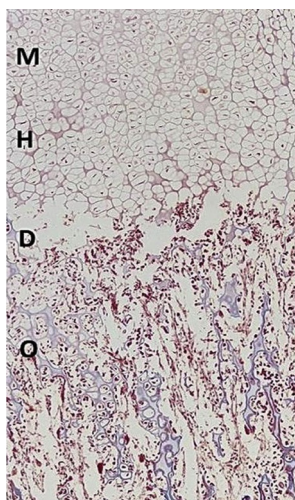

Figure 6. Case 3. Epiphyseal growth plate (femur): enlargement of the maturation (M) and hypertrophy (H) zones. Cartilage degeneration zone (D) is scarcely represented with reduced osteoid deposition in the osteogenic zone (O). Picromallorytrichrome (4×).
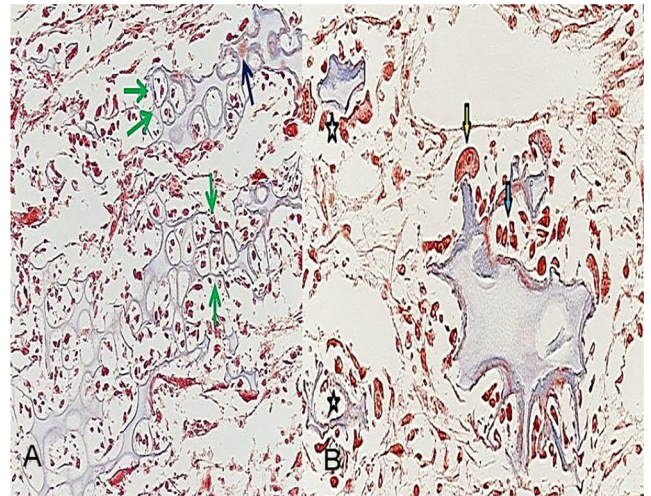

Figure 7. Case 3. (A) Osteogenic zone: within the osteogenic zone, cartilage degeneration is reduced with overall preservation of the chondrocytes (green arrows). Osteoid deposition (red substance, blue arrow) is scarce. (B) Bone remodeling in the osteogenic zone. The osteoclasts are numerous (yellow arrow) and the osteoblasts may be found in clusters (blue arrow). On the left side of the picture there are also two small areas of osteoid remodeling (stars).

campomelic dysplasia, Cumming type, was posited following consultation with the European network for skeletal dysplasia.

Case 4. Femoral hypoplasia—unusual facies syndrome

A 27-year-old G1P0 was referred at 21^+3 weeks for wide metopic suture and micrognathia of the fetus. The femora were short (25.5 mm, < 2.3^rd centile for the gestational age) and bent, especially around the diaphysis. This feature was well detailed using 3D US rendering in surface mode. A presumptive diagnosis of femoral hypoplasia—unusual facies syndrome (FHUFS) was suggested. Radiography confirmed the prenatal diagnosis and revealed splayed transverse vertebral processes in the cervical spine. Maxillary and mandible bone hypoplasia was also identified at *post mortem* examination (Figure 8). Gene sequencing of the *FGFR3* was negative. Histological examination of the epiphyseal growth plate in the femur was essentially normal, and the areas of cartilage differentiation were regularly represented, with orderly piled chondrocytes (Figure 9).

Discussion

A clinical—diagnostic algorithm has been developed (Figure 10) to differentiate and correctly diagnose skeletal dysplasia when bowing of long bones is found at the 2nd trimester US scan. According to Watiker et al. [1], the presence of a narrow and tall pelvis, hypoplastic scapulae, and sex reversal are the key findings in campomelic dysplasia that enable it to be differentiated from Cumming syndrome. Campomelic dysplasia is due to mutations in the *SOX9* gene, which encodes a transcription factor necessary for chondrocyte differentiation, cartilage development, and the production of the Müllerian-inhibiting factor in Sertoli cells [2]. Clinically, the hallmark of campomelic dysplasia is anterior and anterolateral bowing of femurs and tibiae, with dwarfism [3]. Despite the lethality of campomelic dysplasia, the survival rate is estimated at 5—10%. Chondroosseous morphology appears normal in campomelic dysplasia, except at the diaphyseal bend.

According to our algorithm, Cumming syndrome must be considered if campomelic dysplasia molecular testing is negative. A
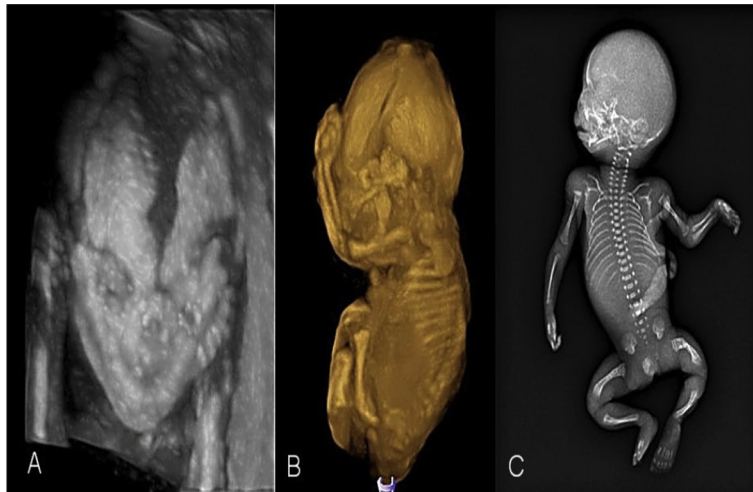
Figure 8. Case 4. (A) Transabdominal two-dimensional ultrasound performed at 21^{+3} weeks documenting a wide metopic suture associated with micrognathia. (B) Three-dimensional ultrasound reconstruction using surface minimum mode showed an extremely bent femur at diaphysis level. (C). Radiography confirmed ultrasound images and disclosed splaying of the transverse vertebral processes in the cervical spine. Maxillary and mandible bone hypoplasia, and bent femora were also identified at *post mortem* examination.

different situation occurs with regard to risk of recurrence, since inheritance of Cumming syndrome is reported as autosomal recessive, whereas campomelic dysplasia is autosomal dominant, with a 25% and 50% risk for future pregnancies, respectively.

The association of skeletal malformations and renal–hepatic–pancreatic cystic disease with polyasplenia complex associated with severe congenital heart disease, in addition to defects in laterality, may (as seen in Cumming syndrome) reflect an underlying condition. It is possible that these conditions represent a developmental field defect with varying manifestations and may be due to a defect in a gene exerting a blastogenetic effect [4].

Another differential diagnosis applicable to fetal bent bones is OI Type II. In the case presented, diagnosis was made early in the second trimester by integrating US findings with molecular findings; in fact, the typical US features of bowed long bones in lower limbs, which are indicative of multiple fractures, were not displayed. Nevertheless, histological examination of the long bones showed features of OI Type II syndrome in its chronological *continuum*.
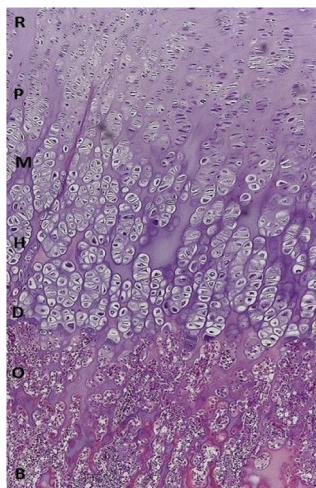

Figure 9. Case 4. Histological examination of the epiphyseal growth plate in the femur was essentially normal and the zones of cartilage differentiation were regularly represented with chondrocytes orderly piled. B = basal zone; D = degenerative; H = hypertrophic; M = maturation; O = osteogenic; P = proliferative; R = resting cartilage.

If US reveals normal thorax and shortening of the lower limbs, a provisional diagnosis of Stüve–Wiedemann syndrome (SWS) and FHUFS should be considered. SWS [5] is characterized by short stature, bowing of the extremities (especially affecting the lower limbs), hypoplastic midface, camptodactyly, respiratory distress/apneic spells, and hyperthermic episodes. SWS is an autosomal recessive disorder caused by a mutation in the *leukemia inhibitory factor receptor* gene 2 on chromosome 5p13 [6]. This disease is associated with a significant increase in neonatal mortality that is principally due to respiratory insufficiency and malignant hyperthermia. It was first reported in two sisters with an early lethal outcome [5].

Clinical features in FHUFS are asymmetric femurs, cleft palate, hypoplastic fibulae, and genitourinary abnormalities. Additional birth defects may include sacral dysgenesis, humeroradial synostosis, renal abnormalities (renal agenesis or dysplasia, multicystic kidneys, or abnormal urinary collection system), and genital abnormalities (cryptorchidism, hypoplastic labia, and microphallus); nevertheless, life expectancy may be normal. Prenatal findings in FHUFS have been reported in 12 fetuses in which femoral hypoplasia varied from minimal shortening and bowing to complete femoral agenesis. The focal femoral defect may be either isolated or part of multiple abnormalities that are typically found in certain rare disorders, such as caudal regression sequence, campomelic dysplasia, Antley–Bixler syndrome, and kyphomelic dysplasia [7–11].

In cases of bent bone osteochondrodysplasias, histological examination of the long bones is highly recommended, with femur sampling being the best option. Bone development differs in each disease. Campomelic dysplasia presents a fairly regular cartilage growth plate.

Primary bone trabeculae show normal architecture, ossification, and remodeling. In the bowed area, a less-organized woven bone replaces the cortical bone, which results in cone-shaped ossification extending into the bone marrow. In OI Type II, the growth plate is also generally normal. However, the bony trabeculae are irregularly organized and narrow in the metaphysis; in the diaphysis, they are severely reduced in dimension and population. Osteocyte hypercellularity is prominent along the spiculae and in the periosteum. Microfractures and remodeling are easily found.

In Cumming syndrome, the epiphyseal growth plate is short, with irregular chondrocyte columns. The chondrocytes are also abnormal, with reduced cytoplasm. In SWS and FHUFS, the
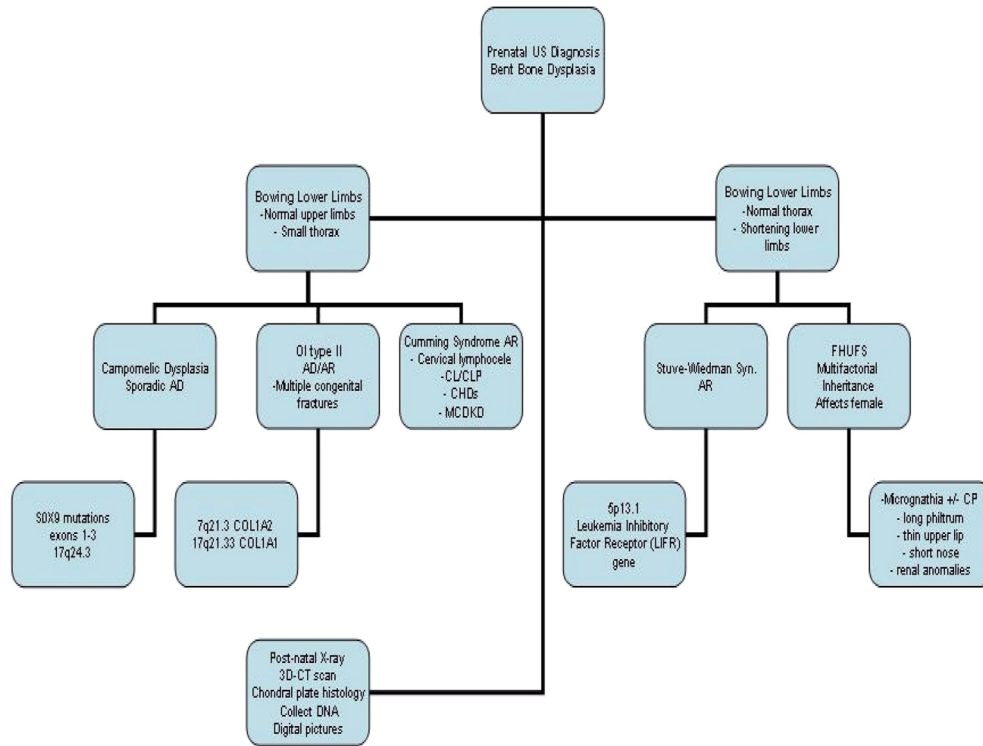
Figure 10. Suggested clinical–diagnostic algorithm in the management of fetal bent bony dysplasias diagnosed by ultrasound at 2nd trimester ultrasound examination. AD, autosomal dominant; AR, autosomal recessive; CL/CLP, cleft lip and palate; CHDs, congenital heart defects; CP, cleft palate; COLA1, COLA2; collagen type alpha1 and alpha2; 3D-CT, three-dimensional computed tomography; DNA, dexossyribonuclei acid; FHUFS, femoral hypoplasia unusual face syndrome.; MCDKD, multycystic dysplastic kidney disease; OI, osteogenesis imperfecta.

Tonni G holds the Copyrigt. This flowchart has never been previously published.

cartilage is apparently normal, with virtually regular proliferative and hypertrophic zones. By contrast, in SWS at the chondroosseous junction, the primary trabeculae are irregular and thickened, and excessive osteoclastic reabsorption is evident. Osteopenia is the foremost feature of SWS and results in weak bones that are easily susceptible to bowing by intrauterine mechanical forces [12–16].

The rapid emergence of next-generation sequencing technology is revolutionizing a broad range of medical research areas, such as rare Mendelian disorders. NGS (next-generation sequencing) now enables clinical investigators to analyze exome and genome from small amounts of DNA/RNA. In the near future, skeletal dysplasia will also be analyzed using this novel approach. A first step may be the use of multigene panel testing for a small subset of genes relevant to a disease phenotype (disease-specific panel). For example, in campomelic dysplasia or Cumming type with microcystic dysplastic kidney disease, a panel could simultaneously analyze the *PKD1* gene and the genes involved in skeletal dysplasia, as was carried out in case presented.

A next or alternative step may include whole-exome sequencing to determine the full range of coding variations present in an individual genome. Whole-exome sequencing is focused not only to find mutations in genes already known to cause disease, but also to identify novel genes, especially in patients with a nonspecific presentation. Finally, the next advancement of NGS technology will be whole-genome sequencing, which will be able to determine the complete DNA sequence of the genome of an organism; however, this technique is not yet practical due to its high cost and the extensive time required for execution.

Detection and characterization of skeletal dysplasias with bowing long bones has a dramatic impact on possible early prenatal diagnosis, selection of targeted molecular testing, genetic counseling of future parents, calculation of recurrent risk, and appropriate obstetrical management. When bowed bones are seen at 2nd trimester US scan, inspection of the thorax and assessment to determine possible involvement of the upper and/or lower limbs are the first sonographic criteria suggested for discriminating between overlapping skeletal dysplasias. Additional recommended steps include radiography, *post mortem* 3D computed tomography and/or magnetic resonance imaging, digital imaging and *post mortem* examination with chondral plate histology. Also, DNA from skin culture fibroblasts should be collected in a bio-bank for subsequent molecular array comparative genomic hybridization, NGS or whole-genome sequencing testing, where clinically indicated.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

References

[1] Watiker V, Lachman RS, Wilcox WR, Barroso I, Schafer AJ, Scherer G. Differentiating campomelic dysplasia from Cumming syndrome. Am J Med Genet 2005;135A:110–2.

[2] Friedrich U, Schaefer E, Meinecke P, Scherer G. SOX9 mutation in a previously published case of campomelic dysplasia without overt campomelia. Clin Dysmorphol 2000;9:233.

[3] Coscia MF, Bassett GS, Bowen JR, Ogilvie JW, Winter RB, Simonton SC. Spinal abnormalities in camptomelic dysplasia. J Pediatr Orthop 1989;9:6–14.

[4] Ming JE, McDonald-McGinn DM, Markowitz RI, Ruchelli E, Zackai EH. Heterotaxia in a fetus with campomelia, cervical lymphocele, polysplenia, and multicystic dysplastic kidneys: expanding the phenotype of Cumming syndrome. Am J Med Genet 1997;73:419–24.

[5] Stüve A, Wiedemann HR. Congenital bowing of the long bones in two sisters. Lancet 1971;2:495.

[6] Dagoneau N, Scheffer D, Huber C, Al-Gazali LI, Di Rocco M, Godard A, et al. Null leukemia inhibitory factor receptor (LIFR) mutations in Stüve–Wiedemann/Schwartz–Jampel type 2 syndrome. Am J Hum Genet 2004;74:298–305.

[7] Tadmor OP, Hammerman C, Rabinowitz R, Fisher D, Itzchaki M, Aboulafia Y, et al. Femoral hypoplasia—unusual facies syndrome: prenatal ultrasono-graphic observations. Fetal Diagn Ther 1993;8:279–84.

[8] Gillerot Y, Fourneau C, Willems T, Van Maldergem L. Lethal femoral–facial syndrome: a case with unusual manifestations. J Med Genet 1997;34:518–9.

[9] Urban JE, Ramus RM, Stannard MW, Rogers BB. Autopsy, radiographic, and prenatal ultrasonographic examination of a stillborn fetus with femoral facial syndrome. Am J Med Genet 1997;71:76–9.

[10] Filly AL, Robnett-Filly B, Filly RA. Syndromes with focal femoral deficiency: strengths and weaknesses of prenatal sonography. J Ultrasound Med 2004;23: 1511–6.

[11] Paladini D, Maruotti GM, Sglavo G, Penner I, Leone F, D'Armiento MR, et al. Diagnosis of femoral hypoplasia-unusual facies syndrome in the fetus. Ul-trasound Obstetr Gynecol 2007;30:354–8.

[12] Pazzaglia UE, Beluffi G. Radiology and histopathology of the bent limbs in campomelic dysplasia: implications in the aetiology of the disease and review of theories. Pediatr Radiol 1987;17:50–5.

[13] Cormier-Daire V, Munnich A, Lyonnet S, Rustin P, Delezoide AL, Maroteaux P, et al. Presentation of six cases of Stüve–Wiedemann syndrome. Pediatr Radiol 1998;28:776–80.

[14] Mortier GR. The diagnosis of skeletal dysplasias: a multidisciplinary approach. Eur J Radiol 2001;40:161–7.

[15] Gonçalves LF, Espinoza J, Mazor M, Romero R. Newer imaging modalities in the prenatal diagnosis of skeletal dysplasias. Ultrasound Obstet Gynecol 2004;24:115–20.

[16] Gilbert-Barness E. Osteochondrodysplasias—constitutional diseases of bone. In: Enid, editor. Potter's pathology of the fetus, infant and child. 2nd ed. Philadelphia: Mosby-Elsevier; 2007. p. 1836.