Early prenatal detection of autosomal dominant skeletal dysplasia using first-trimester ultrasound and cell-free fetal DNA screening: three case reports

Ye Cao[1,2], PhD, FACMG, **Yvonne KY Cheng**[1], MSc (Medical Genetics), FHKAM (Obstetrics and Gynaecology), **TY Leung**[1,2], MD, FHKAM (Obstetrics and Gynaecology), **Shuwen Xue**[1,2], MPhil, PhD, **Yuting Zheng**[1,2], MPhil, **KW Choy**[1,2], MSc (Med), PhD, **Winnie CW Chu**[3], MD, FHKAM (Radiology), **HM Luk**[4], MD, FHKAM (Paediatrics), **KM Law**[1], FRCOG, FHKAM (Obstetrics and Gynaecology), **YH Ting**[1] *, FRCOG, FHKAM (Obstetrics and Gynaecology)

[1] *Department of Obstetrics and Gynaecology, The Chinese University of Hong Kong, Hong Kong SAR, China*
[2] *Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China*
[3] *Department of Imaging and Interventional Radiology, The Chinese University of Hong Kong, Hong Kong SAR, China*
[4] *Department of Clinical Genetics, Hong Kong Children's Hospital, Hong Kong SAR, China*

* Corresponding author: tingyh@cuhk.edu.hk

This article was published on 28 Jan 2026 at www.hkmj.org.

This version may differ from the print version.

Hong Kong Med J 2026;32:Epub
https://doi.org/10.12809/hkmj2513462

Case presentations

Case 1 (Family 1)

A primigravida attended our fetal medicine clinic (FMC) in March 2015 at 12 weeks' gestation for first-trimester (T1) Down syndrome screening. Ultrasound examination revealed an absent nasal bone (NB). A morphology scan at 20 weeks confirmed this finding, along with bilateral non-ossified parietal bones, 11 pairs of ribs, and shortened femur and humerus. Amniocentesis revealed a normal chromosomal microarray. The couple opted for termination of pregnancy at 22 weeks. A computed tomography babygram confirmed the ultrasound findings and also showed bilaterally absent clavicles, hinting at a diagnosis of cleidocranial dysplasia (CCD). Targeted sequencing of the *RUNX2* gene on the amniotic fluid sample revealed a de novo heterozygous pathogenic missense variant, c.674G>A (p.Arg225Gln), confirming the diagnosis. Multimodal prenatal and genetic findings were illustrated in Figure 1.

Cases 2 and 3 (Family 2)

A primigravida attended the FMC in July 2022 at 12 weeks' gestation for non-invasive prenatal screening (NIPS) for fetal aneuploidy. Ultrasound showed non-ossified skull bones (SB) and reduced spine ossification, but both clavicles were present. A review of the paternal history revealed that he had features of CCD, including the ability to approximate his shoulders, similar to a character in an American drama with diagnosed CCD. Molecular testing for CCD showed a pathogenic nonsense variant in the *RUNX2* gene, c.577C>T (p.Arg193Ter), confirming the diagnosis. The fetus was thus suspected to have the same genetic problem. The couple declined invasive genetic testing. Instead, NIPS was performed using a novel technique known as coordinative allele-aware target enrichment sequencing (COATE-seq). This facilitated concomitant screening for chromosomal and monogenic disorders, encompassing 10 aneuploidies, 12 microdeletions and 64 monogenic disorders including *RUNX2*-related diseases (online supplementary Table 1). Results showed that the fetus was at high risk of having a pathogenic variant in the *RUNX2* gene c.577C>T (p.Arg193Ter). Serial ultrasound showed normal SB ossification but with widened sutures, normal spine ossification, and mildly shortened clavicles with a normal S shape. A male infant was delivered at 39 weeks. Skeletal survey showed a persistent metopic suture, widened anterior fontanelle, 11 pairs of ribs, delayed ossification of pubic bones with widely spaced public symphysis, but both clavicles were present. Targeted *RUNX2* variant analysis on the cord blood sample validated the presence of the paternal heterozygous pathogenic variant.

In the same patient's second pregnancy, she attended the FMC at 12 weeks in January 2024 where ultrasound showed hypoplastic clavicles, non-ossified SBs and reduced spine ossification. The NIPS using COATE-seq showed that the fetus was at high risk of having the same pathogenic *RUNX2* variant, c.577C>T (p.Arg193Ter). The couple declined invasive confirmatory testing. Serial ultrasound showed non-ossified SBs with widened sutures and fontanelle, a thin NB, very short clavicles with loss of normal S shape, 11 pairs of ribs, and mildly shortened long bones. A female infant was delivered at 38 weeks. Skeletal survey revealed bilateral hypoplastic clavicles and 11 pairs of ribs. Targeted *RUNX2* variant analysis of the cord blood sample validated the presence of the paternal heterozygous pathogenic variant, confirming the diagnosis. Imaging and genetic findings are illustrated in Figure 2.

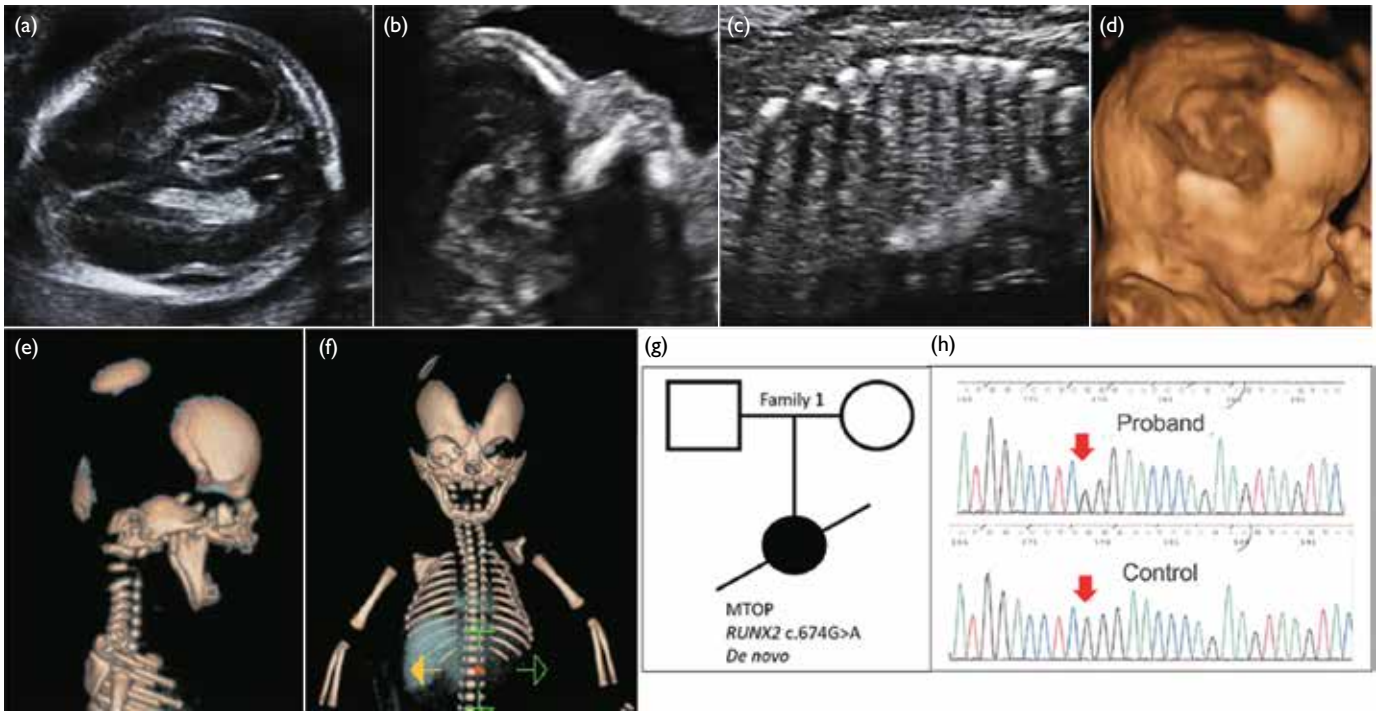

FIG 1. Multimodal prenatal and genetic findings in Case 1, including second-trimester ultrasound. (a) Non-ossified parietal bone. (b) Absent nasal bone. (c) Eleven pairs of ribs. (d) Non-ossified parietal bone on three-dimensional imaging. (e, f) Computed tomography babygram. (e) Non-ossified skull bones with widened fontanelles and sutures. (f) Bilaterally absent clavicles and 11 pairs of ribs. (g) Pedigree. (h) Sanger sequencing showing the heterozygous pathogenic variant *c.674G*>A (arrows)

Discussion

Cleidocranial dysplasia is a rare autosomal dominant skeletal dysplasia characterised by the classic triad of absent or hypoplastic clavicles, delayed ossification of the cranial bones with delayed closure of sutures and fontanelles, and dental abnormalities.[1] Approximately two-thirds of cases are caused by *RUNX2* gene mutations, with the remaining one-third resulting from copy number variations, translocations, or inversions involving the *RUNX2* locus.[2] The *RUNX2* gene, located on chromosome 6p21, encodes a transcription factor that regulates osteoblast differentiation and chondrocyte maturation.[3] Haploinsufficiency of *RUNX2* gene leads to delayed intramembranous and endochondral ossification.[3] The skull and clavicles, formed by intramembranous ossification, are therefore the most frequently affected.[3]

Prenatal diagnosis of CCD is rare. Including our three cases, only 22 cases have been reported to date (online supplementary Table 2). Most had affected family members, hinting at the diagnosis. Most were diagnosed based on clinical findings, with only 10 cases having a molecular diagnosis of *RUNX2* gene defects. This highlights the pivotal role of prenatal ultrasound in identifying the characteristic features, namely, absent or hypoplastic clavicles, absent or inadequate SB ossification with wide fontanelles and sutures, and shortened long bones and absent NB. Among these, clavicular defect is the most characteristic. All three cases in our series had these typical features, detected during the first trimester, with an additional novel finding of 11 pairs of ribs. Nevertheless, the prenatal detection of CCD can be difficult as ultrasound features may be subtle. Although clavicles can be visualised during T1 ultrasound, they are not routinely examined. Conversely, absent NB, a marker for aneuploidy and routinely assessed during T1 nuchal translucency measurement, may be an important clue that prompts further examination of the clavicles and skull. With a positive family history, prenatal detection of inherited CCD by ultrasound may be more feasible. However, this can remain challenging as pathogenic *RUNX2* variants exhibit complete penetrance but variable expressivity.[1] Within the same family, one affected fetus may present with a subtle phenotype while another may show more pronounced manifestations, as illustrated by the two siblings in Family 2. Therefore, meticulous ultrasound is imperative in pregnancies at risk of CCD.

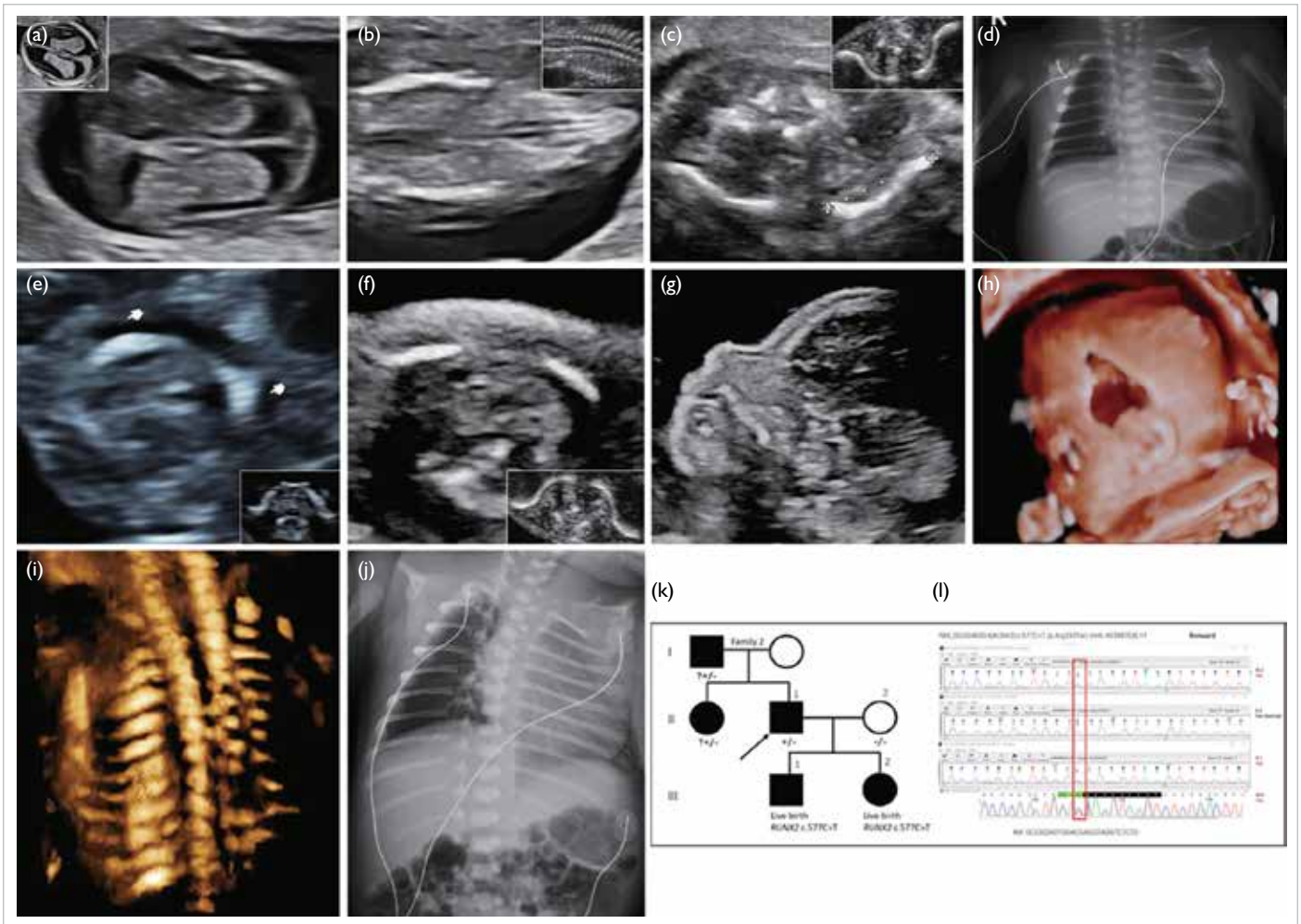

FIG 2. Imaging and genetic findings of Family 2. (a-d) Case 2. (a) Non-ossified skull bones on first-trimester ultrasound (inset: normal skull). (b) Reduced spine ossification on first-trimester ultrasound (inset: normal spine). (c) Slightly shortened clavicles with normal S-shape on second-trimester ultrasound (inset: normal clavicles). (d) Normal clavicles and 11 pairs of ribs on postnatal chest X-ray. (e-j) Case 3. (e) Short clavicles on first-trimester ultrasound (inset: normal clavicles). (f) Short clavicles with loss of normal S-shape on second-trimester ultrasound (inset: normal clavicles). (g) Thin nasal bone on second-trimester ultrasound. (h) Non-ossified skull bones on three-dimensional (3D) ultrasound. (i) Eleven pairs of ribs on 3D ultrasound. (j) Short clavicles and 11 pairs of ribs on postnatal chest X-ray. (k) Pedigree. (l) Sanger sequencing showing the heterozygous pathogenic *RUNX2* variant c.577C>T (highlighted in border)

When CCD is suspected, invasive genetic testing is usually recommended to confirm the diagnosis through targeted *RUNX2* variant analysis. However, invasive testing is associated with 0.1% to 0.2% risk of procedure-related fetal loss.[4] As CCD rarely results in severe disability, many parents, particularly affected ones, may not consider termination of pregnancy and may choose to avoid invasive testing. In such cases, the new NIPS approach, COATE-seq, provides a viable diagnostic alternative.[5] Its performance in high-risk pregnancies has been validated, demonstrating 98.5% sensitivity and 99.3% specificity compared with standard diagnostic methods.[6] The two cases in Family 2 represent the first report of prenatal detection of CCD through the identification of a pathogenic *RUNX2* variant using this novel technique. These cases highlight the great potential of combining T1 ultrasound with NIPS for early, non-invasive prenatal detection. This powerful non-invasive approach may also be applicable to other autosomal dominant skeletal dysplasia and monogenic disorders.

Author contributions

Concept or design: KW Choy, YH Ting.
Acquisition of data: All authors.
Analysis or interpretation of data: All authors.
Drafting of the manuscript: Y Cao, YH Ting.
Critical revision of the manuscript for important intellectual content: Y Cao, YH Ting.

All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

Conflicts of interest

All authors have disclosed no conflicts of interest.

Acknowledgement

The authors thank the affected families for participating in and supporting this study.

Funding/support

This study was supported by the National Key Research and Development Program of China (Grant No.: 2023YFC2705603). The funder had no role in study design, data collection/analysis/interpretation or manuscript preparation.

Ethics approval

This study was approved by the Joint Chinese University of Hong Kong–New Territories East Cluster Clinical Research Ethics Committee, Hong Kong (Ref No.: 2017.442). Written informed consent was obtained from the families for publication of clinical details and images.

Supplementary material

The supplementary material was provided by the authors and some information may not have been peer reviewed. Accepted supplementary material will be published as submitted by the authors, without any editing or formatting. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by the Hong Kong Academy of Medicine and the Hong Kong Medical Association. The Hong Kong Academy of Medicine and the Hong Kong Medical Association disclaim all liability and responsibility arising from any reliance placed on the content. To view the file, please visit the journal online (https://doi.org/10.12809/hkmj2513462).

References

1. Machol K, Mendoza-Londono R, Lee B. Cleidocranial dysplasia spectrum disorder. 3 Jan 2006 [updated 13 Apr 2023]. In: Adam MP, Feldman J, Mirzaa GM, editors. GeneReviews. Seattle (WA): University of Washington; 1993.
2. Motaei J, Salmaninejad A, Jamali E, et al. Molecular genetics of cleidocranial dysplasia. Fetal Pediatr Pathol 2021;40:442-54.
3. Hassan NM, Dhillon A, Huang B. Cleidocranial dysplasia: clinical overview and genetic considerations. Pediatr Dent J 2016;26:45-50.
4. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: a systematic review and meta-analysis. Ultrasound Obstet Gynecol 2015;45:16-26.
5. Xu C, Li J, Chen S, et al. Genetic deconvolution of fetal and maternal cell-free DNA in maternal plasma enables next-generation non-invasive prenatal screening. Cell Discov 2022;8:109.
6. Zhang J, Wu Y, Chen S, et al. Prospective prenatal cell-free DNA screening for genetic conditions of heterogenous etiologies. Nat Med 2024;30:470-9.

