SHORT REPORT

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Compound heterozygosity for two variants in *BMP5* in human skeletal dysostosis with atrioventricular septal defect

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Abstract

The growth and development of the skeleton is regulated by bone morphogenetic proteins of which several are linked to genetic skeletal disorders. So far, no human skeletal malformations have been associated with variants in *BMP5*. Here, we report a patient with biallelic loss of function variants in *BMP5* and a syndromic phenotype including skeletal dysostosis, dysmorphic features, hypermobility, laryngo-tracheo-bronchomalacia and atrioventricular septal defect. We discuss the phenotype in relation to the known tissue-specific expression of Bmp5 and similar morphological abnormalities previously reported in experimental animal models. Our findings suggest a new association between *BMP5* variants and a range of developmental anomalies, involving ears, heart and skeleton, thereby increasing understanding of BMP5's role in human development.

1 | INTRODUCTION

Bone Morphogenetic Proteins (BMPs) belong to the transforming growth factor-beta (TGF-beta) superfamily and are essential for development and growth.[1–4] BMP5 acts in the extracellular matrix, where it is released, cleaved, and dimerized to obtain the final active protein that is encoded only by exons 5, 6, and 7. Only a few BMP-related genetic skeletal disorders have been reported, for example, those related to *BMP1*, *BMP2*, *BMPR1B*, and *BMPER*.[5] In addition, pathogenic variants in several other genes involved in the BMP-signaling pathway give rise to genetic skeletal disorders through their effect on ligands, receptors (*ACVR1*), mediators (*SMAD4*) and inhibitors (*NOG*).[5,6] To our knowledge, no genetic skeletal disease has been linked to *BMP5* in humans.[5,7] However, studies in mice have shown that *Bmp5* has multiple roles in skeletal, cartilage, ear and heart development.[8–10] This report presents a child, compound heterozygous for two variants in *BMP5*, and a complex syndromic phenotype involving skeletal dysostosis, dysmorphic features, hypermobility, laryngo-tracheo-bronchomalacia, and atrioventricular septal defect (AVSD).

2 | METHODS

2.1 | Ethics

This study was approved by the Regional Ethical Review Board Karolinska University Hospital and Karolinska Institutet (protocol numbers 2014/983-31/1, 2012/2106-31/4, 2018/2207-32, 2021-05360). Written informed consent was obtained.

2.2 | Genetic analyses

Genomic DNA was extracted from blood from the patient and her parents, and buccal swabs from her unaffected siblings.

Genome sequencing was performed as a singleton to identify structural and single nucleotide variants (SNVs) in all known skeletal dysplasia genes using a gene panel according to PanelApp[11] and the Nosology of Genetic Skeletal disorders.[5] Furthermore, we performed family-trio analysis for all known human genes. Induction of BMP5 expression in primary fibroblasts and RNA sequencing were performed as previously described.[12] Further details are provided in S1.

3 | RESULTS

3.1 | Clinical data

The patient is a 7-year-old girl and the second child of healthy non-consanguineous Danish parents. The pregnancy was conceived naturally. At gestational age (GA) 13 + 0, routine ultrasound showed an increased nuchal translucency (3.9 mm, ref. <3.5 mm); chorionic villus sampling was normal (Elucigene QF-PCR, Agilent 180 K). Fetal ultrasound scans at GA 16 + 2 and 20 + 5 were normal. Birth was uncomplicated at GA 39 + 3 (birth weight 3716 g [+1 standard deviation (SD)], birth length 50 cm [median], head circumference 36 cm [+1SD][13]). At day 12, she was admitted to hospital due to dyspnea. Echocardiography showed an AVSD that was surgically repaired at age 6 weeks. Surgery was complicated by third-degree AV block necessitating pacemaker. At 6 months, she was admitted to hospital due to an upper airway infection. An echocardiography showed dyssynchronous left ventricle and reduced ejection fraction. Treatment with anti-asthmatic and diuretic medication was successful. At 1 year and 5 months, she had tachypnea and sparse weight gain and was diagnosed with severe left AV valve insufficiency requiring surgery.

Until age 2.5 years, the girl suffered from several wheezing episodes and airway infections, but spirometry and bronchodilatator response were normal. A trachea-bronchoscopy at 1 year and 11 months, revealed moderate laryngo-tracheo-bronchomalacia, a stalked omega-shaped epiglottis, and a short right main bronchus. She is hypomimic with no cranial nerve palsies, has malar hypoplasia, short palpebral fissures, short nose, low nasal bridge, anteverted nares, long philtrum, small ears with abnormally folded antihelix (Figure 1), redundant but not hyperextensible skin, asymmetrical *pectus carinatum*, and thoracolumbar scoliosis. She has severe hypermobility of shoulders (100° rotation), elbows (20° hyperextension), knees (30° hyperextension), and ankles. She has a minor conductive hearing loss due to serous otitis media, mild muscular hypotonia and delay of gross motor development (independent walk at 25 months). Her cognitive

FIGURE 1 Photographs of ears (7 years, 6 months). Note small, dysplastic ears.
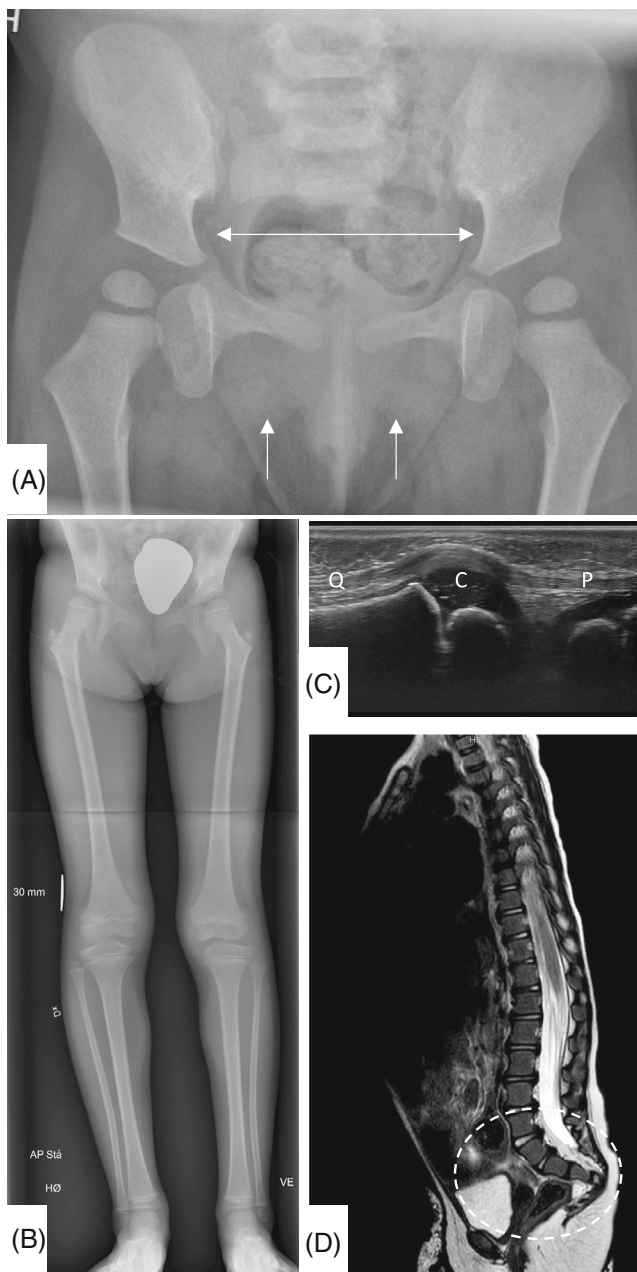
FIGURE 2 (A) Pelvic radiograph (1 year, 8 months). Note absent ossification of the inferior pubic rami, the adjacent part of the pubic body and the ischial rami. The central part of the pelvis is broad, with horizontal superior pubic rami. (B) Frontal standing radiograph of legs (6 years, 0 months). Mild anisomelia. Pelvic findings as described above. Bilateral *coxa valga*. Ossification centers in the greater trochanter small for age. (C) Sonography left knee (1 year, 10 months). Absence of patellar cartilage. Quadriceps tendon (Q), patellar ligament (P), cartilaginous epiphysis distal femur (C). (D) Sagittal T2 weighted spine MRI (2 years, 2 months). Horizontal sacrum, and anterior angulation of the coccyx.

development is normal but below the mean. She is doing well in a regular school program (further information in Supplementary Material).

Skeletal survey showed mild thoracolumbar scoliosis, four sacral segments, absent ossification of the inferior pubic rami, the adjacent

part of the pubic body and the ischial rami, and suspicion of patellar aplasia. Sonography showed absence of patellar cartilage anlagen bilaterally and of the cartilage anlagen of the inferior pubic and the ischial rami. MRI showed four sacral segments and anterior angulation of the coccyx (Figure 2).

3.2 | Genetic findings

Chromosomal microarray and clinical exome trio analyses were normal. Genome sequencing trio analysis identified compound heterozygosity for two variants in *BMP5* (NM_021073.4): c.88_89del, p.(Gly30Argfs*11) (paternal) and c.1104+2del, p.(?) (maternal). Splice prediction for the c.1104+2del variant indicates loss of donor splice site (SpliceAI delta score 0.98 and Pangolin delta score 0.84).[14,15] Her two healthy siblings were heterozygous for the maternal c.1104+2del variant.

3.3 | Confirmation of abnormal splicing on the maternally inherited allele

Alternative splicing of *BMP5* in maternal fibroblasts resulted in multiple annotated isoforms with a single aberrant transcript due to skipping of exon 5 (NM_021073.4:r.1028_1104del) (Figure S1A). The resulting frameshift was predicted to introduce a premature termination codon early in the penultimate exon (NP_066551.1:p.(Asp343Glyfs*15)) expected to trigger nonsense-mediated decay (NMD). Sanger sequencing and RT-PCR using primers in exon 5 and the 3′UTR region for two on genomic sequence heterozygous 3′UTR downstream variants indicated complete loss of activity at the donor splice site of exon 5 in the allele containing the c.1104+2del variant (Figure S1B). BMP5 is a highly evolutionarily conserved protein in different species as well as among paralogues (Figure S2A,B). In addition, exon 5, that is deleted in the maternal allele, encodes the conserved cysteine 353 of the TGF-β-like domain (Figure S2C,D) that is important for the proper folding required for receptor binding. In addition, in silico analysis of the in BMP5 signaling pathway proteins showed interactions with BMPER, TBX4 and PITX1 (Figure S3); genes associated with dysostoses involving pubic and ischiatic bones, sacrum and patella.

4 | DISCUSSION

We report a patient with biallelic loss of function variants in *BMP5* presenting with a rare phenotype including skeletal dysostosis, dysmorphic features, hypermobility, laryngo-, tracheo-bronchomalacia and AVSD. Searching for similar patients in GeneMatcher and our collaborative networks did not lead to identification of other affected individuals, indicating that this diagnosis is ultrarare.

The variant inherited from the father (c.88_89del) leads to a premature stop codon and is predicted to lead to nonsense mediated

decay. The maternal variant (c.1104+2del) leads to abnormal transcript due to skipping of exon 5 that encodes the TGF-β-like domain. This is also predicted to trigger NMD, but if the abnormal RNA transcript results in synthesis of a truncated protein, it will lack the functionally important TGF-β-like domain. This domain is involved in BMP5 interaction with bone morphogenetic protein receptors BMPR1A and BMPR2 to initiate the signaling cascade.[4]

In mice, loss of function mutations in this locus reduce outer ear growth due to disrupted formation and activity of the surface perichondrium around the ear.[8] Monozygotic twins with unilateral ear hypoplasia were reported to be heterozygous for variants in *BMP2* (p.-Ser111Ile) and *BMP5* (c.833-4C>G). However, segregation or cDNA sequencing were not performed,[10] and the pathogenicity of those variants remains unclear. Previous studies of chicken embryonic heart development showed BMP5 expression in the endoderm underlying the precardiac mesoderm, the myocardium of the atrioventricular canal and outflow tract regions.[9] These are all sites where the valvuloseptal endocardial cushion tissue and the primordia of the valves and septa of the adult heart are induced.[16,17] These findings suggest that BMP5 is essential for the development of myocardial and endocardial cushion tissue.[9]

BMP5 expression is confined to specific parts of the skeleton and cartilage in mice and is tightly regulated by different enhancers.[18,19] The skeletal abnormalities observed in our patient, including dysostosis of ischial, pubic and sacral bones, and bilateral patellar aplasia, are similar to those seen in the known human syndromes diaphanospondylodysostosis, *BMPER*-related, mandibular-pelvic patellar syndrome, *PITX1*-related and ischiocoxopodopatellar syndrome, *TBX4*-related, as well as in campomelic dysplasia, *SOX9*-related.[7] The *BMP5* locus controls development of several bones, including processes on vertebrae, xiphoid, the fibula, the number of ribs along vertebra, and the volume of the thoracic cavity.[18] In addition, several individuals with *BMPER*-related conditions,[20,21] as well as our patient, show laryngotracheo-bronchomalacia, indicating the importance of the BMP-signaling pathway for cartilage maturation in the respiratory tract. Previous in vitro studies showed that BMP5 modifies chondrogenesis.[22] Considering the multiple malformations in our patient, and the molecular interactions of BMP5 with TBX4, PITX1, LMX1B and BMPER pathways, (Figure S3), it is plausible that the loss of function variants in *BMP5* may cause our patient's ischio-pubic-patellar dysostosis and cardiac malformation. However, this study includes a single patient with an ultrarare condition. Further exploration including characterization of additional patients and studies of the disease mechanisms behind *BMP5*-related conditions are necessary to confirm and expand the findings of this study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The identified variants are submitted to ClinVar database (accession numbers SCV005038946 and SCV005038947). Other data are available from the authors upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.