

Extension of the phenotypic spectrum of BMP1-related disorders through a novel homozygous variant identified in a foetus with intrauterine growth retardation

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Short Report

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Abstract

Background Osteogenesis imperfecta encompasses a heterogeneous group of heritable connective tissue disorders characterised by bone fragility and skeletal deformities. Autosomal recessive forms of OI may result from defects in the *BMP1* gene, which encodes two main metalloproteinases, BMP1 and its longer isoform mTLD, both essential for type I procollagen processing. To date, *BMP1*-related OI has only been reported postnatally.

Results We report a complex homozygous *BMP1* variant identified by prenatal whole-exome sequencing in a foetus presenting a polymalformative phenotype at gestational week 23 + 2. Ultrasound findings included severe intrauterine growth restriction, bilateral clubfeet, cranial bone anomalies, and signs of cerebral hypoplasia. At this developmental stage, histological examination did not show features of osteoporotic bone structure. A combination of molecular and biochemical strategies led us to characterise the variant and the consequences on BMP1 protein expression as its biological activity in the processing of several substrates.

Functional in vitro assays demonstrated that the c.835A > G variant, located within the protease domain of BMP1, leads to (1) a deleterious missense substitution (p.Arg279Gly) affecting protease activity toward multiple BMP1 substrates, including procollagen I, thrombospondin-1, and chordin and (2) aberrant splicing, inducing exon 6 skipping, and a subsequent frameshift of the open reading frame.

Conclusion This case broadens the phenotypic spectrum of *BMP1*-related disorders and suggests a role for BMP1 in early human development beyond skeletal fragility. The findings support the inclusion of *BMP1* in prenatal diagnosis gene panels for severe syndromic foetal presentations and underscore the importance of functional validation in variant interpretation.

BACKGROUND

Osteogenesis imperfecta (OI) also known as ‘brittle-bone disease’ encompasses a heterogeneous group of heritable collagen-related disorders, primarily characterised by bone fragility, recurrent fractures, bone deformities and growth deficiency [1, 2]. Disease severity ranges from moderate to severe forms, including intrauterine fractures and perinatal lethality [2]. To date, more than twenty forms of OI have been described with an estimated global incidence between 1:15,000 and 1: 20,000 births [2–4]. OI results from abnormalities in the synthesis or post-translational modification of type I collagen, the main structural protein of the bone matrix and a key component of the extracellular matrix in various connective tissues, including skin, lung, heart, cornea or tendon [2].

During bone formation, type I collagen is mainly produced by osteoblasts as a heterotrimeric precursor molecule (called procollagen) with two $\alpha 1$ and one $\alpha 2$ polypeptide chains. Following secretion and cleavage of the amino- (N-) and carboxyl (C-) terminal propeptides, mature type I collagen molecules assemble into highly-ordered collagen fibrils [5, 6]. While heterozygous mutations of the *COL1A1* and *COL1A2* genes, encoding the $\text{pro}\alpha 1$ and $\text{pro}\alpha 2$ chains, account for approximately 90% of autosomal

dominant OI cases, over twenty additional genes have been implicated in other autosomal dominant, recessive or more rarely X-linked forms. These genes encode proteins involved in various steps of type I collagen biosynthesis, such as post-translational modification, folding, secretion or proteolytic maturation [2]. Among these, the *BMP1* gene, located on chromosome 8p21.3, encodes two main structurally-related protein isoforms: bone morphogenetic protein-1 (BMP1; 730 amino acids) and mammalian tolloid (mTLD; 986 amino acids). The proteolytic activity of both BMP1 and mTLD proteins - conferred by an identical astacin-like metalloprotease domain, also conserved with high identity in other mammalian tolloid-like proteins encoded by the *TLL1* and *TLL2* genes - is essential for cleaving the C-terminal propeptide of type I procollagen, a critical step in the formation of mature collagen fibrils [5, 7]. These proteases also contribute to other proteolytic maturation required for extracellular matrix assembly or de-assembly, and to the activation of growth factors belonging to the TGF- β /BMP superfamily [8]. A prominent example is the release of BMP2, BMP4 and BMP7 through the cleavage of the chordin antagonist, a process which must be tightly regulated during embryonic development, osteogenesis and organogenesis [9]. Similarly, TGF- β activation can be enhanced upon cleavage of thrombospondin-1 and contribute to the regulation of cell phenotypes and of extracellular matrix production [10].

To date, approximately twenty-five patients with *BMP1* biallelic mutations have been reported across various ethnic backgrounds, with onset ranging from early postnatal development to young adulthood. Most of them presented with recurrent fractures and/or skeletal deformities [11–15]. Identified mutations typically affected both BMP1 and mTLD isoforms and were found in signal peptide, prodomain, protease domain, and CUB (Complement C1r/C1s-Uef-BMP1) domains, although some variants appear to affect BMP1 exclusively [16].

In this study, we provide evidence that the spectrum of *BMP1*-related disorders appears to be broader than initially recognised. We report the diagnosis of a foetus presenting with second-trimester ultrasound abnormalities, primarily severe intrauterine growth restriction (IUGR). A novel homozygous missense variant in the *BMP1* gene was identified by Whole Exome Sequencing (WES) of DNA from amniotic cells and seems to be the first variant described in the prenatal period. We further investigated the molecular consequences of this variant on BMP1 protein expression and its biological activity in the processing of several prominent substrates.

METHODS

Patient information, legal requirements and use of artificial intelligence

Clinical and genetic data were retrospectively extracted from medical records and from investigations performed as part of standard clinical care. The genetic data required informed written consent from parents. This study was conducted in accordance with the French MR-004 regulatory framework, which governs the secondary use of health data for research.

ChatGPT (OpenAI) was used solely for English language editing, stylistic revision and text improvement. It was not used for data analysis, result generation, figure production, or scientific interpretation. All scientific content was reviewed and validated by the authors.

Genetic analysis and computational annotation

WES was performed on foetal amniotic cells and parental blood samples after genomic DNA extraction using a standard protocol (QIAGEN). Library preparation was performed using SureSelect Human All Exon V7 capture (Agilent Technologies), and sequencing was conducted using the paired-end short-read technology on a NextSeq550 DNA sequencer (Illumina). A trio-based strategy was applied.

Bioinformatic analysis including alignment, variant calling, and annotation was performed using an inhouse pipeline. Single nucleotide variants (SNVs) and copy number variations (CNVs) were analysed. Variant were filtered based on allele frequency within inhouse and public databases (gnomAD v4.1.0, [17]), variant allele fraction (VAF), and segregation pattern.

In silico pathogenicity predictions combined multiple tools, algorithms and meta-predictors including MetaRNN, MISTIC, BayesDel_addAF, ClinPred, and Dynamut2 [18–22]. Splice-site predictions were obtained using MaxEntScan, NNSplice, GeneSplicer, SPiP, and dbSCSNV [23–27].

Splicing functional exploration

To assess splicing alterations, minigenes were constructed using pSpliceExpress vector [28]. Each minigene included 286 bp of the 3' terminal region of intron 5, the full sequence of exon 6, and the first 480 bp of intron 6 (Supplementary Fig. 1). Wild-Type (WT) and mutant DNA fragments were cloned into separate minigene constructs.

To further investigate potential splicing defects, RT-PCR (reverse transcription PCR) was carried out. Total RNA was extracted using RNable (Eurobio) on primary amniotic fluid culture cells, following the manufacturer's protocol, and RT-PCR analysis was performed using random primers, M-MLV Reverse Transcriptase (Promega) and AmpliTaq Gold DNA polymerase (Thermo Fisher Scientific). PCR primers are listed in supplementary table 1.

Western blot

For the detection of BMP1 and mTLD in cell supernatants, protein concentrations were measured with the Coomassie Plus Reagent (Pierce) with bovine serum albumin diluted in culture medium as a standard. Seventeen micrograms of total protein per lane were loaded in the gel after precipitation with 10% trichloroacetic acid and washing of the pellet with ice-cold acetone. Recombinant BMP1 and mTLD proteins, expressed in 293-EBNA cells and purified as previously described [29], were used as controls

(20 and 24 ng respectively). For the detection of procollagen I cleavage products, only 0.5 µg/lane was used with no precipitation.

Proteins were separated by SDS-PAGE in 25 mM Tris pH 8.5, 0.2 M Glycine, 0.1% SDS buffer (Euromedex) and electroblotted for 2 h onto polyvinylidene difluoride (PVDF; 0.45 µm) membranes (Millipore) in 10 mM CAPS (N-cyclohexyl-3-aminopropanesulfonic acid) pH 11 with 10% ethanol. UV activation was performed on the acrylamide gel containing 2,2,2 trichloroethanol for 30 s using a Fusion FX system (Vilber Lourmat) prior to electroblotting to allow the visualisation of transferred proteins (Stain-Free detection) on the PVDF membrane. Ten percent skim milk in PBS for 1 h was used as blocking buffer before overnight incubation at 4°C with primary antibodies. After three washes in PBS containing 0.05% Tween 20, horseradish peroxidase (HRP)-coupled secondary antibodies were added for 1 h at room temperature. Proteins of interest were detected by chemiluminescence with Amersham ECL Select Western blotting detection reagent (Cytiva) using the Fusion camera (with the FusionCapt Advance FX7 16.16b software). When applicable, quantification of band intensities was performed with the ImageQuant TL software v8.2 (Cytiva).

BMP1 and mTLD were detected with the MAB1927 rat monoclonal antibody (1/1,000; Bio-Techne), procollagen I C-propeptide with the LF-41 rabbit polyclonal antibody (1/1,000; kind gift of Larry fisher, [30], procollagen I N-propeptide with the SP1.D8 mouse monoclonal antibody (1/1,000; DSHB Cat# SP1.D8, RRID:AB_528438).

Recombinant proteins

Mini-procollagen I sequence, production and purification were as described in Mariano *et al.* [31]. Native human thrombospondin-1 (TSP1) was purified from human platelets as described in Anastasi *et al.* [10]. Human recombinant chordin was purchased from Bio-Techne [32]. BMP1 with a C-terminal FLAG tag (WT-FLAG) was obtained as reported [33] and the same construct in pCEP4 vector was used as a template to insert the Arg279Gly mutation using NEB's Q5 Hot PCR site-directed mutagenesis kit, according to the manufacturer's protocol (Arg279Gly-FLAG). Overlapping oligonucleotides (forward sequence: CAC ATT CTC CGG GGG CAT CTT; reverse sequence: TTC CGA GCG TAA TGC ATG) were purchased from Sigma. The correct insertion of the mutation was confirmed by sequencing (Microsynth). The resulting plasmid was transfected in 293-EBNA cells using X-tremeGENE HP DNA (Merck) as a transfection agent and transfected cells were selected with 300 µg/ml hygromycin B (Merck) for 3 weeks. Then, cells were plated in CellSTACK culture chambers (6,360 cm²; Corning) with complete medium (DMEM high glucose with stable glutamine and sodium pyruvate, 10% foetal calf serum, 300 µg/ml hygromycin B). At confluency, they were switched to serum-free DMEM with 50 µg/ml gentamycin and conditioned medium was collected after 48 h, supplemented with 0.25 mM AEBSF (Merck) and 2 mM N-Ethyl-maleimide (Merck) and centrifuged for 10 min at 10,000 g.

The BMP1 mutant (Arg279Gly-FLAG) was first purified on a Reactive Red 120-agarose (Sigma) column equilibrated in Buffer A (20 mM HEPES, pH 7.4, 2.5 mM CaCl₂) with 0.15 M NaCl. The protein was eluted

in Buffer A containing 2 M NaCl and 25% ethylene glycol and dialyzed against Buffer A with 0.5 M NaCl. Then, it was loaded onto an Anti-FLAG M2-agarose affinity column (Sigma) equilibrated in Buffer A with 0.5 M NaCl and 0.02% octyl- β -D-glucopyranoside (β OG) and eluted with 0.25 mg/ml FLAG peptide dissolved in the same buffer. After removal of the FLAG peptide by dialysis, the protein was flash-frozen and stored at -80°C .

In vitro cleavage assays

All cleavage assays were performed at 37°C in 50 mM HEPES pH 7.4, 5 mM CaCl_2 and 0.02% β OG. The fluorescence emitted during the cleavage of the fluorogenic peptide Mca-YVADAPK(Dnp)-OH (20 μM , Covalab, France) was recorded for 20 min (excitation: 320 nm; emission: 390 nm) in a Tecan Spark fluorimeter equipped with the SparkControl software v5.2.4. The slope of the curve was used to calculate BMP1 (WT-FLAG or Arg279Gly-FLAG) activity. All other cleavage assays were performed in a total volume of 30 μl by mixing the protease and its substrate at the concentrations and for the duration indicated in figure legends. The reactions were stopped by adding Laemmli buffer with 10 mM DTT and boiling for 5 min at 95°C . Cleavage products were analysed by SDS-PAGE with home-made or commercial 4–20% Mini-Protean (Bio-Rad) acrylamide gels.

Protein modelling

The BMP1 Arg279Gly mutant was modelled with AlphaFold 2 [34] and superimposed on the crystal structure of BMP1 catalytic domain (PDB 6BSM) with ChimeraX [35] and BPM1 co-crystal structure with substrate interaction [36]

RESULTS

Clinical information

A foetus with severe IUGR identified on ultrasound imaging at Gestational Week (GW) 23 + 2 days, associated to microcephaly, microretrognathia, abnormal cardiac axis and bilateral clubfoot, was referred to our multidisciplinary centre of prenatal diagnosis. At the first trimester, ultrasound screening, including nuchal translucency measurement and maternal serum biomarkers, was unremarkable, without any obvious morphological anomalies. The primigravid mother of French origin was 23 year old and the father, of Iraqi origin, was 36 year old. The parents were healthy, non-consanguineous, and had no history of exposure to toxins or medications. There was no known family history of skeletal disorders, such as recurrent fractures. Medical termination of pregnancy was performed at GW 25 + 1. Thereafter, foetal autopsy confirmed a female, small foetus for gestational age, weighing 555 g (5th percentile for GW 25, 750 g expected). Microcephaly of at least 3.5 standard deviation was reported, with abnormal Sylvian fissures, brachycephaly, and bilateral temporal lobe hypoplasia. External craniofacial examination showed hypertelorism, exophthalmos, low-set and posteriorly rotated ears, smooth philtrum, oblique eyelid fissures, microretrognathia and an ogival palate. Dextrocardia was observed without associated

structural cardiac anomalies. Bilateral clubfoot was also present (Fig. 1). In addition, some early slight deformities of long bones were identified on skeletal radiography, including small curved femora and irregular tibiae (Fig. 1). Long bone lengths measurements corresponded to an estimated GW 23. Macroscopic cranial bone fractures were also reported. Bone mineralisation appeared to be normal although osteodensitometry was not available. Bone histology did not support a diagnosis of foetal osteoporosis. Fragility of skin and others tissues was underlined during foetal examination, without reliable histological characterisation.

Placenta examination revealed no specific pathological features on either gross or histological analysis.

Identification of a novel likely-pathogenic variant in BMP1 gene

Prenatal diagnosis detected no foetal abnormalities in chromosomal number or structure. However, trio-WES involving foetus and both parents identified, a homozygous missense variation in exon 6 of the *BMP1* gene (NM_006129.5: c.835A > G, p.(Arg279Gly)). This variation was absent in gnomAD v4.1.0 reference database, suggesting a novel extremely rare alteration in the *BMP1* gene (Fig. 2A). Both parents were heterozygous carriers of this variation. Foetal and parental genotypes were confirmed by Sanger sequencing (Fig. 2B).

This missense variation is localised in a mutational hotspot affecting the functional protease domain of BMP1/mTLD (astacin-like metalloprotease domain). Most of *in silico* prediction tools, based on several meta-scores including MetaRNN (score: 0.8637; prediction: pathogenic moderate), Mistic (0.74; damaging), BayesDel addAF (0.3282; pathogenic moderate), ClinPred (0.976 ; damaging), argued for a pathogenic missense variant which may destabilise protease three-dimensional structure. Based on the crystal structure of the catalytic domain of BMP-1, Arg279 is located on a flexible surface loop, near the entrance of the active site cleft of the enzyme. AlphaFold2 modelling showed that its mutation to glycine should not alter the overall folding but is likely accompanied by loss of hydrogen bonds / polar interactions with residues Glu239 and Ile241 (Fig. 2C).

Moreover, this variation affects the penultimate base of exon 6 and several splicing predictions tools (MaxEntScan, NNSplice, GeneSplicer, SPIP, dbSCSNV) suggested disruption of the adjacent donor splice site. According to ACMG guidelines [37], the variant c.835A > G / p.(Arg279Gly) could be classified as likely-pathogenic variation : moderate pathogenic criterion 1 and 2 (PM1 and PM2), supporting pathogenic criterion 3 (PP3).

Impact of c.835A > G on splicing mechanism

Due to the position of the mutation at the penultimate nucleotide of exon 6, a potential effect of c.835A > G on RNA splicing was evaluated using a combination of *in silico* prediction tools and minigene assays. Splice sites were analysed across the entire exon 6 and intron 6 sequences of the *BMP1* gene using multiple algorithms, including MaxEntScan, NNSplice, GeneSplicer, SPIP, and dbSCSNV. Most algorithms predicted a strong alteration of the native donor splice site in intron 6 and no relevant alternative cryptic

donor sequence. These data suggested exon skipping, ultimately leading to a shift of the open reading frame.

In the minigene assay, RT-PCR amplification of the wild-type construct yielded a single 367 bp product (Fig. 2C), consistent with expected splicing. In contrast, the mutant minigene construct carrying the c.835A > G variant produced a single 261 bp product, corresponding to alternative splicing between exons 2 and 3 of the rat insulin gene contained in pSpliceExpress, indicating exon 6 skipping.

To investigate whether any residual splicing at the intron 6 natural donor splice site could occur, a nested PCR was performed using primers targeting exon 2 of the rat insulin gene and exon 6 of *BMP1*. This amplification yielded a 279 bp product in both wild-type and mutant conditions (Fig. 2D1), suggesting that the natural donor site of intron 6 remained partially functional, although likely used at a very low frequency. This residual splicing would result in the inclusion of exon 6 and preservation of the Gly279 codon in a minority of transcripts.

As exons 5 and 7 of *BMP1* are not in frame, skipping of exon 6 would generate a frameshift and a premature termination codon in exon 7, expected to trigger nonsense-mediated mRNA decay (NMD). To assess this mechanism in a physiological context, RT-PCR was performed on RNA extracted from cultured foetal amniotic fluid cells. The predominant transcript lacked exon 6 (Fig. 2D2), consistent with the minigene assay results. However, in contrast to the *in vitro* system, a significant proportion of the full-length transcript containing exon 6 was also detected. Direct sequencing of the RT-PCR products obtained from both wild-type and mutant foetal cells confirmed the presence of alternative splicing events involving exon 6 (Fig. 2F).

Biochemical characterisation of the BMP1/mTLD p. (Arg279Gly) variant

Amniotic cells from the proband's foetus were cultured and compared to control cells under the same conditions. Proband's foetus-derived cells exhibited slower proliferation rates, although sufficient amounts of conditioned medium were collected for protein analysis.

Western blot of control cell supernatants revealed that the major secreted product of the *BMP1* gene was the longer isoform mTLD. In two out of three controls, additional minor bands corresponding to BMP1 were also detected (Fig. 3A). In contrast, no BMP1-derived protein was detectable in the supernatant of proband's cells, suggesting either an absence of protein production or levels below the detection threshold.

Procollagen I processing was then assessed by immunoblot using antibodies specific to its C-propeptide and pN-collagen cleavage products (Fig. 3B). While the amount of unprocessed procollagen appeared similar between proband and control cells (except for one control displaying higher secretion), the expected cleavage products were markedly reduced in the proband cells. Quantification of C-propeptide

and pN-collagen confirmed the reduced extent of processing, consistent with a global decrease in collagen maturation in the proband's conditioned medium.

To determine whether this defect could be explained solely by the absence of BMP1/mTLD isoforms or by a loss of enzymatic function of the p.(Arg279Gly) variant, further analyses were conducted. Wild-type (WT-FLAG) and mutant (Arg279Gly-FLAG) BMP1 proteins were expressed in 293-EBNA cells and purified to homogeneity (Fig. 4A). During production, it was noted that cells transfected with the Arg279Gly-FLAG construct remained adherent, unlike those expressing wild-type BMP1, suggesting a reduced proteolytic activity of the mutant protease. To confirm this, their enzymatic activity was evaluated *in vitro* on representative substrates.

Using a fluorogenic peptide containing a canonical BMP1 cleavage motif, the concentration of Arg279Gly-FLAG mutant needed to achieve the same level of cleavage as with the wild-type enzyme was two-fold higher (Fig. 4B). This reduced activity was confirmed on human substrates closer to native substrates, including a recombinant mini-procollagen I, TSP1, and recombinant chordin (Fig. 4C-E). Processing of mini-procollagen I and TSP1 by the Arg279Gly-FLAG mutant was diminished by at least twofold compared to wild-type, while chordin cleavage remained partially preserved but suboptimal.

These results collectively suggest a dual mechanism combining decreased protein production and impaired proteolytic activity of the BMP1: p.(Arg279Gly) variant.

DISCUSSION

To date, approximately twenty-five individuals harbouring *BMP1* variants have been reported in the literature and reference databases. Most of them developed a severe form of OI during childhood or later in life. This occurred despite an initially relatively normal general habitus and neuromotor development. The typical clinical phenotype includes multiple fractures, bone deformities, short stature and scoliosis at older ages, despite normal or increased bone mineral density. Other rarer features such as muscle weakness, dysmorphic facial features, blue sclerae (which tends to become white with age), and motor delays have also been described [11–15]. Here, we report the clinical, molecular, and functional characterisation of a novel *BMP1* variant in a foetus presenting with a severe polymalformative phenotype at GW 23 + 2. Beyond the detection of a severe IUGR, during the second trimester, using ultrasound, the foetus exhibited bilateral clubfoot, slight deformities of long bones, macroscopic fractures of cranial bones associated with a severe microcephaly and brachycephaly, and bilateral temporal lobe hypoplasia. Facial dysmorphic features were also observed. No intrauterine fracture was documented at this early foetal development stage. While a formal osteodensitometry could not be performed, the visual analysis of the radiographs and bone histology did not reveal features suggestive of osteoporosis.

This severe antenatal phenotype was associated with a homozygous variant in the *BMP1* gene: c.835A > G located within the protease domain. This variant exerts a complex dual molecular effect, introducing a missense change while also disrupting normal splicing and resulting in the skipping of exon 6. Although

the aberrant transcript introduces a frameshift and a premature stop codon in exon 7, a minor but significant proportion of transcripts retaining exon 6 is still expressed, thereby encoding the missense variant p.(Arg279Gly). To date, no similar homozygous truncating variants in *BMP1* gene had been reported. In fact, all truncating variants previously described occurred in compound heterozygosity, typically alongside a missense variant on the second allele [13].

Like the five previously reported variants located within the BMP1 protease domain [13, 14], the novel homozygous c.835A > G (or p.(Arg279Gly)) variant could be classified as likely-pathogenic variation according to ACMG guidelines [37]. This homozygous variant is also very likely to result in miscarriage or early neonatal death.

The incomplete exon skipping allows a residual expression of the full-length BMP1 and mTLD mRNA carrying the p.(Arg279Gly) substitution in the proband's foetus although the corresponding proteins were below the detection threshold in our western blot assay. To test the activity of a possible residual amount of mutant proteases, three major substrates were assayed with the Arg279Gly mutant, including a model substrate derived from procollagen I, TSP1 and chordin. These substrates play important roles in developmental patterning, organogenesis and response to injury [5, 38, 39]. In vitro assays revealed that the cleavages of mini-procollagen I and TSP1 by Arg279Gly-FLAG were reduced by at least twofold compared to wild-type BMP1 control. Chordin cleavage was also impaired although partially preserved. Together, the results support a dual mechanism combining decreased protein production and impaired proteolytic activity of the Arg279Gly mutant, that may support the range and severity of the observed phenotype despite the presence of some residual activity.

While previous studies have mainly focused on the role of BMP1 in procollagen maturation and extracellular matrix assembly [11, 15], our results suggest that its significant decrease may also disrupt key embryological processes beyond collagen processing. Notably, BMP1 is essential for the proteolytic cleavage of chordin, a major extracellular antagonist of BMP2/4/7 signalling [40]. In the early embryonic development, this cleavage plays a pivotal role in establishing the dorsal–ventral axis and regulating BMP gradients critical for tissue patterning. The persistence of uncleaved chordin due to BMP1 deficiency may therefore lead to aberrant BMP/TGF- β signalling dynamics, potentially contributing to the complex developmental anomalies observed in our case [38, 40, 41]. These observations underscore the need for further functional studies to explore how impaired regulation of BMP signalling, through defective chordin processing, may underlie part of the prenatal phenotype associated with BMP1 deficiency. In this context, BMP1 should be considered not only as a procollagen C-proteinase, but also as a key modulator of morphogenetic signalling pathways during early human development.

Interestingly, the Arg279 residue lies within the astacin-like catalytic domain that is conserved across the BMP1/tolloid-like protein family including BMP1, mTLD but also two other mammalian proteins encoded by the *TLL1* and *TLL2* genes which also contribute to various extents to the cleavage of the C-terminal propeptide from procollagens [5, 7]. A knock down of TLL1 in mice has also been associated with lethal cardiac or skeletal phenotypes in embryos [42].

Limitations

Although no experimental structure of BMP1 in complex with a natural substrate is currently available, its position near the entrance of the active cleft suggests that Arg279 could contribute to the correct folding of the access tunnel and/or to substrate recognition. The observed decreased activity toward multiple substrates in our assays supports this hypothesis.

CONCLUSION

Our findings support the involvement of a biallelic BMP1 variant in a syndromic presentation characterized by growth restriction, bone and connective tissue anomalies. The identification of this homozygous variant with combined effects (missense and splicing) broadens the known phenotypic spectrum of BMP1-related disorders, particularly in the prenatal setting. Our case highlights the relevance of including *BMP1* in prenatal diagnostic gene panels for the differential diagnosis of early-onset and severe polymalformative foetal syndromes.

Declarations

Ethics approval and consent to participate

Clinical and genetic data were retrospectively extracted from medical records and from investigations performed as part of standard clinical care. Written informed consent for genetic analyses was obtained from the parents. This study was conducted in accordance with the French MR-004 reference methodology, approved by the CNIL Délibération n° 2018 – 155 du 3 mai 2018, governing the processing of personal data for research purposes in the context of research not involving the human person and based on data collected during routine care. As the study was non-interventional and did not fall within the scope of Article L.1121-1 of the French Public Health Code, formal approval from an institutional review board or research ethics committee was not required.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

XLGH and CD contributed equally to this work. XLGH conceived the study, collected and interpreted the clinical and genetic data, and wrote a substantial part of the manuscript. CD assembled, synthesised and interpreted all data from the functional experiments, and wrote the sections relating to the functional studies. SVLG, CM, MB, PL, QRP and FB contributed to clinical, molecular and/or biological data acquisition and interpretation. AM contributed to manuscript revision and stylistic editing. All authors critically revised the manuscript, approved the final version, and agree to be accountable for all aspects of the work.

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Data Availability

The datasets generated and/or analysed during the current study are not publicly available because they concern an individual rare prenatal case and public sharing could compromise participant privacy. Relevant data supporting the findings of this study are included in this published article and its supplementary information files. Additional de-identified data are available from the corresponding author on reasonable request, subject to applicable French legal and ethical restrictions.

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Figures

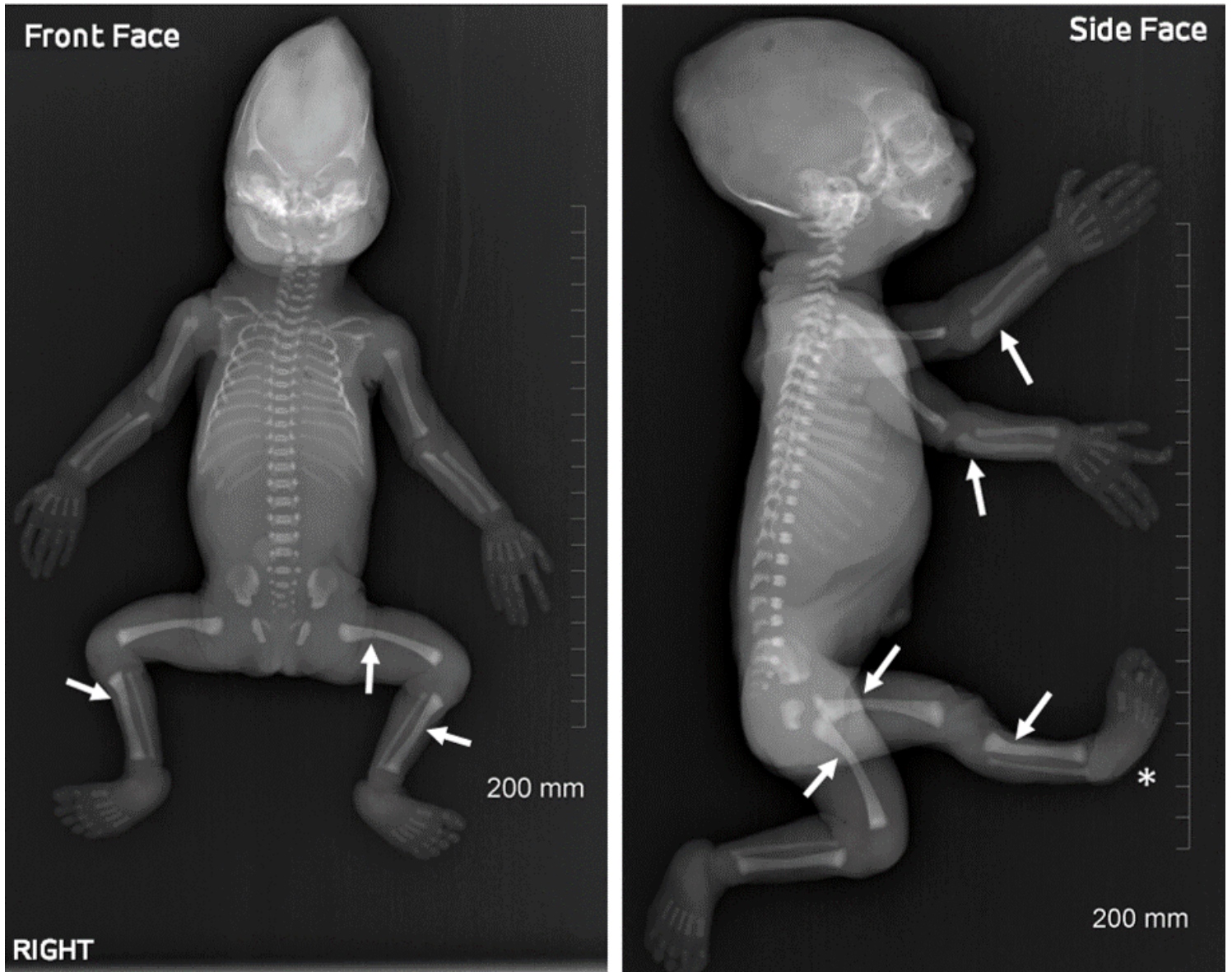


Figure 1

X-ray radiographs of the foetus at 25 weeks and 1 day of gestation. The radiographs display several skeletal anomalies: bilateral clubfoot (indicated by asterisks, *), and irregular and curved long bones (highlighted by arrows)

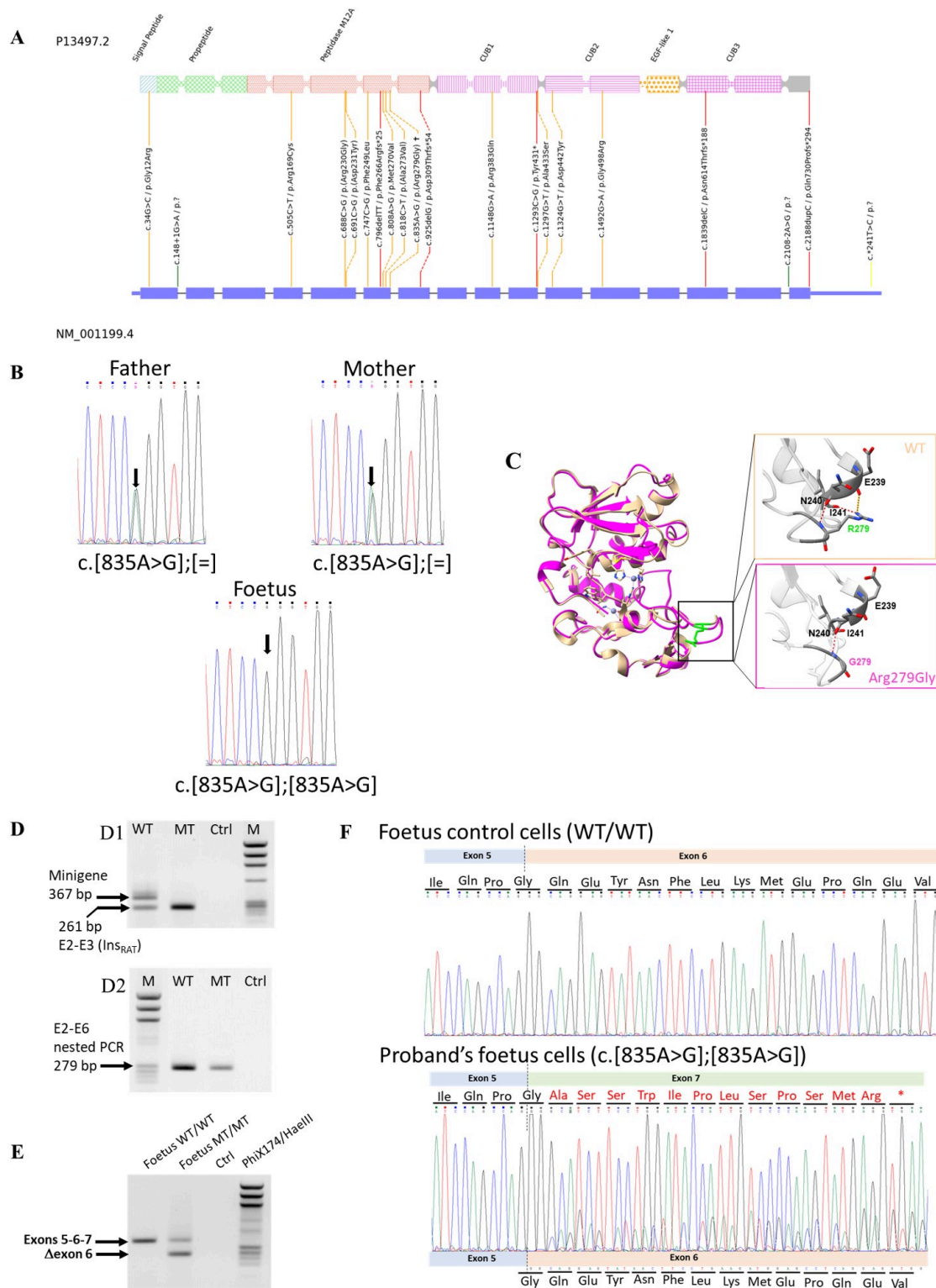


Figure 2

(A) Schematic representation of the BMP1 transcript and protein with variant locations. The lower part shows the *BMP1* transcript (NM_001199.4) with its coding exons. In the upper part, the corresponding BMP1 protein (Uniprot P13497.2) is depicted. The positions of previously described pathogenic variants are indicated. Our novel identified variant is specifically marked by a dagger symbol (†), showing its location relative to the transcript and protein domains. **(B) Sanger sequencing of DNA samples from the**

foetus and parents. Chromatograms display the identified variant (i.e. c.835A>G) in a homozygous state in the foetus and in a heterozygous state in both parents, confirming its segregation within the family. **(C) Structural modelling of the BMP1 catalytic domain carrying the p.(Arg279Gly) substitution** Superimposition of the wild-type structure (beige) and the AlphaFold2 model of the mutant (pink) highlights the position of residue 279 near the entrance of the catalytic cleft. Insets show the local environment of Arg279 in the wild-type protein and the predicted consequences of substitution by glycine (residue Arg279 highlighted in green) **(D) Migration analysis of minigene constructs.** Migration of the PCR product amplified using forward and reverse primers targeting the rat insulin exons, demonstrated successful insertion of *BMP1* exon 6 between exons 2 and 3 of the rat insulin gene and an apparent BMP1 exon 6 skipping in mutated minigene construction (D1). A primary amplification was performed using primers within the rat insulin exons, followed by a nested PCR using primers specific to the inserted BMP1 exon 6, confirming the correct integration and presence of the exon within the minigene (D2). WT: Wild Type; MT: proband's foetus mutation BMP1: c.835A>G ; M: Marker. **(E) RT-PCR product migration of *BMP1* transcript.** RT-PCR was performed using primers located in BMP1 exons 5 and 7. The migration pattern demonstrates the presence of a transcript construct lacking exon 6, indicating aberrant splicing (exon 6 skipping). **(F) Sanger sequencing of RT-PCR product from the *BMP1* transcript.** Sequencing of the RT-PCR product from panel D reveals a frameshift induced by exon 6 skipping, resulting from small part of normal transcript construct and a majority of misread sequence

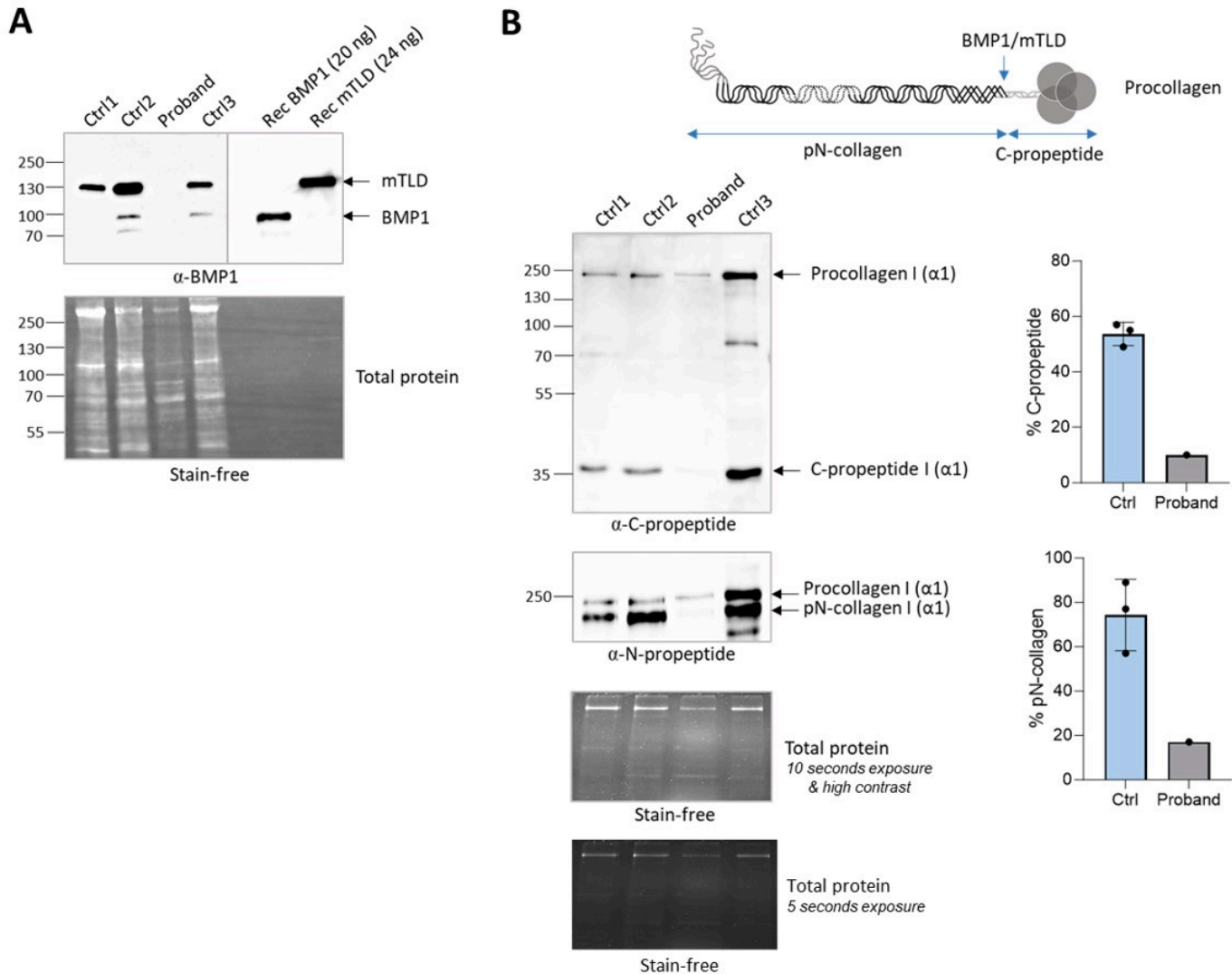


Figure 3

Immuno-detection of BMP1/mTLD and procollagen I in the conditioned medium of foetal cells. (A) Western blot analysis of BMP1 and mTLD in the supernatant of proband's foetus-derived cells and control cells (17 μ g total protein/lane), detected with the MAB1927 antibody (10 % acrylamide gel with stain-free reagent; reducing conditions). Recombinant BMP1 and mTLD (amounts as indicated) were loaded on the same gel but image acquisition was shorter to avoid signal saturation. **(B)** Western blot analysis of procollagen I and BTP-dependent cleavage products (see schematic; 0.5 μ g/ total protein/lane) in the foetal cell supernatants (10 % acrylamide gel with stain-free reagent; reducing conditions). Quantification of signal intensity with ImageQuantTL is also shown (% C-propeptide or pN-collagen are calculated relative to the sum of uncleaved procollagen and C-propeptide or pN-collagen)

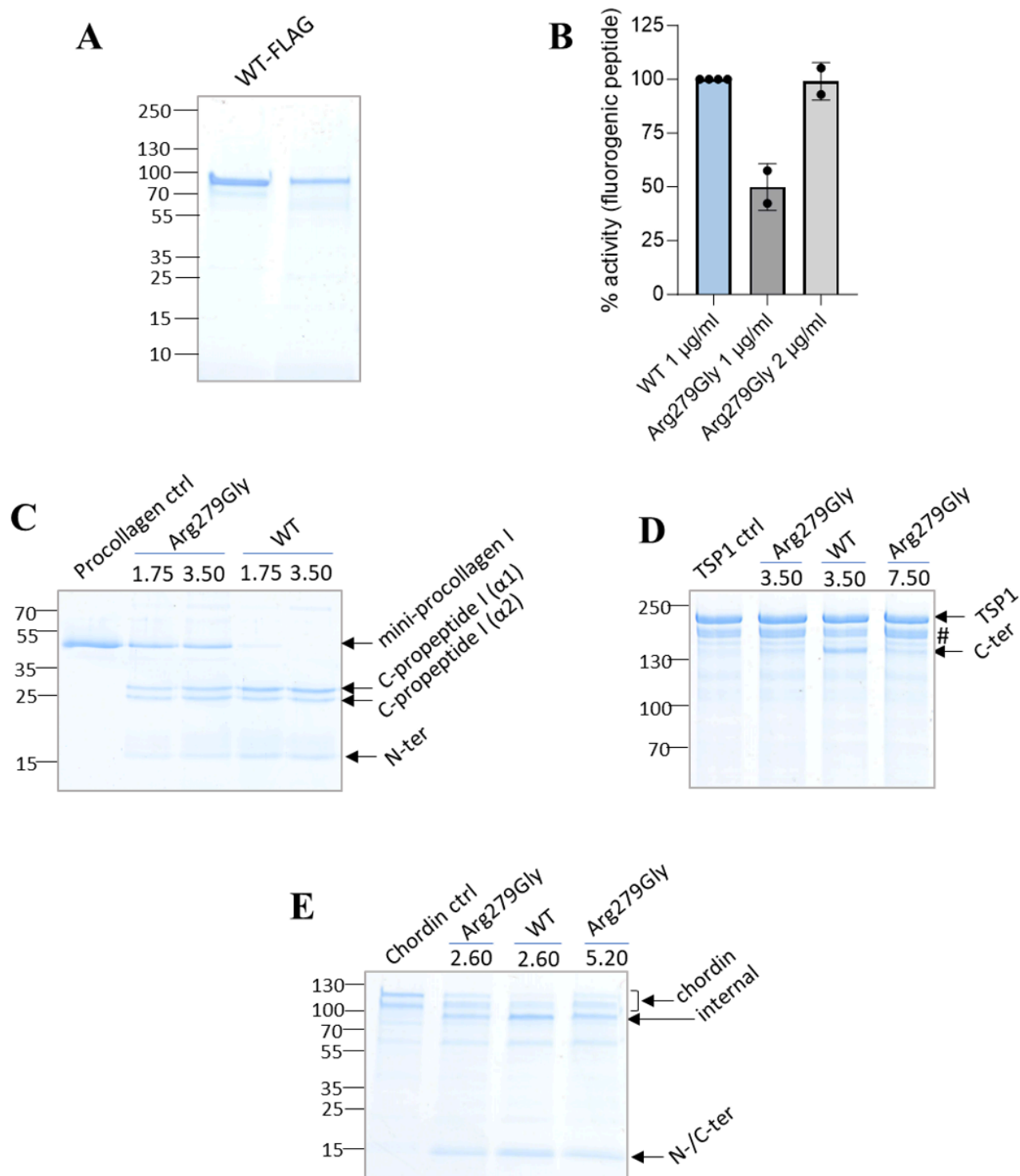


Figure 4

Production and activity of the p.(Arg279Gly) recombinant mutant of BMP1. (A) SDS-PAGE analysis of 1 µg of WT-FLAG BMP1 and its Arg279Gly-FLAG mutant after production in 293-EBNA cells and purification (4-20 % acrylamide; reducing conditions) (B) Comparison of the activity of BMP1 and Arg279Gly at the indicated concentrations with 20 µM of Mca-YVADAPK(Dnp)-OH fluorogenic peptide. (C-E) SDS-PAGE analysis of the cleavage of three substrates by WT-FLAG BMP1 and Arg279Gly-FLAG

(concentrations in µg/ml as indicated); (C) Cleavage of mini-procollagen I (350 nM) after 1 h (15 % acrylamide; reducing conditions), (D) Cleavage of TSP1 (513 nM) after 1 h (8 % acrylamide; reducing conditions). # degradation product of TSP1 due to processing by thrombin during platelet activation. (E) Cleavage of chordin (363 nM) after 1 h (4-20 % acrylamide gradient gel; reducing conditions)

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