The American Journal of Human Genetics, Volume *101*

Supplemental Data

Mutations in Fibronectin Cause a Subtype of Spondylometaphyseal Dysplasia with "Corner Fractures"

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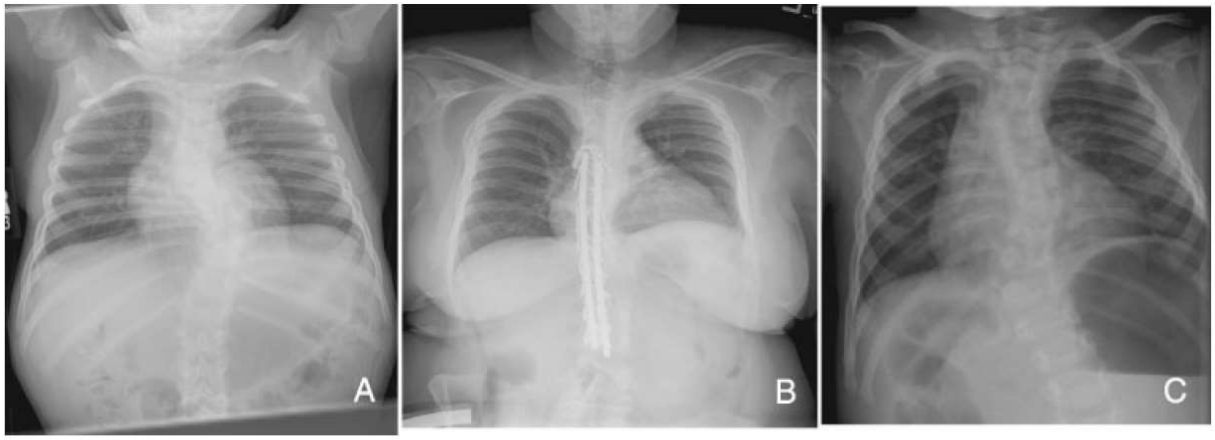
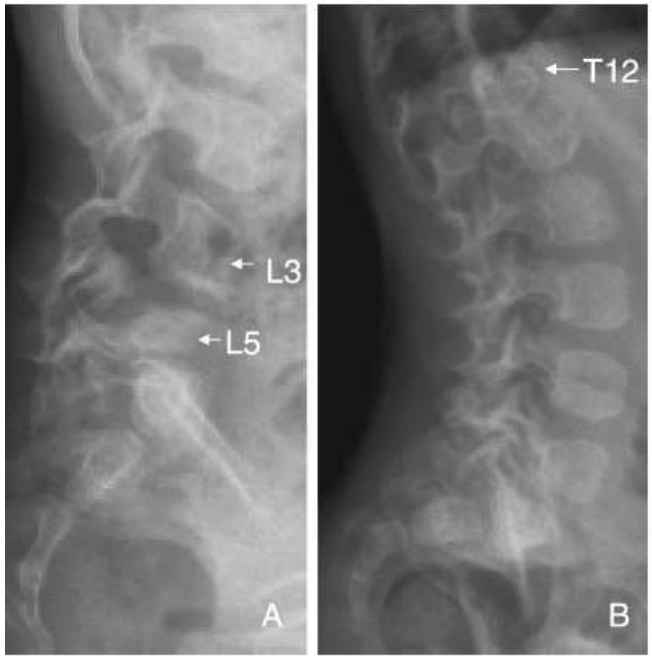
Figure S1. Radiographs of all individuals with FN1 mutations.

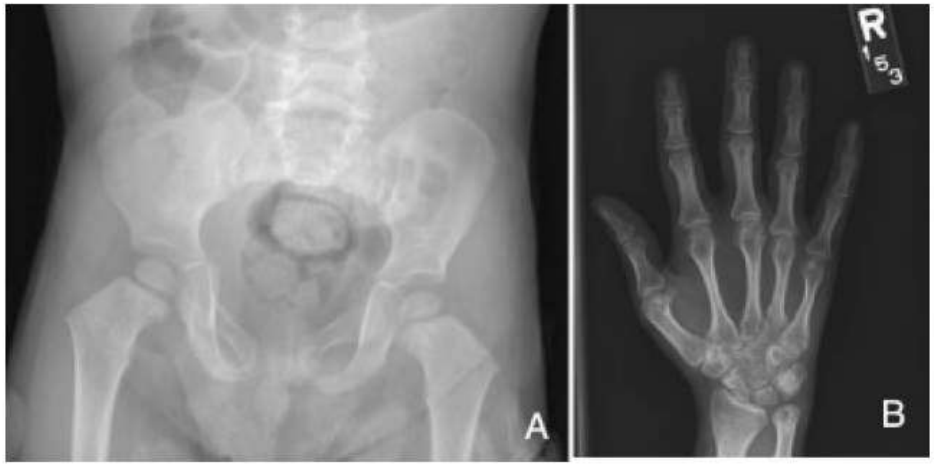
Family 1. Reproduced with publisher's permission from Sutton et al.[2]



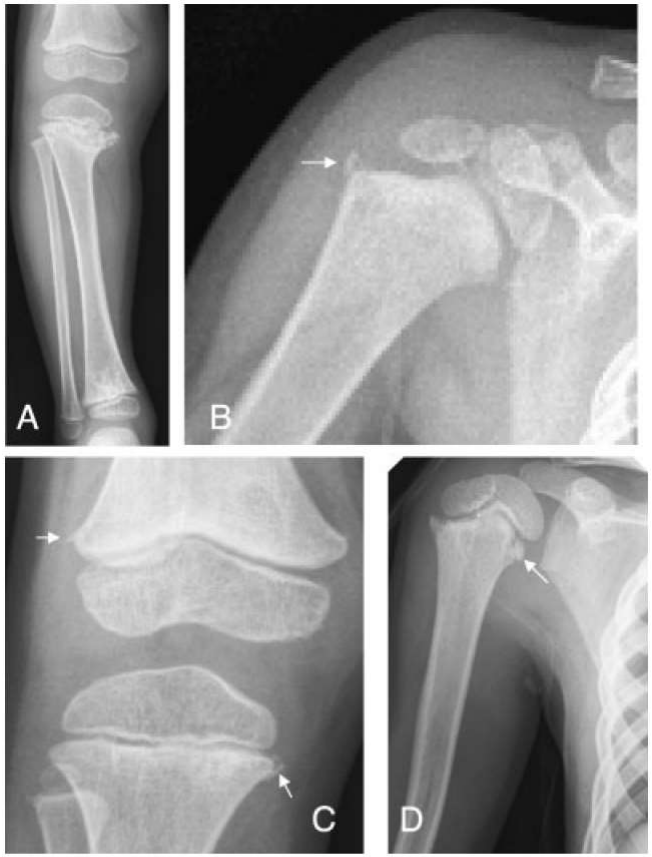
AP thoracic spine radiographs of the older boy (**A**), mother (**B**), and younger boy (**C**) showing severe scoliosis.



Lateral views of the lumbar spine. **A**: Younger boy at 3 years of age; L4 vertebral body is almost completely aplastic with only posterior elements present and L5 is hypoplastic. **B**: Older boy at 5 years of age showing hypoplasia of T12 and ovoid vertebral bodies. S1 has a triangular configuration.

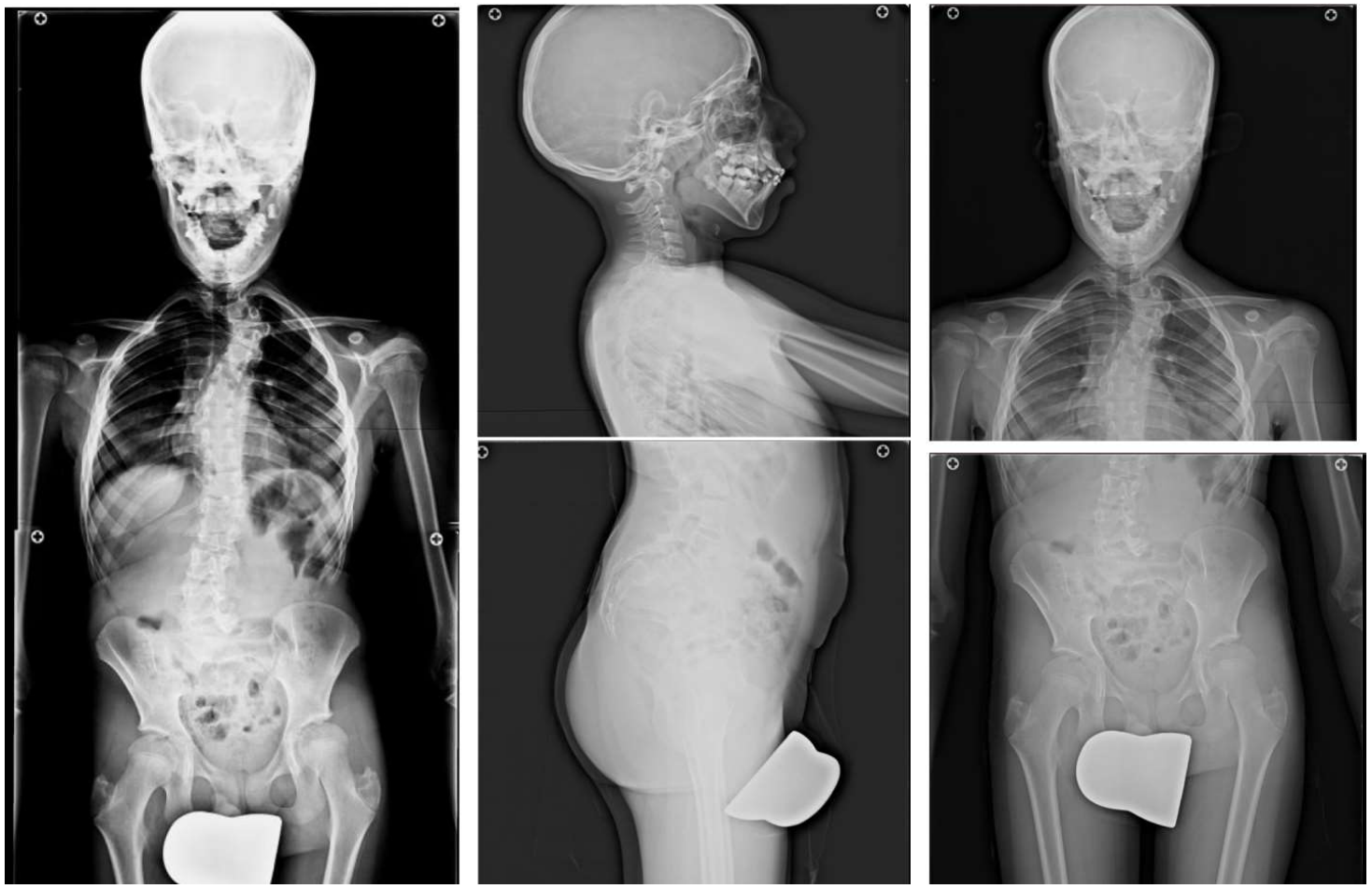


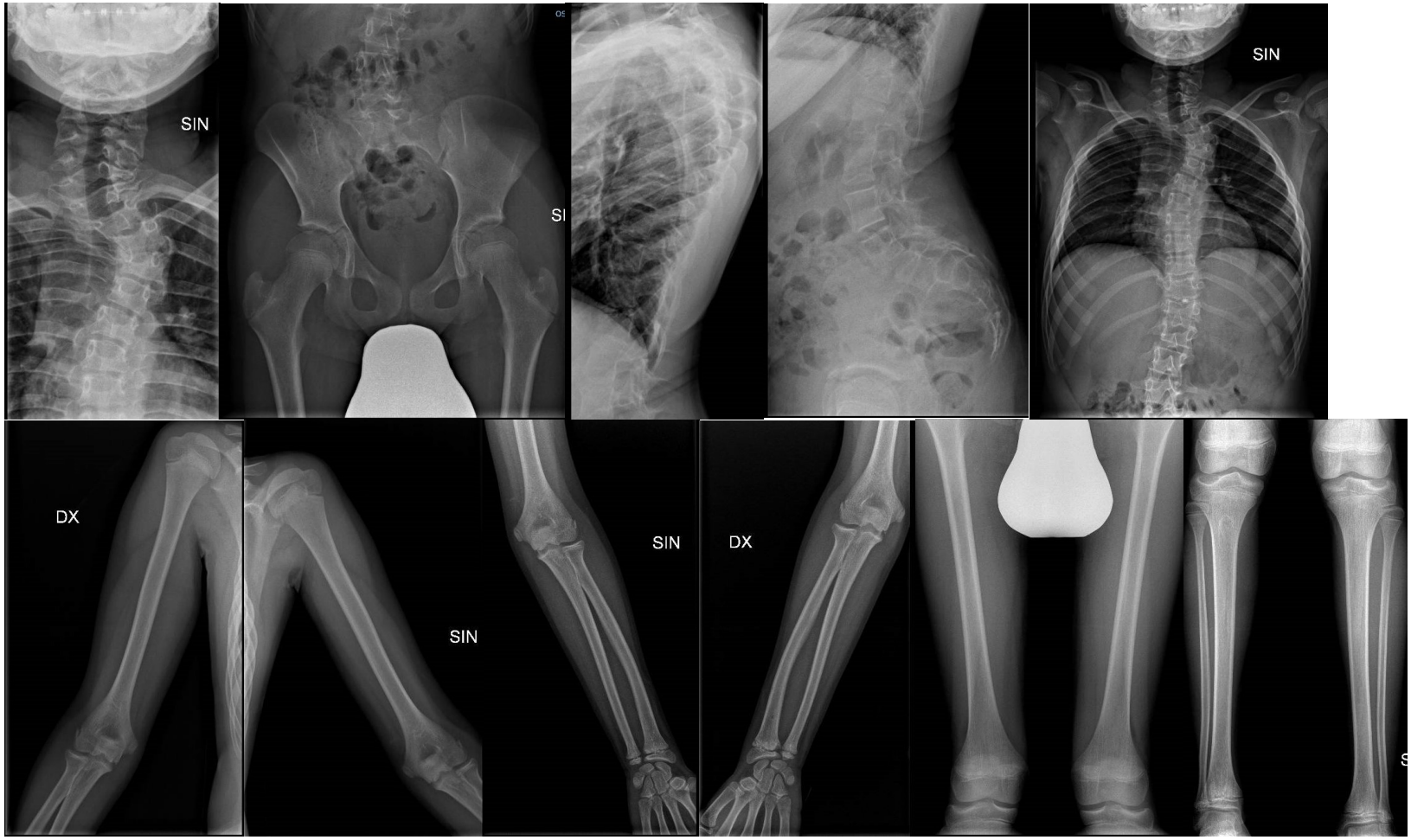
A: AP pelvis and hips demonstrating absence of coxa vara in older boy. **B**: Hand image of mother demonstrating absence of brachydactyly.



Arrows in the following panels point to metaphyseal fragmentation or "corner fractures". **A**: Right knee and leg radiograph of younger boy at 3 years of age showing metaphyseal dysplasia. **B**: Proximal humerus of younger boy at 3 years of age. **C**: Right knee of older boy at 5 years of age. **D**: Right shoulder radiograph of older boy at 5 years of age showing fragmented ossification and metaphyseal dysplasia.

Family 2

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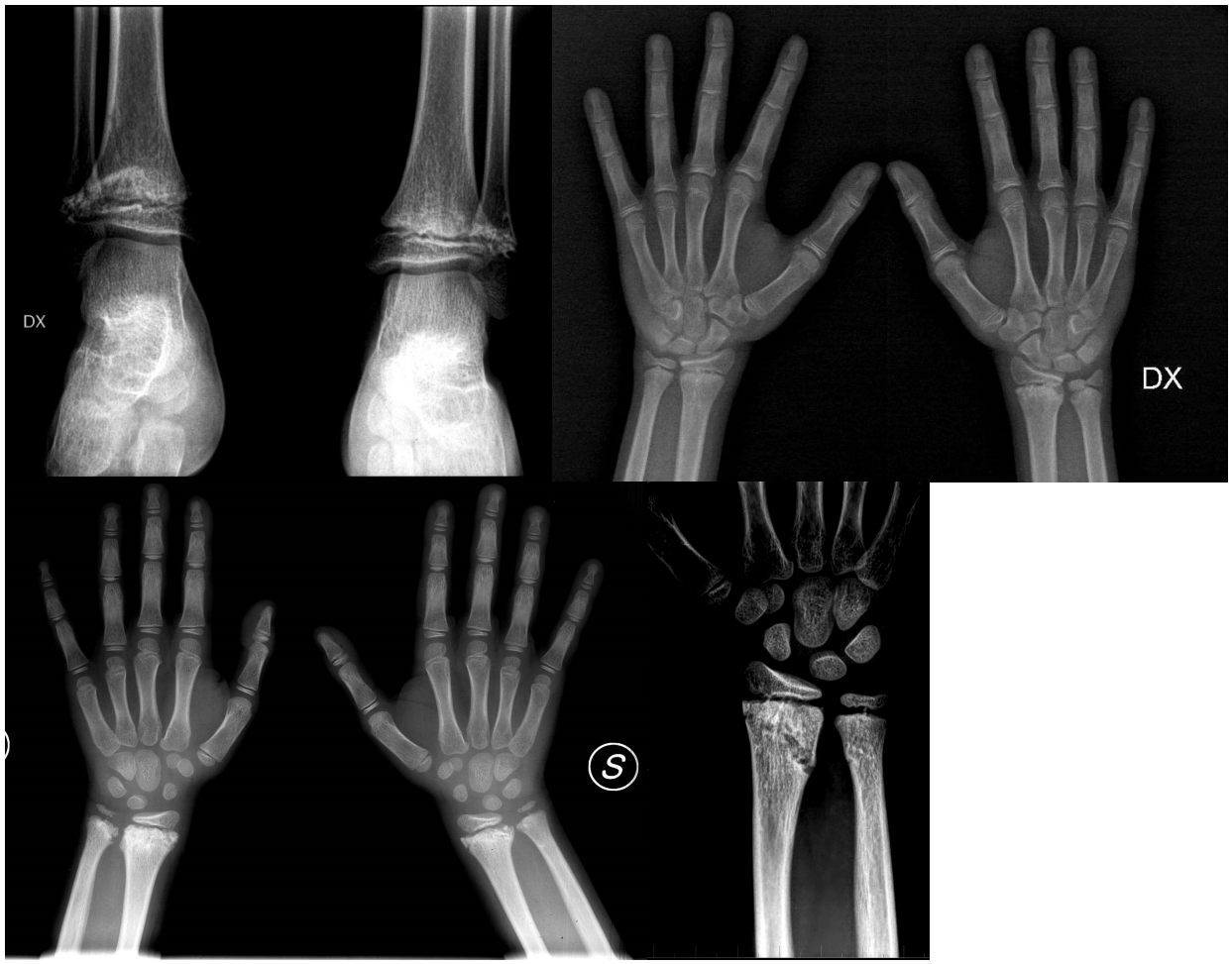
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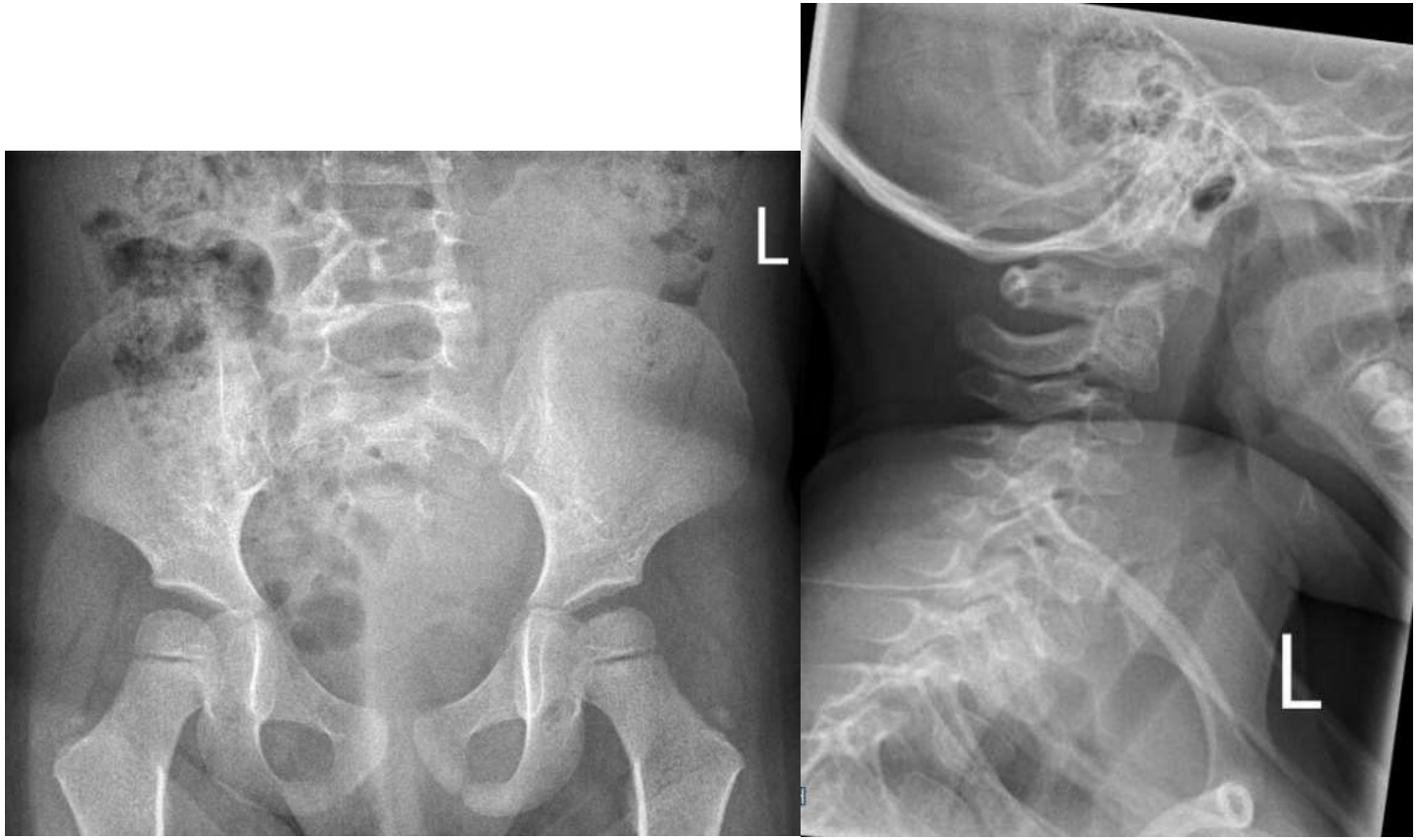

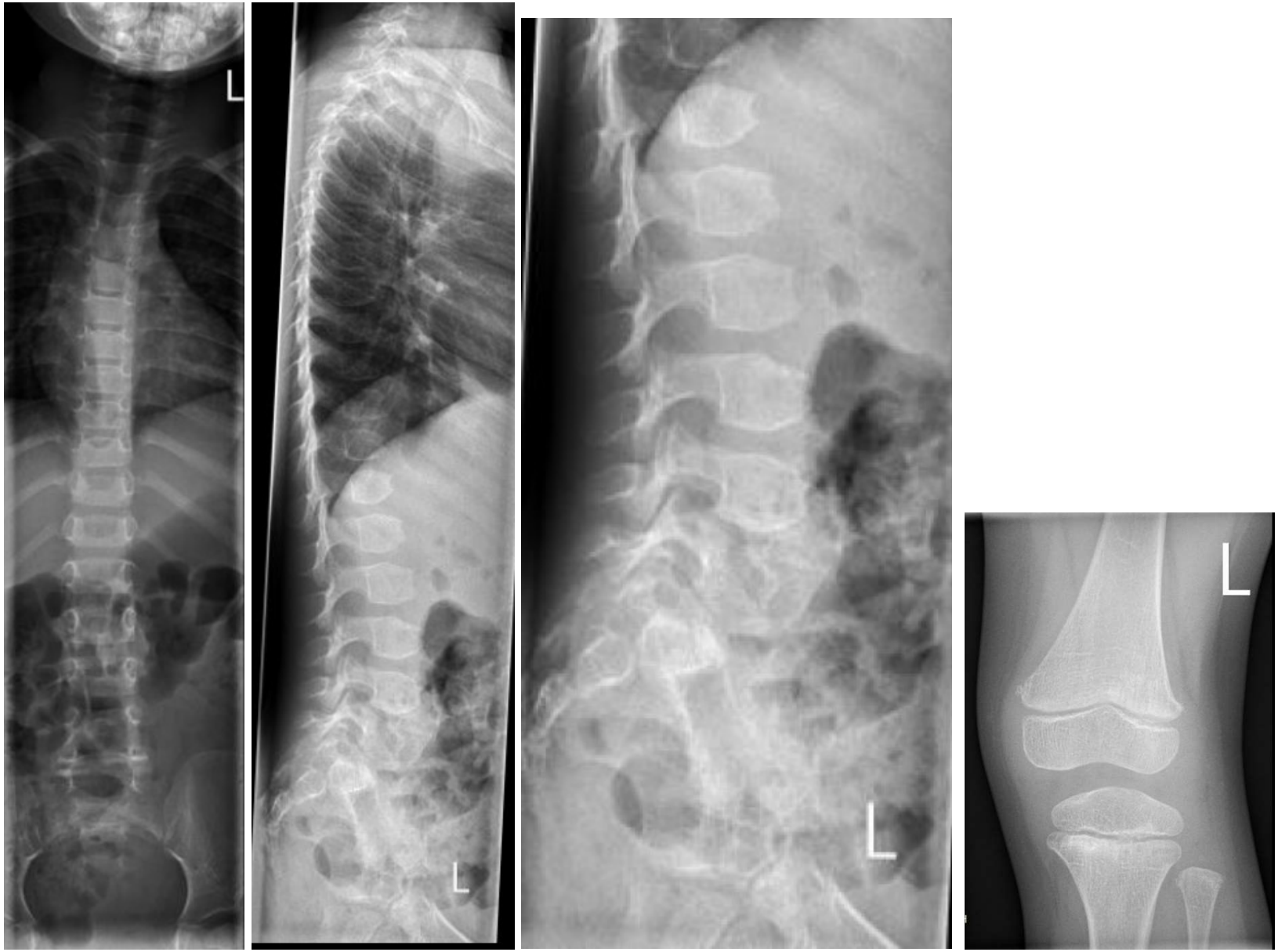
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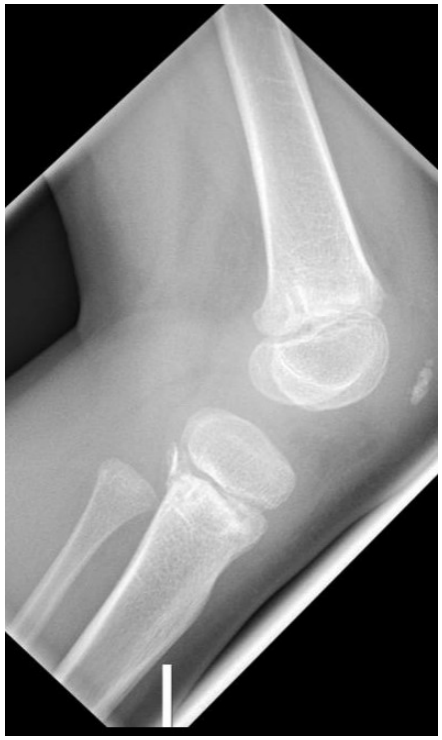
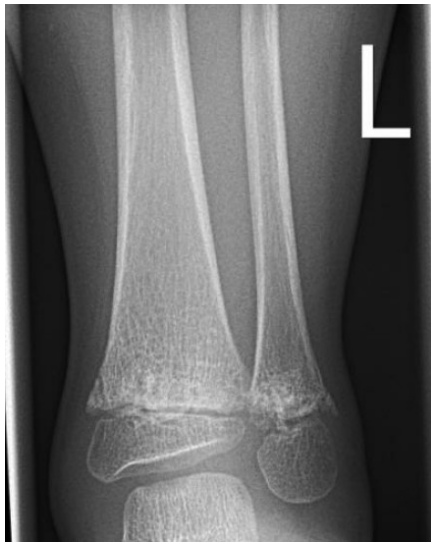
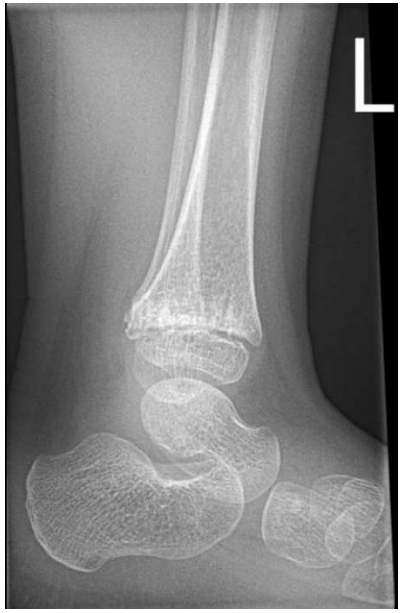
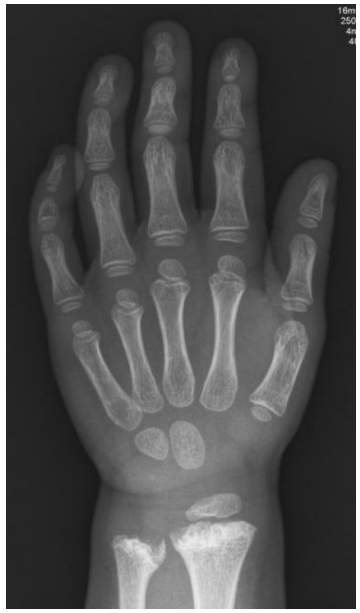

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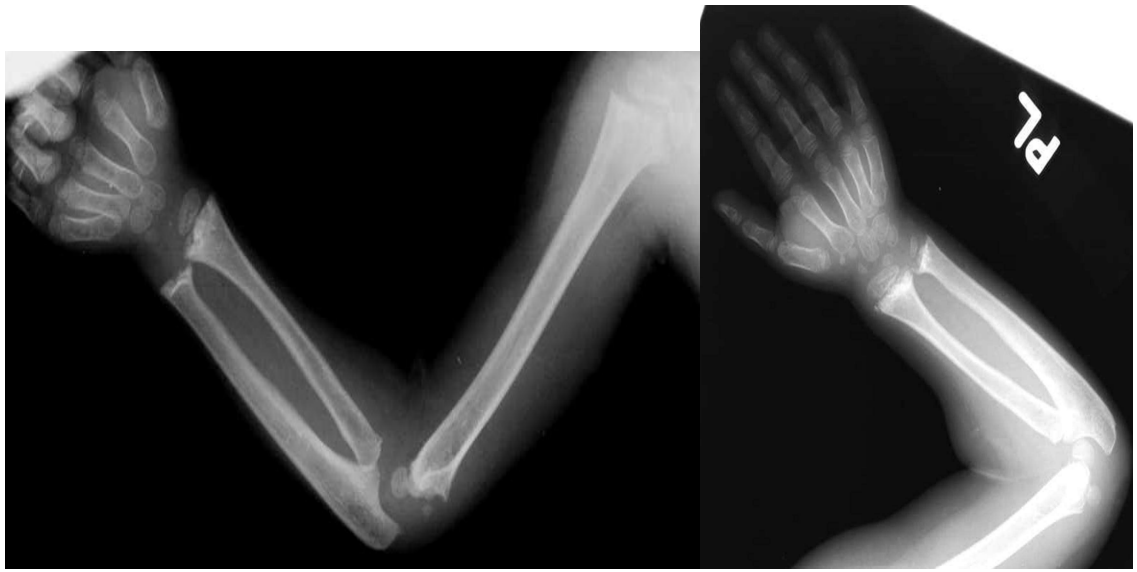

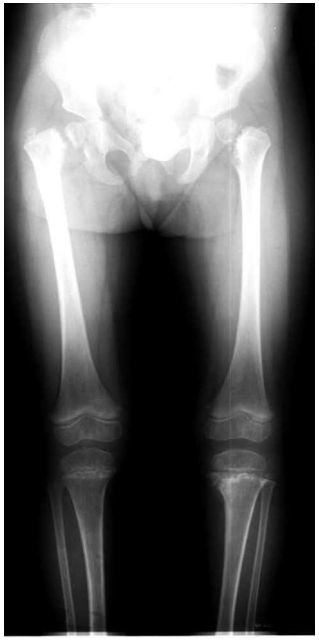
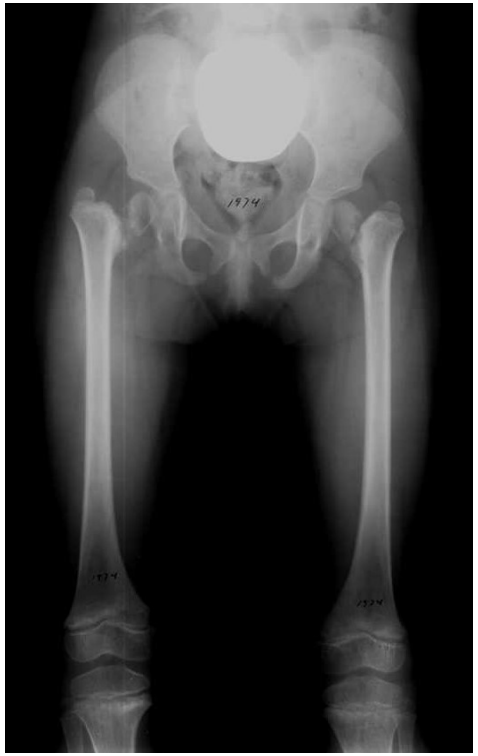
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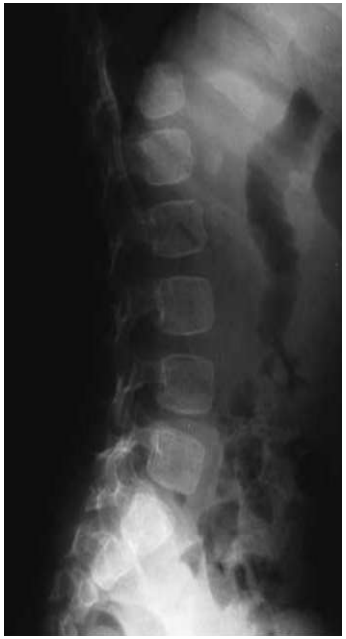
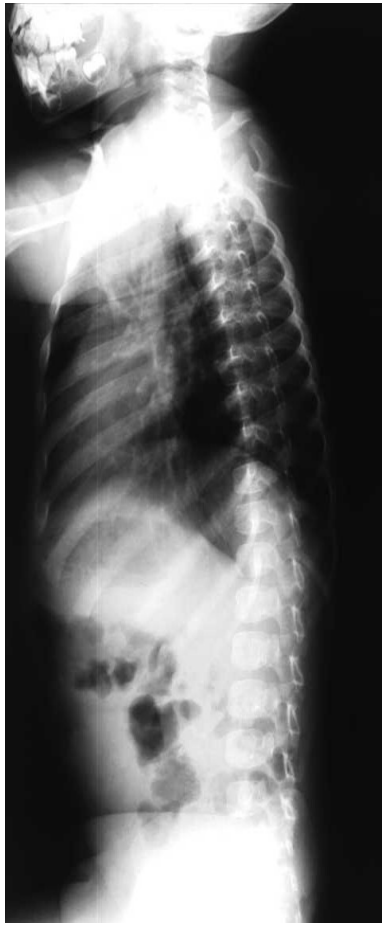

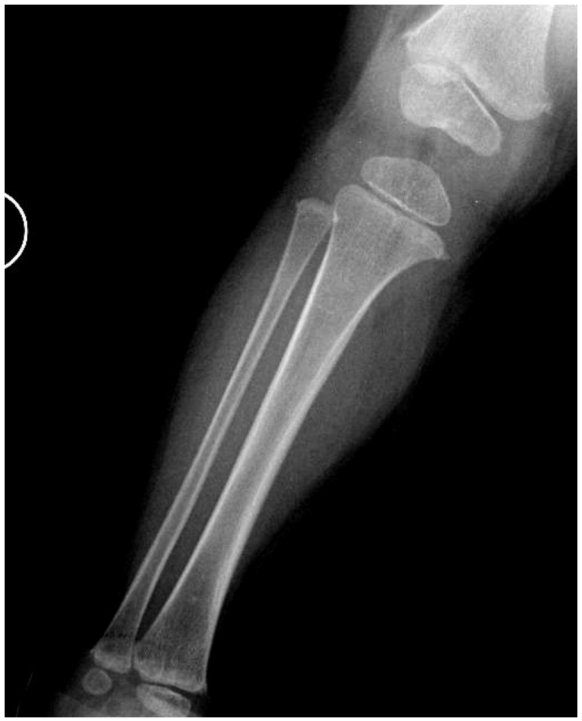

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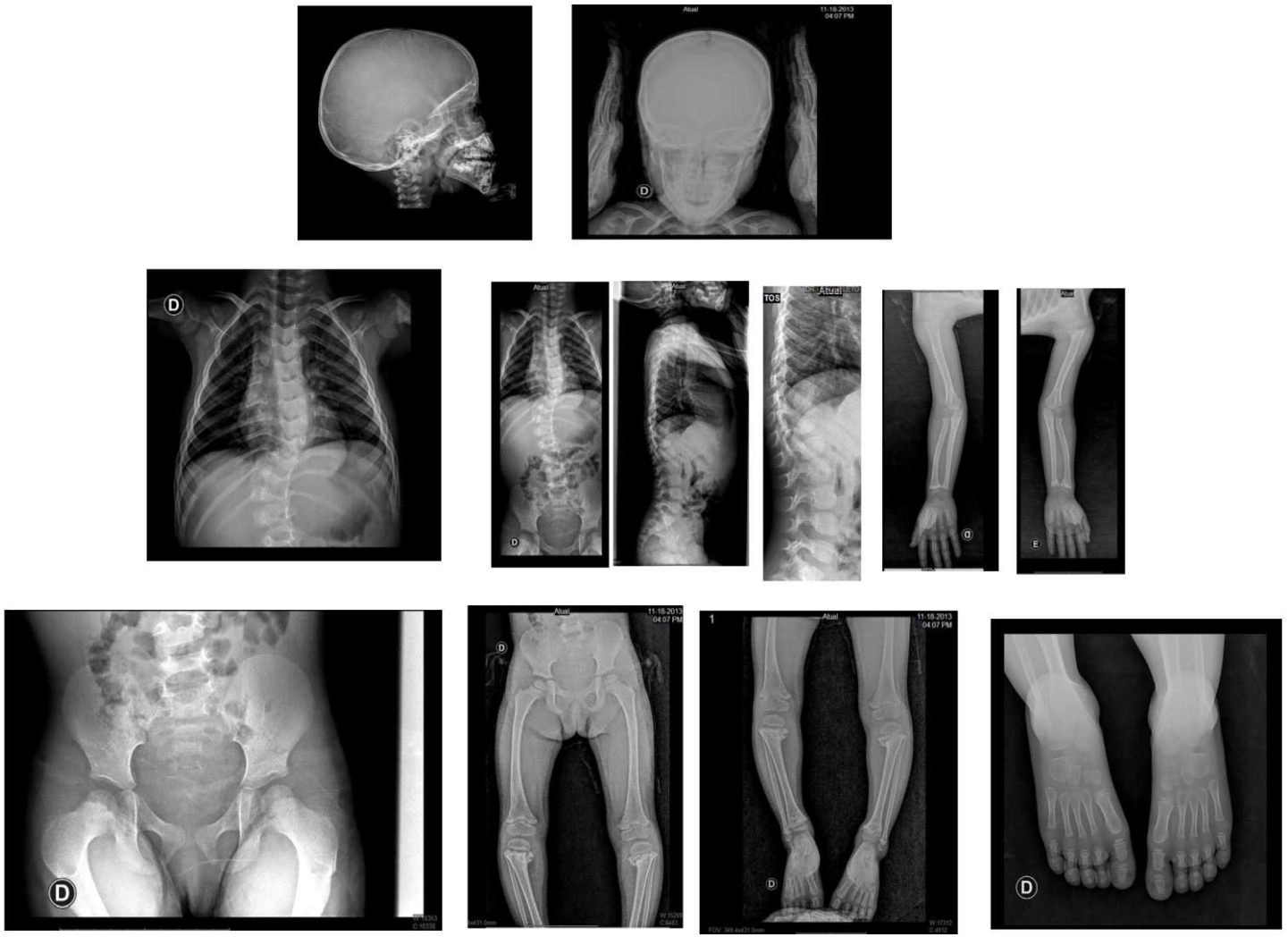

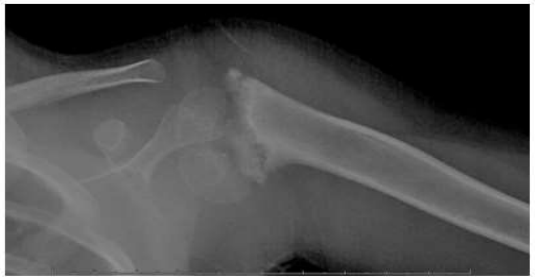

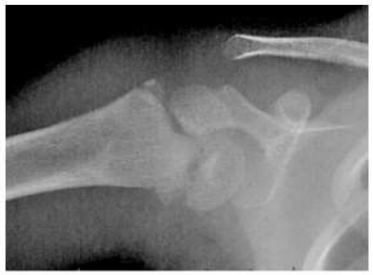

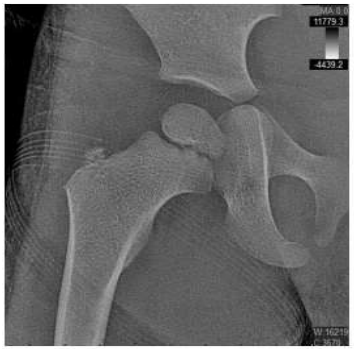
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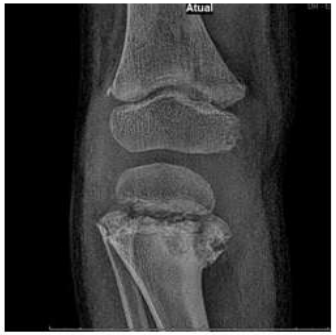
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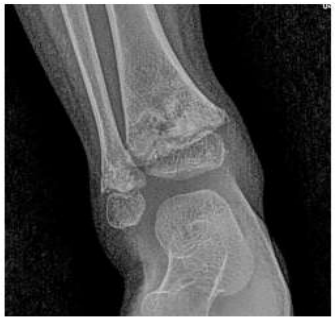

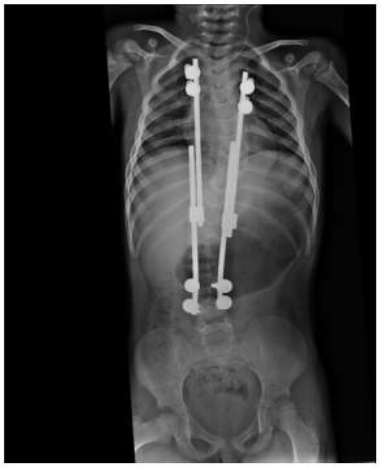

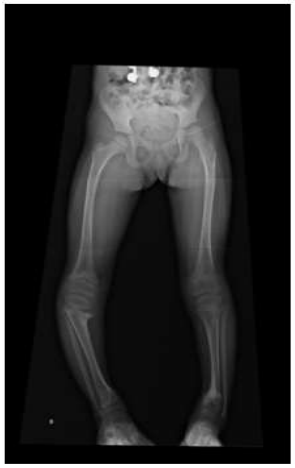

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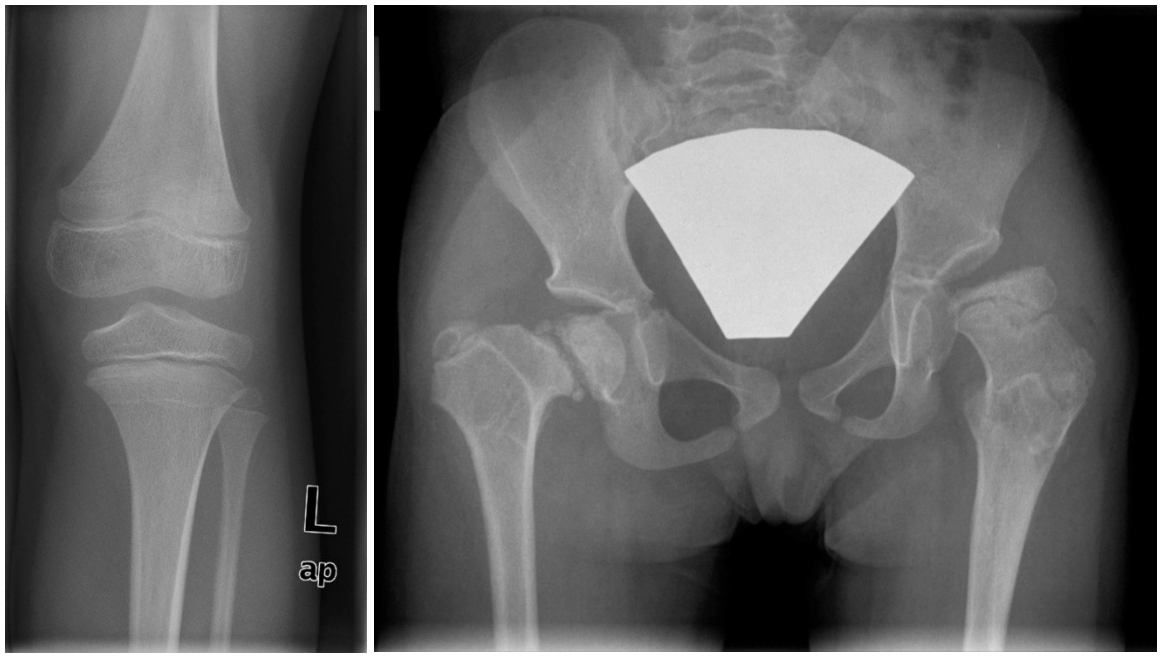

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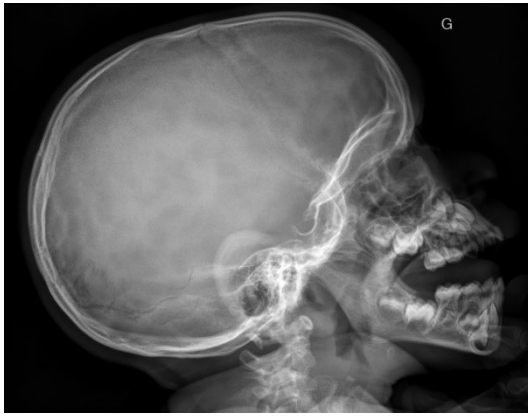

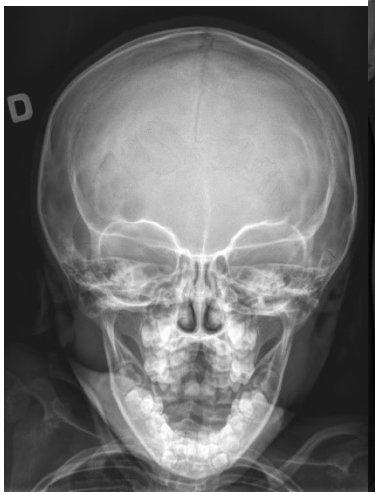

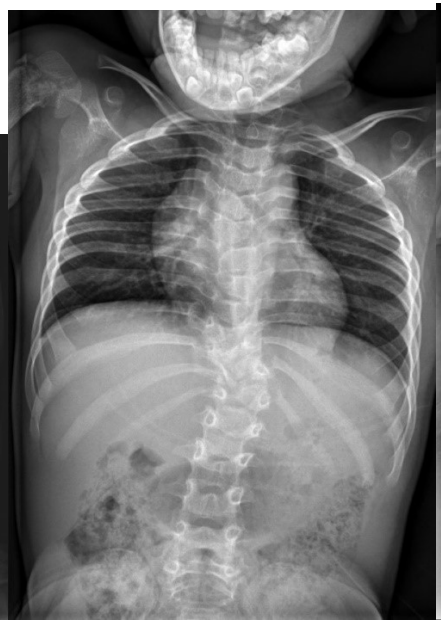

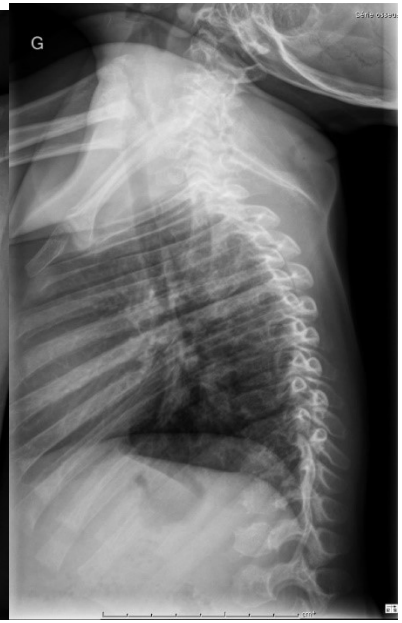

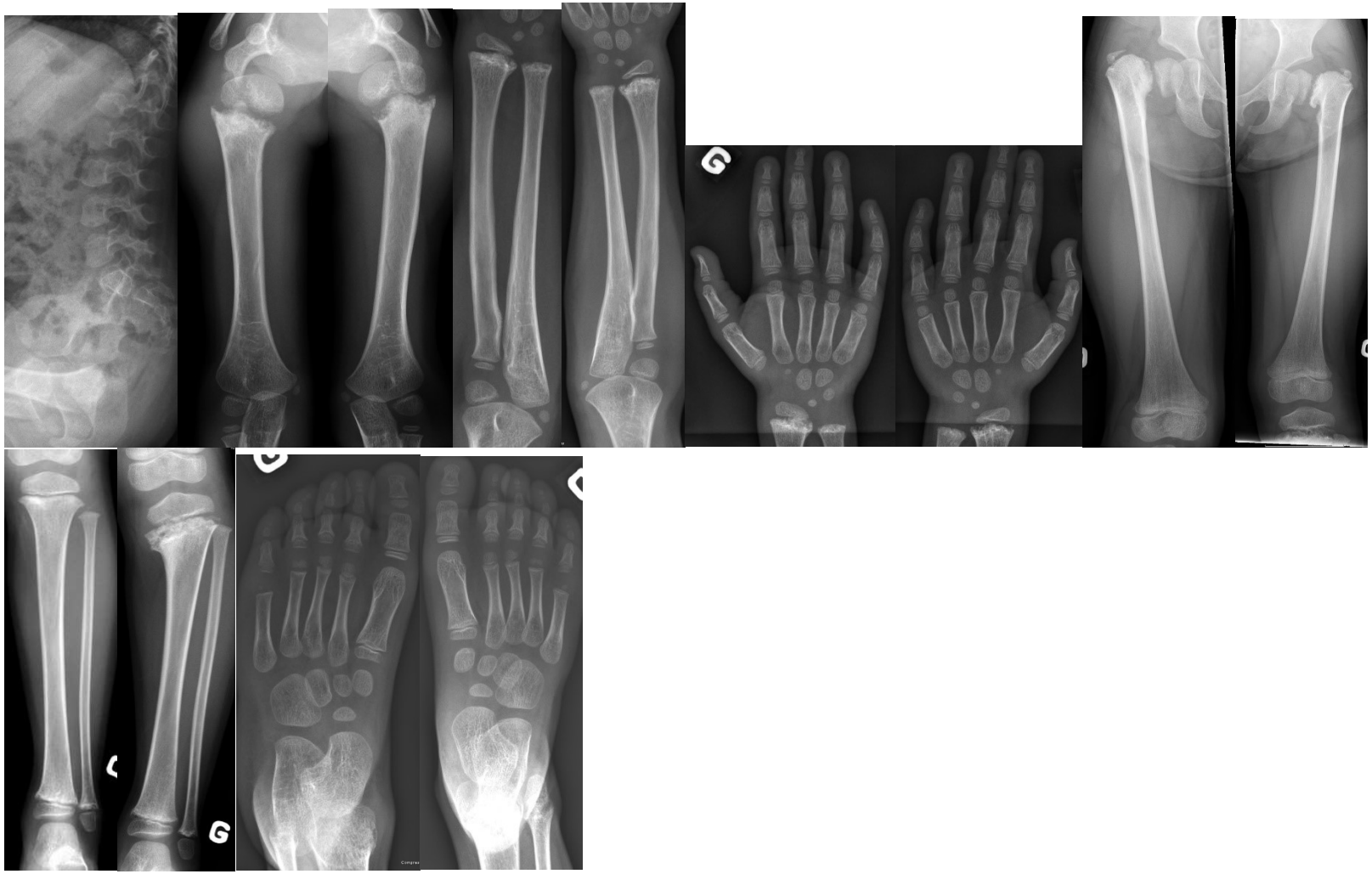

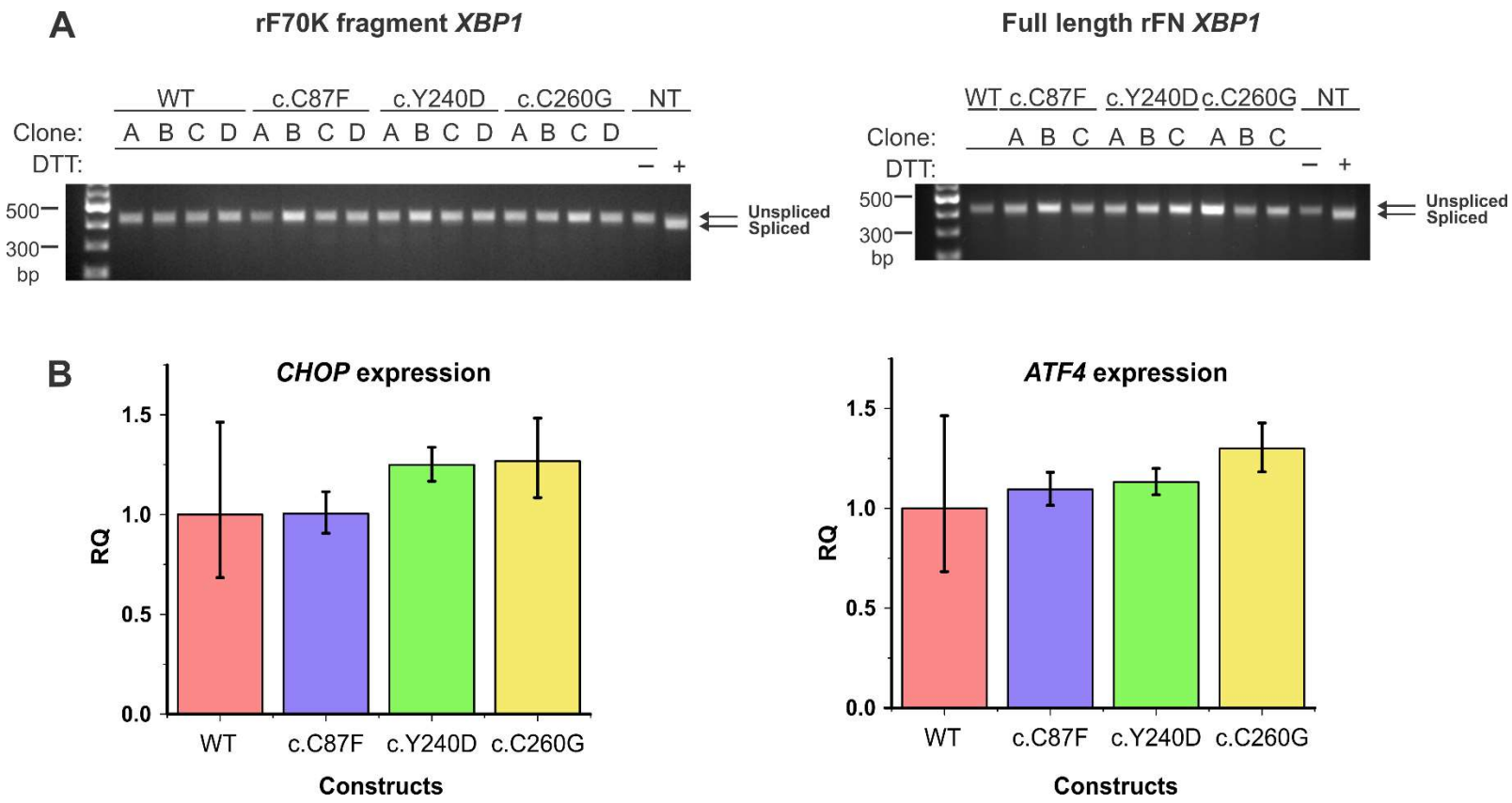

Figure S2. Analysis of the unfolded protein response of the recombinant rF70K and rFN wild-type and mutant proteins.

A) *XBP1* mRNA analysis. Mutations do not induce *XBP1*-mediated unfolded protein response. rF70K wild-type (WT) and mutants were analyzed in the left panel, and full length rFN WT and mutants in the right panel. NT represents non-transfected HEK293 cells as controls. Addition of dithiothreitol (DTT) for 1 h induces the unfolded protein response and causes *XBP1* splicing and is included as a positive control. The unspliced amplification product is 424 bp, the spliced product is 398 bp. Note that all analyzed mutations, both in rF70K and in full length rFN, do not induce the *XBP1*-mediated unfolded protein response (absence of the 398 bp band). **B**) qPCR mRNA expression analysis of *CHOP* and *ATF4* from full length rFN WT and rFN mutant cells. Both analyses showed no significant changes between rFN WT and the rFN mutants. RQ represents relative quantification of mRNA levels normalized to *GAPDH* levels. The rFN mutants were compared to the rFN WT, set to 1. Quantitative real time PCR was performed in a StepOne Real-Time PCR System (Applied Biosystems) using SYBR Select Master Mix (ThermoFisher Scientific), according to the manufacturer's protocol. Melt-curve analysis was performed after each run to determine the specificity of the detected product. Relative quantification of mRNA levels (fold change) was calculated using the $2^{-\Delta\Delta Ct}$ method. Error bars represent standard deviation. The values of all mutants were not statistically different from the wild-type ($p \geq 0.05$; Student's t-test).

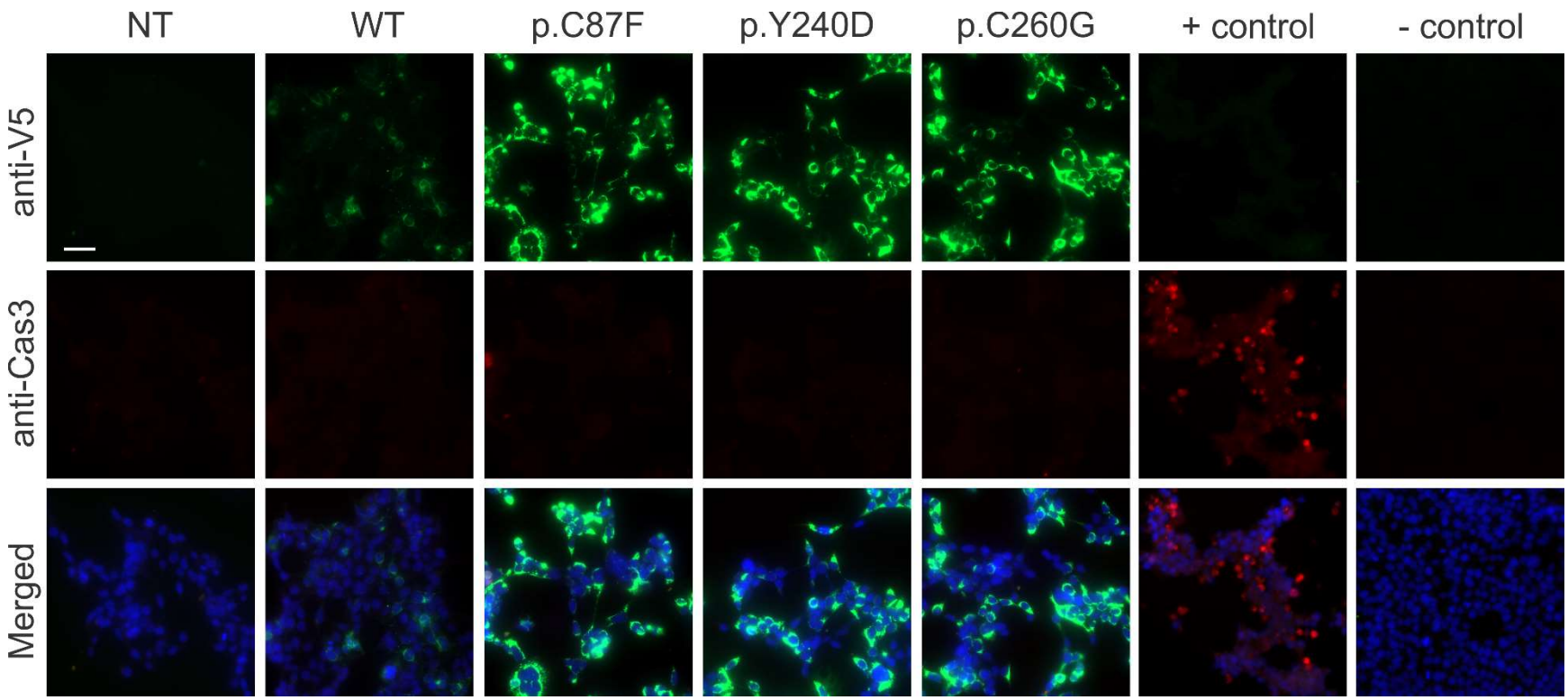

Figure S3. Analysis of caspase-3 mediated apoptosis of the recombinant rFN wild-type and mutant proteins.

Immunofluorescence of full length rFN WT and mutant cell clones with antibodies against cleaved caspase-3. Cleavage of caspase-3 is an indication of unfolded protein response mediated apoptosis. Cells were analyzed 3 days post-seeding. Note that both non-transfected HEK293 cells (NT) and transfected cells showed no staining of cleaved caspase-3 (Cas3; no red signal, primary antibody Cell Signaling; 9661S, secondary Alexa-488 conjugated antibody Thermo Fisher Scientific; A-11008), indicating the absence of detectable apoptosis. Non-transfected HEK293 cells treated for 12 h with 2µM staurosporine (Abcam; ab120056) in DMSO (0.2% DMSO final concentration) served as a positive control for apoptosis (+ control; red signal). Non-transfected HEK293 cells treated with 0.2% DMSO alone (- control) did not induce apoptosis. The rFN constructs were stained with an anti-V5 antibody (green signal). Cell nuclei were counterstained with DAPI (blue signal in the merged images). The scale bar indicates 50µm. Images were recorded at 400× magnification.

Table S1. Phenotypes of cases without a *FN1* mutation.

Family	**8[a]**	**9[a]**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
Age at last assessment	12 yrs	13 yrs	4 yrs 6 mos	NA	6 yrs 6 mos	3 yrs 11 mos	1 y 4 m	5yrs 7 mos	7 yrs, 2 mos
Gender	M	M	M	F	F	F	M	F	F
Height in cm (and SD)	75.7 cm (-3 S.D,)		95.2 cm	NA	94.0 cm (ca. -4 S.D.)	99.5 cm (25th centile)	NA	unknown	101 cm (<5th centile)
Ovoid vertebral bodies	Some	Some	NA	NA	+	+	+	+	-
Other vertebral Changes (e.g. platyspondyly)	platyspondyly	platyspondyly	NA	NA	Platyspondyly ; disproportion-ate decreased height dorsally	-	-	Anterior wedging	End plate sclerosis; anterior wedging
Scoliosis	Mild	Mild	-	- (but excessive lordosis)	+ (progressive, requiring casting)	- (mild lumbar lordosis	+	unknown	+ (mild S-shaped)
Odontoid hypoplasia	+	-	NA	NA	-; but craniocervical stenosis requiring decom-pression	-	NA	-	-
Developmental coxa vara	-	+	-, slipped capital femoral epiphyses	+	-	+	+	+	+
Irregular metaphyses	+	+	NA	+	+	+	+	+	+
“Corner fractures”	+	+	+	+	+	+	+	+/-	+/-
Knee anomalies	Vara then valga	-	-	NA	Abnormal patellar tracking	Left varum	Genu varum	Dislocated patella	-

Short trunk	-	+	+	NA	-	-	-	+	unknown
Short long bones (rhizo, meso or micromelia)			-	NA	Mesomelic disproportion	-	+	(rhizo, meso	
Chest or rib anomaly (e.g. pectus)	-	-	broad	NA	AP flattening	-	NA	Short sternum	Mild anterior rib cupping
Hand and feet anomalies	-	-	-		Profound brachydactyly; short metacarpals and phalanges	-	-	-	Cupped metacarpal and phalangeal metaphyses
Other			Pes planus, hypospdias, speech delay,		Dentinogene-sis imperfecta; multiple fractures; hip uncoverage; tibia varus and internal tibial torsion	mild leg length discrepancy	relative macrocephaly, high forehead, hypertelorism, shawl scrotum, slightly bowed forearms	Unossified CFE, other epiphyses nl; metaphyseal irregularities throughout	

[a] Published (Machol K et al.[1])

Table S2. Details on targeted enrichment, sequencing and WES data analysis

Family	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Connection for enrollment in project	Texas Children's bone program	Genematcher	SkelDys listserv	ISDR	Genematcher	Collaboration	Shriners Canada Skeletal Dysplasia Clinic
Site for sequencing	BCM-HGSC	OPBG	Radboud	BCM-HGSC	CEGH-CEL-Universidade de São Paulo	BGI	Calgary
Exome library capture	Roche SeqCap EZ HGSC VCRome	Illumina Nextera v1.2	No exome done, *FN1* Sanger sequencing only.	Roche SeqCap EZ HGSC VCRome	Illumina TrueSeq kit	Agilent SureSelect Human All Exon v4	Illumina TruSight One Sequencing Panel
Sequencing	Illumina HiSeq	Illumina HiSeq		Illumina HiSeq	Illumina HiSeq	Illumina HiSeq	Illumina HiSeq
Alignment	Mercury pipeline	In-house pipeline (GATK)		Mercury pipeline	Burrows-Wheeler Aligner (BWA)	Novoalign software	In-house pipeline (GATK)
Variant calling, filtering and annotation	Mercury pipeline	In-house pipeline (GATK, SnpEff, CADD/dbNSFP_SVM)		Mercury pipeline	GATK/ ANNOVAR	In-house pipeline (GATK, ANNOVAR, Perl and Bash scripts)	In-house pipeline

Supplemental References

1. Machol, K., Jain, M., Almannai, M., Orand, T., Lu, J.T., Tran, A., Chen, Y., Schlesinger, A., Gibbs, R., Bonafe, L., et al. (2017). Corner fracture type spondylometaphyseal dysplasia: Overlap with type II collagenopathies. Am J Med Genet A *173*, 733-739.
2. Sutton, V.R., Hyland, J.C., Phillips, W.A., Schlesinger, A.E., and Brill, P.W. (2005). A dominantly inherited spondylometaphyseal dysplasia with "corner fractures" and congenital scoliosis. Am J Med Genet A *133A*, 209-212.