Spondylo-epiphyseal dysplasia in two sibs due to a homozygous splicing variant in COL2A1

Manuscript Details

Manuscript number	EJMG_2020_394_R2
Title	Spondylo-epiphyseal dysplasia in two sibs due to a homozygous splicing variant in COL2A1
Article type	Correspondence

Abstract

Type 2 collagenopathies encompass a large group of chondrodysplasias ranging from the perinatally lethal achondrogenesis type 2 and hypochondrogenesis at the severe end of the spectrum to early-onset osteoarthritis with normal stature at the milder end of the spectrum. With the exception of a few reported cases, these dysplasias are predominantly caused by heterozygous variants in the COL2A1 gene and hence show an autosomal dominant inheritance pattern. Here we report on two siblings, originating from a consanguineous family, who presented with disproportionate short stature, ocular abnormalities, cleft palate and hearing impairment. The radiographic study showed signs of a spondyloepiphyseal dysplasia, compatible with a type 2 collagen disorder. Indeed, both siblings were homozygous for a c.3111+2T>C splice site variant in the COL2A1 gene. cDNA analysis performed on skin fibroblasts from the affected sibs revealed the co-occurrence of the wild-type transcript and an aberrant splice product, the latter believed to be degraded by nonsense-mediated mRNA decay. The parents who were heterozygous for this variant were phenotypically normal. This paper confirms that type 2 collagenopathies can show an autosomal recessive inheritance.

Keywords	Type 2 collagenopathies Spondylo-epiphyseal dysplasia COL2A1

Corresponding Author	Nouriya Al-Sannaa
Corresponding Author's Institution	Johns Hopkins Aramco Healthcare
Order of Authors	Nouriya Al-Sannaa, Kristien Hoornaert, Lut Van Laer, Hind AlAbdulwahed, Geert Mortier
Suggested reviewers	Andrea Superti-Furga, Mouna BARAT-HOUARI

Submission Files Included in this PDF

File Name [File Type]

cover letter.docx [Cover Letter]

Response to the reviewers 1.docx [Response to Reviewers]

Report of a Saudi Family with Kniest dysplasia_version_070420.docx [Manuscript File]

Figure 1.pptx [Figure]

Figure 2.pptx [Figure]

Figure 3.pptx [Figure]

CRediT Author Statement.docx [Author Statement]

statement.PDF [Supplementary Material]

Supplements.pptx [Supplementary Material]

To view all the submission files, including those not included in the PDF, click on the manuscript title on your EVISE Homepage, then click 'Download zip file'.

Title: Spondylo-epiphyseal dysplasia in two sibs due to a homozygous splicing variant in COL2A1

This paper supports the autosomal recessive inheritance of type 2 collagenopathies

Corresponding author

Dr. Nouriya Abbas Al-Sannaa

Johns Hopkins Aramco Healthcare

Dhahran, Saudi Arabia

31311

Pediatrics Services Division

Telephone-00966138708290

Fax-00966138703792

Email-nouriya.sannaa@jhah.com

Co-authors

Dr. K.P. Hoornaert

General Hospital Maria Middelares

kristienhoornaert@oogziekenhuisgent.be

Dr. L. Van Laer

University of Antwerp and Antwerp University Hospital, Antwerp, Belgium

lut.vanlaer@uantwerpen.be

Ms. Hind AlAbdulwahed

Johns Hopkins Aramco Healthcare

Dhahran, Saudi Arabia

Hind.abdulwahed@jhah.com

Professor-G. Mortier

University of Antwerp and Antwerp University Hospital, Antwerp, Belgium

geert.mortier@uantwerpen.be

Response to the reviewers

Editor's comments

Response-the variant was submitted to Clin Var (http://www.ncbi.nlm.nih.gov/clinvar)

Accession number-SCV001429665

Reviewer 1

Arrange the figures and supplementary figures

Response-done

Adding agarose gel electrophoresis

Unfortunately, cultured fibroblasts are not available for all family members and this might not be feasible within the time frame

Spondylo-epiphyseal dysplasia in two sibs due to a homozygous splicing variant in *COL2A1*

N. A. Al-Sannaa (1), K.P. Hoornaert (2), L. Van Laer (3) H. Y. Al-Abdulwahed (1), G. Mortier (3).

(1) Johns Hopkins Aramco Healthcare, Pediatric Services Division, Saudi Arabia, (2) General Hospital Maria Middelares, Ghent, Belgium, Ghent, Belgium, (3) University of Antwerp and Antwerp University Hospital, Antwerp, Belgium

Abstract

Type 2 collagenopathies encompass a large group of chondrodysplasias ranging from the perinatally lethal achondrogenesis type 2 and hypochondrogenesis at the severe end of the spectrum to early-onset osteoarthritis with normal stature at the milder end of the spectrum. With the exception of a few reported cases, these dysplasias are predominantly caused by heterozygous variants in the *COL2A1* gene and hence show an autosomal dominant inheritance pattern. Here we report on two siblings, originating from a consanguineous family, who presented with disproportionate short stature, ocular abnormalities, cleft palate and hearing impairment. The radiographic study showed signs of a spondyloepiphyseal dysplasia, compatible with a type 2 collagen disorder. Indeed, both siblings were homozygous for a c.3111+2T>C*p.(Glu1033Lysfs*5)* splice site variant in the *COL2A1* gene. cDNA analysis performed on skin fibroblasts from the affected sibs revealed the co-occurrence of the wild-type transcript and an aberrant splice product, the latter believed to be degraded by nonsense-mediated mRNA decay. The parents who were heterozygous for this variant were phenotypically normal. This paper confirms that type 2 collagenopathies can show an autosomal recessive inheritance.

Introduction

Type II collagen is the major collagenous component of hyaline cartilage. It consists of three identical polypeptide chains (known as the α1(II)-chains) encoded by the *COL2A1* gene. Heterozygous variants in the *COL2A1* gene can cause a broad spectrum of chondrodysplasias known as the type 2 collagenopathies. They range from perinatally lethal disorders such as achondrogenesis type 2 and hypochondrogenesis (MIM# 200610), over phenotypes with severe disproportionate short stature such as spondyloepiphyseal dysplasia congenita (MIM# 183900), spondyloepimetaphyseal dysplasia Strüdwick type (MIM# 184250), spondyloperipheral dysplasia (MIM# 271700), and Kniest dysplasia, to conditions with normal stature but early-onset degenerative joint disease such as Stickler syndrome (MIM# 156550) (MIM# 108300) (MIM# 609508) and familial precocious osteoarthropathy (Carlson et al 2006, Warman et al 2011 & Barat-Houari et al 2016). Autosomal dominant inheritance has been considered for many years the mode of inheritance for type 2 collagen disorders. However, in the past years, a few consanguineous families have been reported showing evidence that homozygous missense variants in *COL2A1* can cause a spondyloepiphyseal dysplasia phenotype with autosomal recessive inheritance (Tham E et al 2015, Barat-Houari M et al. 2016, Girisha et al 2019). Here, we report on a brother and a sister born to healthy first cousins of Saudi-Arabian origin. Both sibs presented with a clinical and radiographic phenotype reminiscent of a type 2 collagen disorder. Molecular analysis revealed the presence of a homozygous splice site variant (c.3111+2T>C)p.(Glu1033Lysfs*5) in the *COL2A1* gene in both affected sibs. The phenotypically normal parents were confirmed to be heterozygous for the same variant supporting an autosomal recessive pattern of inheritance.

Case reports

Patient-1

The proband was born at term by a normal vaginal delivery after an uneventful pregnancy. The mother was 24 years old and the father was 24 years old. Postnatal examination revealed a weight of 3.1 kg (0 SDS), a length of 43 cm (-3 SDS), and a head circumference of 35 cm (0 SDS). He presented at birth with prominent eyes, clefting of the soft palate, micrognathia, rhizomelic shortening of the limbs and left clubfoot. There was restricted mobility in the elbow, hip and knee joints. Other systemic examination was normal.

The child's medical course was complicated by recurrent ear infections and feeding difficulties requiring nasogastric tube feeding. At 2.5 years of age, he was diagnosed with bilateral high myopia (-16.00 diopters). At the age of 3 years severe sensorineural hearing loss requiring hearing aids was noted. He had achieved a normal motor and social development. However, he was enrolled in a special education program due to his severe hearing loss. His clinical phenotype at the age of 13 years is shown in Fig 1 (A-D).

Physical examination at the age of 22 years showed a relatively obese young man with disproportionate short stature. His height was 149 cm (-4.0 SDS), his weight was 70 kg (0 SDS) and his head circumference was 56 cm (0 SDS). He had a flat face with supraoribital hypoplasia, depressed nasal bridge, short nose, midfacial hypoplasia, and microretrognathia. The trunk showed pectus carinatum, thoracic kyphoscoliosis, and lumbar hyperlordosis. Limited range of movements was noted in the elbows, knees and hips. The knees and elbows were rather broad. Other systemic examination was normal.

Patient 2

The affected sister was born at term by a normal vaginal delivery after an uneventful pregnancy with a birth weight of 2.8 kg (-2 SDS), length of 43 cm (-3 SDS) and head circumference of 35.5 cm (0 SDS). Her Apgar scores were 9 and 9 at respectively 1 and 5 minutes. She was noticed to have a similar phenotype as her brother with short extremities, relative macrocephaly, prominent eyes, midfacial hypoplasia, cleft soft palate, microretrognathia, and hip contractures (Fig 1 E-F).

The girl's initial clinical course was complicated by poor feeding and failure to thrive requiring nasogastric tube feeding. At 4 months of age she was diagnosed with a high myopia (-16.00D/-17.00D) and progressed to complete retinal detachment and loss of vision of the left eye at 7 years of age. She had recurrent episodes of ear infection and subsequently developed severe sensorineural hearing loss requiring hearing aids. Her motor and social developmental milestones were appropriate for age apart from delayed speech. She was enrolled in a special educational program due to her hearing and visual impairment and had sustained some learning difficulties. She had her menarche at 13 years of age.

Physical examination at 17 years of age showed a relatively obese young lady with a disproportionate short stature. Her weight was 57 kg (0 SDS), her height 139 cm (-4.05 SDS) and head circumference 55 cm (0.43 SDS). Craniofacial features included supraorbital hypoplasia, prominent eyes, depressed nasal bridge, short nose, and microretrognathia. In addition, she had a short neck, pectus carinatum, mild

thoracic kyphosis, and lumbar hyperlordosis. There was limited extension in the elbows, broad prominent knees and limited mobility in the ankles. The other systemic examination was normal.

Parents

The parents had a full clinical evaluation including ophthalmologic examination, skeletal radiographs (Supplement E, F), and hearing test that revealed no abnormalities. The father height was 175 cm (+2 SDS) and the mother was 153 cm (- 1SDS) for the Saudi population.

Radiographic Study

The radiographic changes in both siblings were very similar (Figure 2 A-D & supplement A-D). Radiographs of the pelvis and lower limbs in the boy (patient 1) at the age of 3 months showed short femora and tibiae with widened metaphyses and flat knees epiphyses. The femora had a mild dumbbell appearance, typically seen in Kniest dysplasia. Other radiographic features included an abnormal pelvis with small iliac wings, flat acetabular roofs and broad ischial bones. Subsequently, the boy developed dysplastic hip and knee epiphyses and thoracic kyphoscoliosis with anterior wedging of thoracic vertebrae. The girl (patient 2) also showed shortening of the long tubular bones with dumbbell appearance of the femora in infancy. Later she developed dysplastic hip and knee epiphyses. The spine at the age of 5 years was normal.

Molecular Study

Blood samples for DNA extraction were collected from all seven family members. Genomic DNA was extracted by standard procedures, followed by touchdown PCR amplification of the *COL2A1* gene using forward and reverse primers located in the flanking introns (primer sequences available upon request). PCR fragments were sequenced on the ABI PRISM 3730 automated sequencer (Applied Biosystems, Foster City, CA) using the BigDye terminator cycle sequencing chemistry. These obtained sequences were compared to the wild-type sequence as submitted to GenBank Accession number NM_001844. The nucleotides were numbered starting from the first base of the start codon (ATG) of the cDNA reference sequence. Amino acid residues were numbered from the first methionine (start codon for translation) of the procollagen α1(II) chain (GenBank Accession number L10347). These molecular studies revealed that both sibs (patient 1 & 2) were homozygous for a splice site variant c.3111+2T>C in intron 44 of the *COL2A1* gene (Clin Var accession number-SCV001429665). Both parents and two unaffected sibs were heterozygous for the variant, whereas one unaffected sister was homozygous for the normal sequence (Figure 1). Because of the presence of a splice site variant, a skin biopsy was requested for analysis of mRNA splicing. In order to stabilize mutant *COL2A1* mRNA, cycloheximide (Sigma, www.sigmaaldrich.com) was added to the cultures, followed by mRNA isolation and cDNA preparation. Nested PCR (primers available upon request) was used to obtain sufficient PCR fragments for direct sequencing. cDNA analysis in IV:2 and IV:6 revealed the co-occurrence of the wild-type transcript and of a transcript generated from a cryptic splice site, 17 nucleotides upstream of the donor splice site, resulting in a frameshift and most likely degraded by nonsense-mediated mRNA decay (Figure 3).

Discussion

Type 2 collagen disorders refer to a clinically heterogeneous group of skeletal disorders characterized by variable degrees of disproportionate short stature, ocular abnormalities (myopia, vitreous abnormalities, retinal detachment), hearing impairment and orofacial features (flat face, cleft palate and Pierre Robin sequence). The tissue-specific expression of the *COL2A1* gene in connective tissues such as hyaline cartilage, intervertebral discs, vitreous tissue and inner ear accounts for these pleiotropic manifestations. The spectrum of clinical severity ranges from perinatal lethality to normal survival with premature arthrosis (Gregersen et al 1993).

The vast majority of type 2 collagenopathies are caused by heterozygous variants in the *COL2A1* gene and show autosomal dominant inheritance. Heterozygous missense variants that substitute a glycine residue in the triple helical domain usually cause a severe phenotype with short stature whereas heterozygous nonsense or frameshift variants result in rather milder phenotypes with normal stature such as the Stickler syndrome. The former variants act in a dominant negative way whereby the mutant chains adversely influence the function of the normal chains. The latter variants on the other hand are inactivating variants causing haploinsufficiency for type II collagen in the extracellular matrix.

The two siblings described in this report presented at birth with short length, short limbs, flat face and Pierre-Robin sequence. Both developed moderate disproportionate short stature, high myopia and severe hearing impairment. The initial radiographs with dumbbell appearance of the femora were suggestive of Kniest dysplasia but later on the radiographic phenotype evolved more into that of a classical form of spondyloepiphyseal dysplasia. In both affected siblings a homozygous splice site variant (c.3111+2T>C)p.(Glu1033Lysfs*5) in the *COL2A1* gene was identified. Subsequent cDNA analysis showed that this variant resulted in either normal splicing or abnormal splicing by using a cryptic donor site upstream of the variant resulting in a frameshift. It has been shown earlier that splice site alterations or variants in the COL2A1 can cause multiple RNA isoforms in patients with Stickler syndrome (KP Hoornaert et al 2010). Although both the wild-type and the aberrant splice product seemed to occur in approximately equal amounts, we hypothesize that the homozygous splice site variant found in the affected sibs reduces the amount of normal type II collagen by more than 50%. Therefore, it results in a phenotype more severe than that seen with pure haploinsufficiency caused by heterozygous inactivating variants such as in Stickler syndrome. Because of the persistence of the wild-type transcript in the presence of the splice site variant, heterozygous individuals are hypothesized to have sufficient wildtype transcript to prevent the occurrence of clinical symptoms. Indeed, both parents were clinically and radiographically investigated and did not show signs of a type 2 collagenopathy.

Other examples of homozygous variants in the *COL2A1* gene causing a spondyloepiphyseal dysplasia phenotype have been reported in the recent literature. In 2015, Tham E. et al. reported on a male proband with normal birth length who was born to second cousins of Indian origin (Tham E et al 2015). The parents were of average stature and had normal vision and hearing. The boy presented at the age of 2 years with short stature. Skeletal survey showed signs of a spondyloepiphyseal dysplasia. At the age of 12 years he had severe short stature, kyphoscoliosis, barrel-shaped chest, short neck, flat face, brachydactyly, myopia (-6.5/-7.5 diopters) and normal hearing. He underwent surgery for genua valga.

Genetic analysis revealed homozygosity for the c.1309C>T (p.Arg437Trp) missense variant in the *COL2A1* gene. The parents and the unaffected brother were heterozygous for this variant. In 2016, a second case was reported by Barat-Houari M. et al (Barat-Houari M et al 2016). These authors described a boy who presented at 24 weeks gestation with shortening of the long bones. Birth length was severely reduced (-5,7 SDS) and a Pierre-Robin sequence was present at birth, compromising neonatal feeding. The parents were related. The father had myopia (- 5 diopters) and the mother measured 154 cm. At the age of 3 years severe myopia (-10 diopters) and hearing loss were detected. Sanger sequencing revealed homozygosity for the c.1373C>T (p.Pro458Leu) missense variant in the *COL2A1* gene. The parents were heterozygous for this variant supporting the autosomal recessive mode of inheritance. Recently, Girisha et al. described four patients, originating from two unrelated Indian consanguineous families, who presented with variable degree of short stature (mild to severe) with short limbs, normal hearing and no myopia (Girisha et al 2019). Skeletal survey showed signs of a spondyloepimetaphyseal dysplasia. Two different homozygous missense variants in the *COL2A1* gene were identified: the c.4135C>T (p.Arg1379Cys) variant in family 1 and the c.3190C>T (p.Arg1133Cys) variant in family 2. Interestingly, all these reported variants are missense variants that only cause a phenotype in the homozygous state. In the heterozygous state they are apparently well tolerated and do not exert a dominant negative effect like the commonly observed glycine substitutions. In the homozygous state, on the other hand, they affect all polypeptide chains and therefore prevent the formation of any normal type II collagen homotrimers.

In conclusion, we have confirmed the possibility of autosomal recessive inheritance for type 2 collagenopathies and have provided some data on how bi-allelic splice site variants may cause the abnormal phenotype. Of note is the observation that bi-allelic variants in *COL2A1* do not result in lethal disorders nor in phenotypes with normal stature. Disproportionate short stature with spondylo-epiphyseal dysplasia on skeletal survey seems to be the common phenotypic feature so far.

Acknowledgement

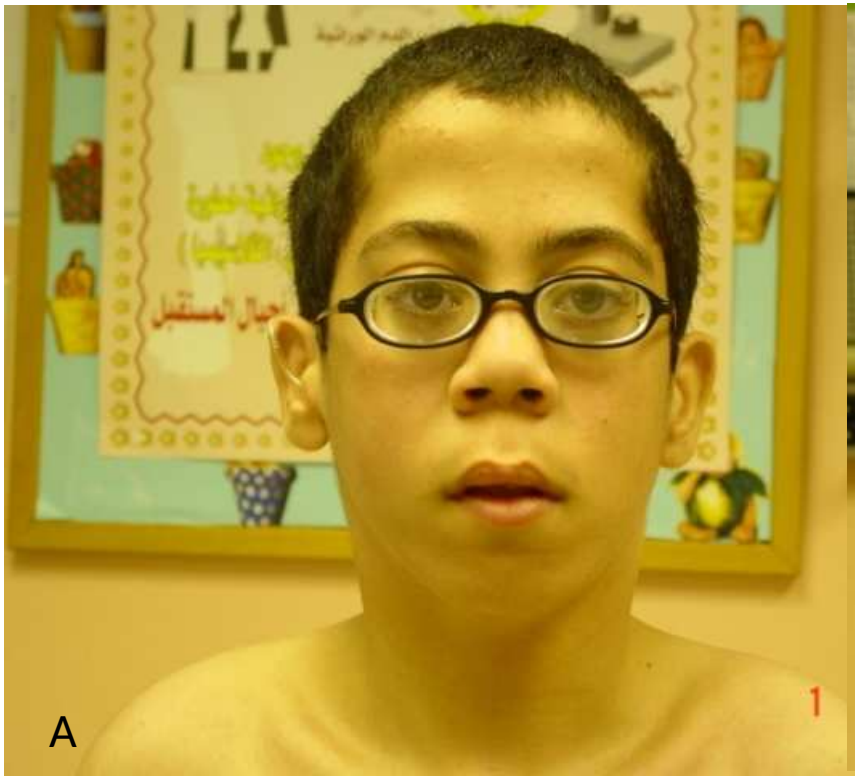
We are grateful to the patients and the family for participation and the institutional review board to approve the study for publication. No funding was received for this project.

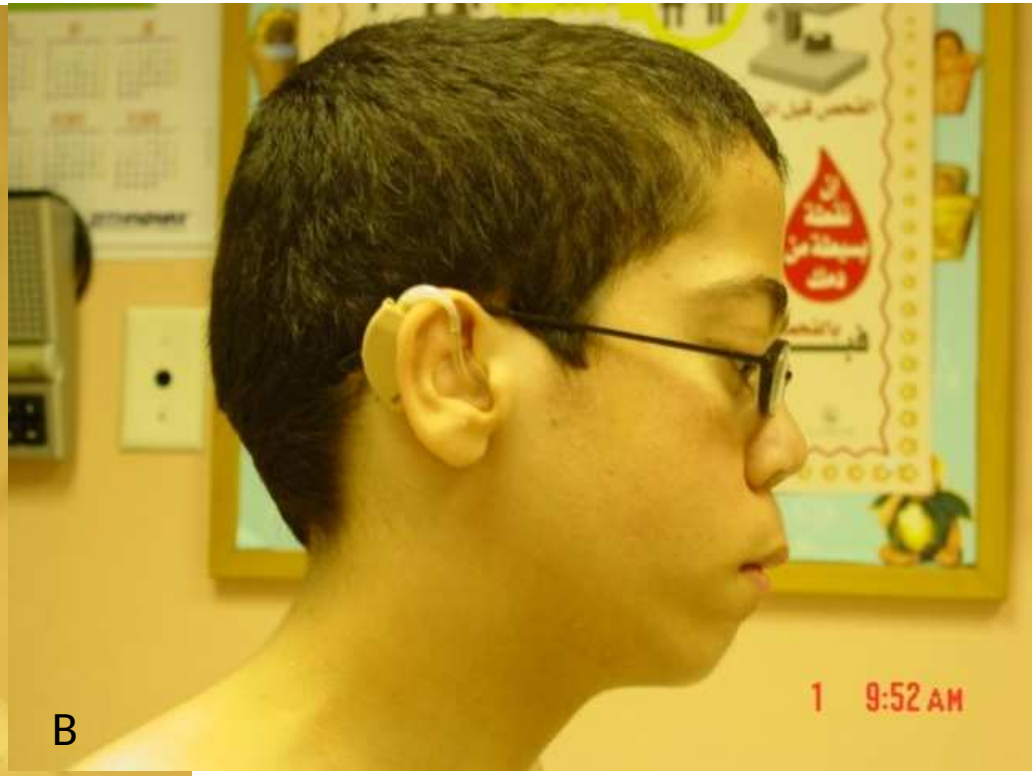
References

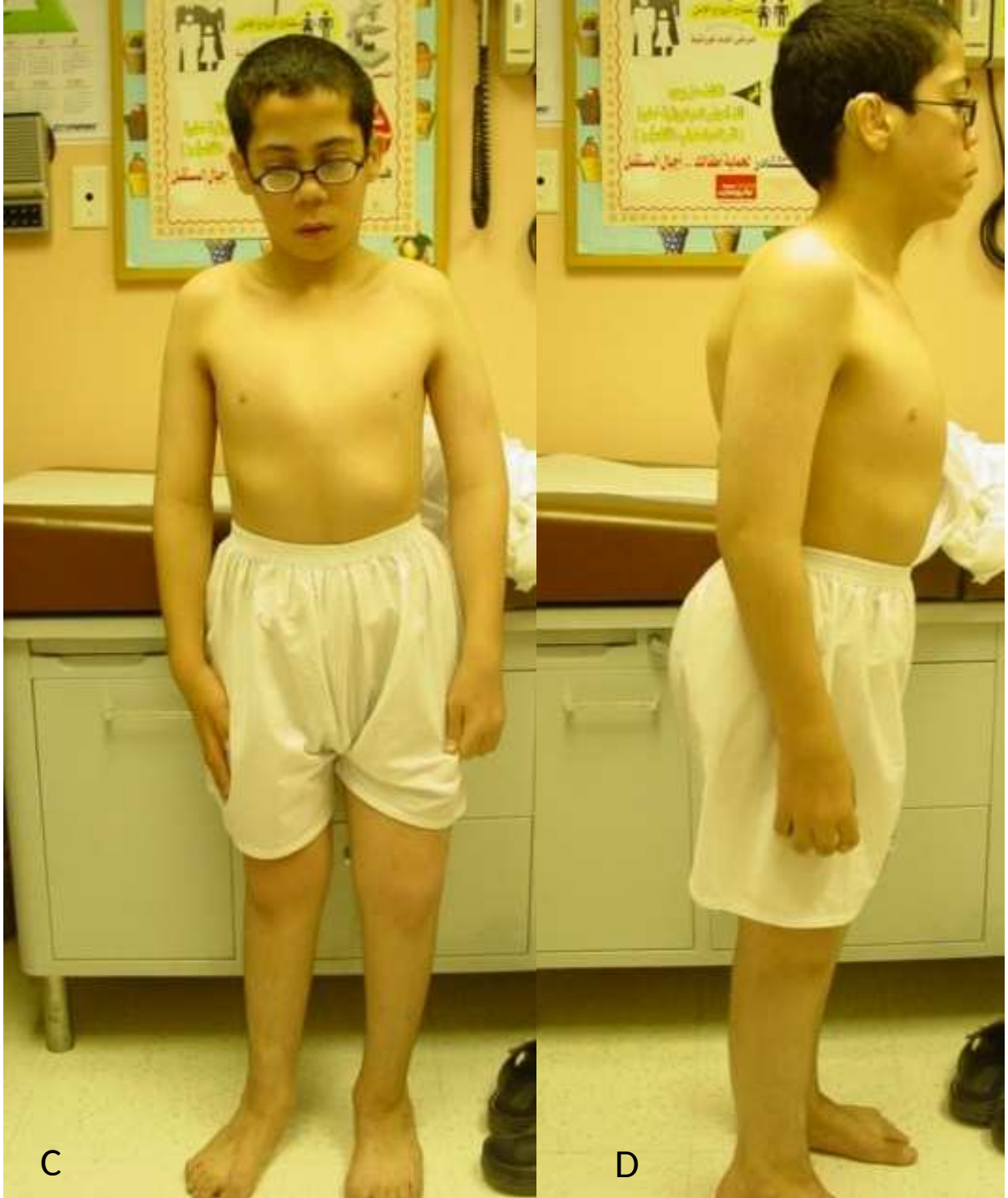
1. Barat-Houari M, Baujat G, Tran Mau Them F, Fabre A, Geneviève D, Touitou. Confirmation of autosomal recessive inheritance of COL2A1 mutations in spondyloepiphyseal dysplasia congenita: Lessons for genetic counseling. Am J Med Genet A. 2016 Jan;170A(1):263-5
2. Barat-Houari M, Sarrabay G, Gatinois V, Fabre A, Dumont B, Genevieve D, Touitou I. Mutation Update for COL2A1 Gene Variants Associated with Type II Collagenopathies. Hum Mutat. 2016 Jan;37(1):7-15.
3. Barat-Houari M,, Dumont B, Fabre A, Them FT, Alembik Y, Alessandri JL, Amiel J, Audebert S, Baumann-Morel C, Blanchet P, Bieth E, Brechard M, Busa T, Calvas P, Capri Y, Cartault F, Chassaing N, Ciorca V, Coubes C, David A, Delezoide AL, Dupin-Deguine D, El Chehadeh S, Faivre

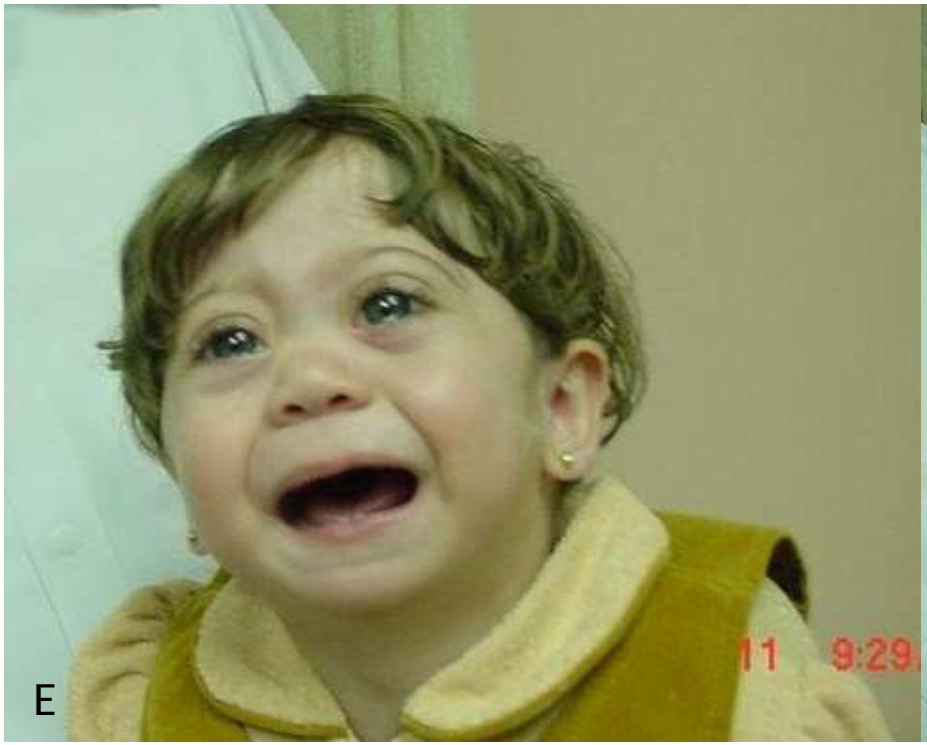
L, Giuliano F, Goldenberg A, Isidor B, Jacquemont ML, Julia S, Kaplan J, Lacombe D, Lebrun M21, Marlin S, Martin-Coignard D, Martinovic J, Masurel A, Melki J, Mozelle-Nivoix M, Nguyen K, Odent S, Philip N, Pinson L, Plessis G, Quélin C, Shaeffer E, Sigaudy S, Thauvin C, Till M, Touraine R, Vigneron J, Baujat G, Cormier-Daire V, Le Merrer M, Geneviève D, Touitou I. The expanding spectrum of COL2A1 gene variants IN 136 patients with a skeletal dysplasia phenotype. European Journal of Human Genetics, (2016) 24, 992–1000

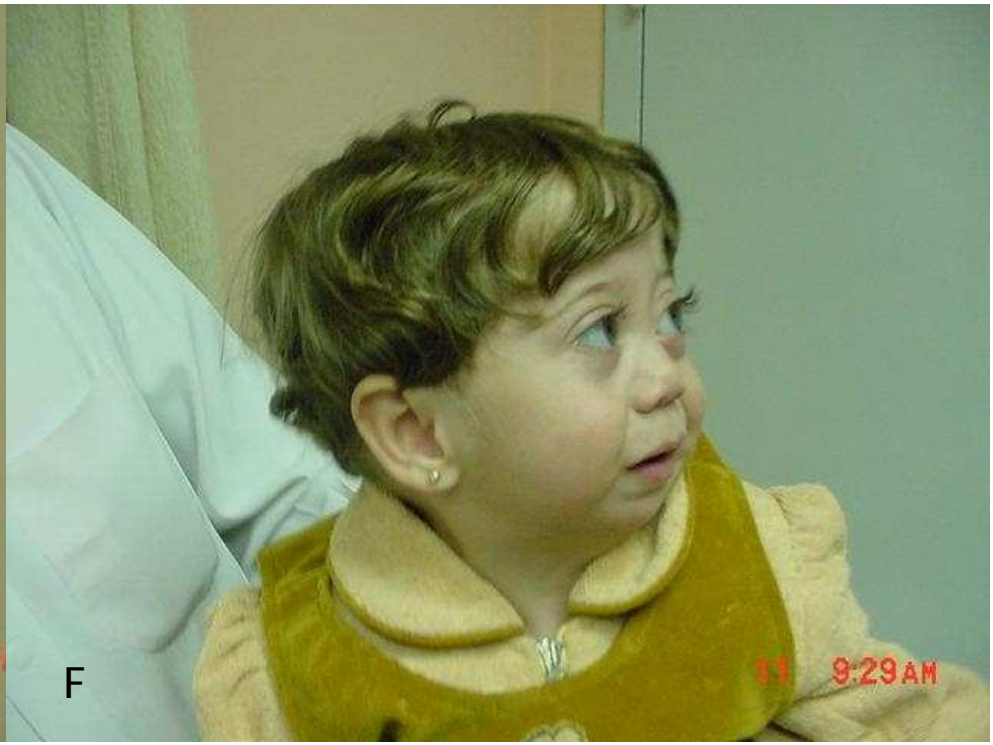
4. Carlson KM1, Yamaga KM, Reinker KA, Hsia YE, Carpenter C, Abe LM, Perry AK, Person DA, Marchuk DA, Raney EM. Precocious osteoarthritis in a family with recurrent COL2A1 mutation. J Rheumatol. 2006 Jun;33(6):1133
5. Girisha KM, Bhavani GS, Shah H, Moirangthem A, Shukla A, Kim OH, Nishimura G, Mortier GR. Biallelic variants p.Arg1133Cys and p.Arg1379Cys in COL2A1: Further delineation of phenotypic spectrum of recessive Type 2 collagenopathies. Am J Med Genet A. 2019 Nov 22. doi: 10.1002/ajmg.a.61414. [Epub ahead of print].
6. Gregersen, P. A., Savarirayan, R. (1993). Type II Collagen disorders overview. In M. P. Adam, H. H. Ardinger, R. A. Pagon, S. E. Wallace,L. J. Bean, K. Stephens, et al. (Eds.), GeneReviews® [Internet]. Seattle, WA: University of Washington. Retrieved from http://www.ncbi.nlm. nih.gov/books/NBK540447/.
7. Kristien P Hoornaert, Inge Vereecke, Chantal Dewinter, Thomas Rosenberg, Frits A Beemer, Jules G Leroy, Laila Bendix, Erik Bjo¨rck, Maryse Bonduelle, Odile Boute, Valerie Cormier-Daire, Christine De Die-Smulders, Anne Dieux-Coeslier, He´le`ne Dollfus, Mariet Elting, Andrew Green, Veronica I Guerci, Raoul CM Hennekam, Yvonne Hilhorts-Hofstee, Muriel Holder, Carel Hoyng18, Kristi J Jones, Dragana Josifova, Ilkka Kaitila, Suzanne Kjaergaard, Yolande H Kroes, Kristina Lagerstedt, Melissa Lees, Martine LeMerrer, Cinzia Magnani, Carlo Marcelis, Loreto Martorell26, Miche`le Mathieu, Meriel McEntagart, Angela Mendicino, Jenny Morton, Gabrielli Orazio, Ve´ronique Paquis, Orit Reish, Kalle OJ Simola34, Sarah F Smithson35, Karen I Temple36, Elisabeth Van Aken, Yolande Van Bever, Jenneke van den Ende, Johanna M Van Hagen, Leopoldo Zelante, Riina Zordania, Anne De Paepe, Bart P Leroy, Marc De Buyzere, Paul J Coucke and Geert R Mortier. Stickler syndrome caused by COL2A1 mutations: genotype–phenotype correlation in a series of 100 patients. European Journal of Human Genetics (2010) 18, 872–881.
8. Tham E, Nishimura G, Geiberger S, Horemuzova E, Nilsson D, Lindstrand A, Hammarsjö A, Armenio M, Mäkitie O, Zabel B, Nordgren A, Nordenskjöld M, Grigelioniene G. Autosomal recessive mutations in the COL2A1 gene cause severe spondyloepiphyseal dysplasia. Clin Genet. 2015 May;87(5):496-8.
9. Warman ML, Cormier-Daire V, Hall C, Krakow D, Lachman R, LeMerrer M, Mortier G, Mundlos S, Nishimura G, Rimoin DL, Robertson S, Savarirayan R, Sillence D, Spranger J, Unger S, Zabel B, Superti-Furga A. Nosology and classification of genetic skeletal disorders: 2010 revision. Am J Med Genet A. 2011 May; 155A (5):943-68.

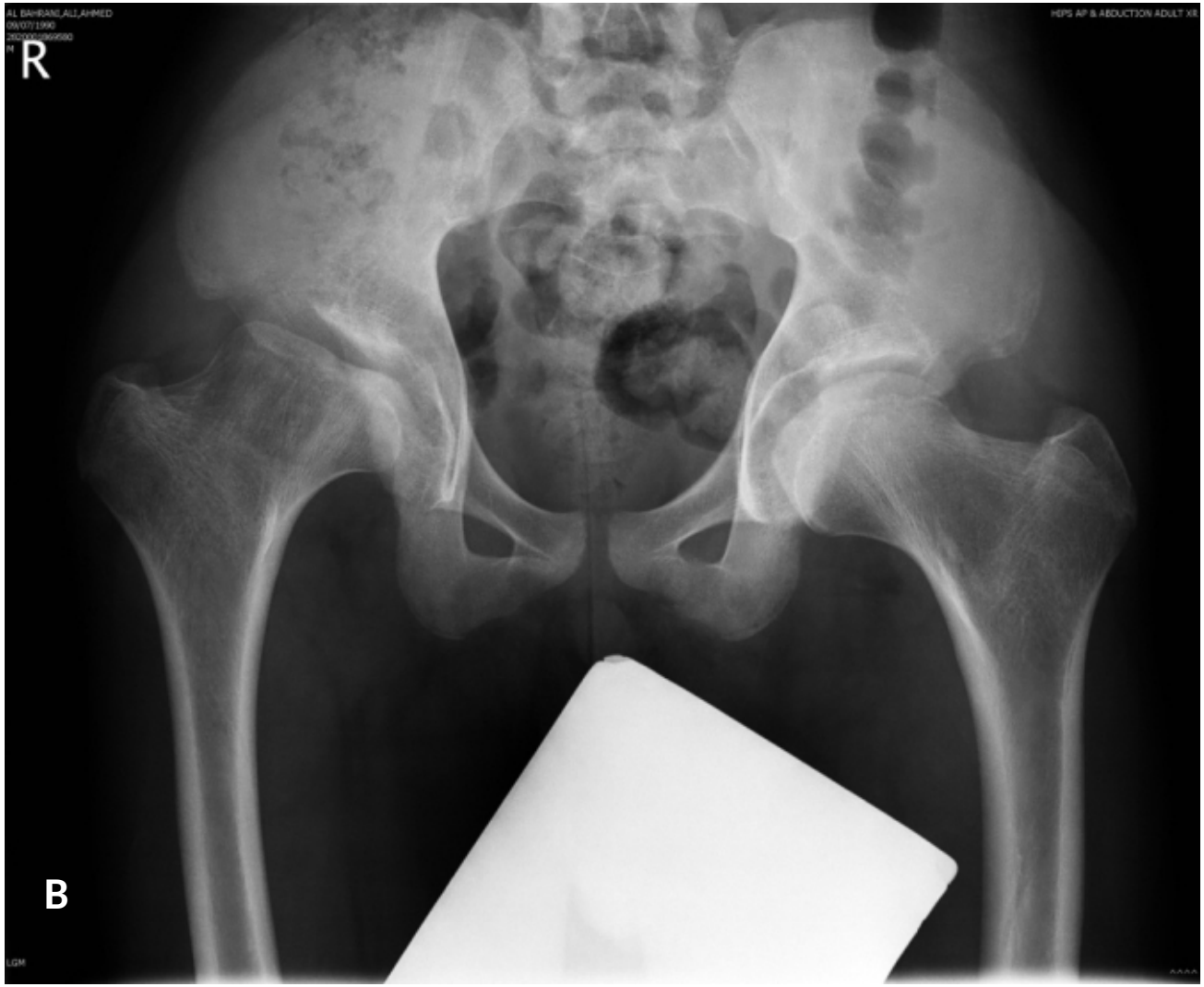

A
1

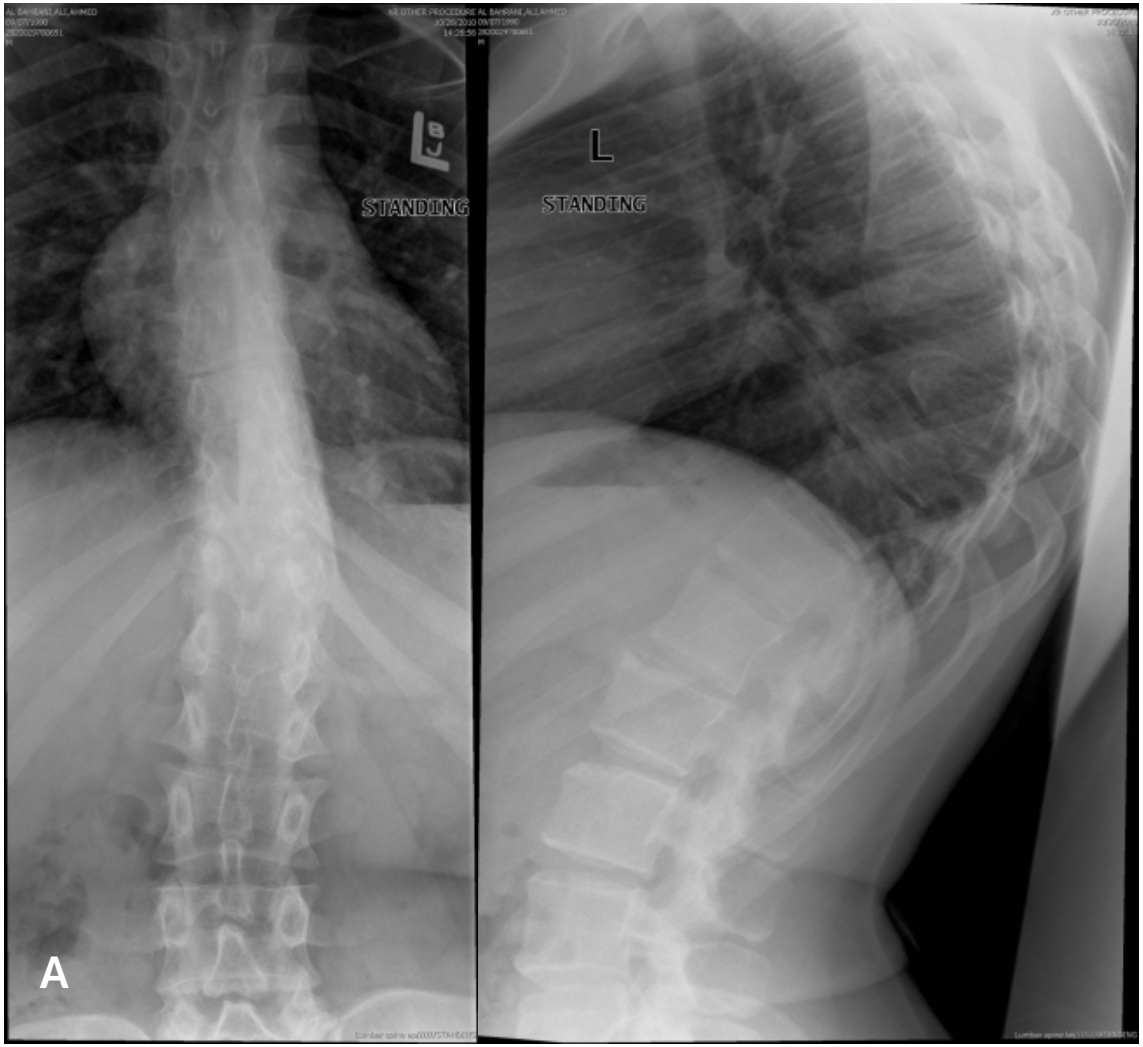

B
1 9:52 AM

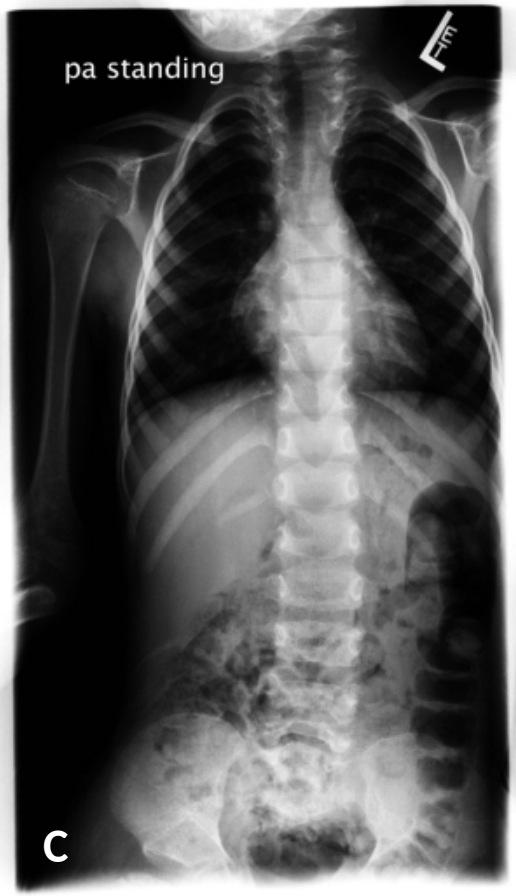

C
D

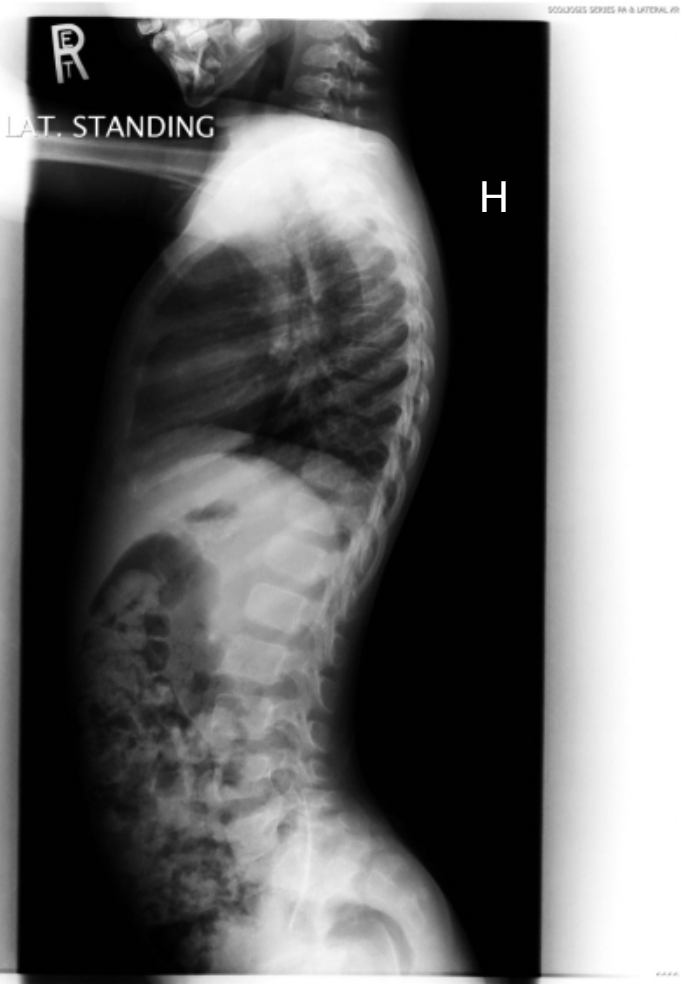

E

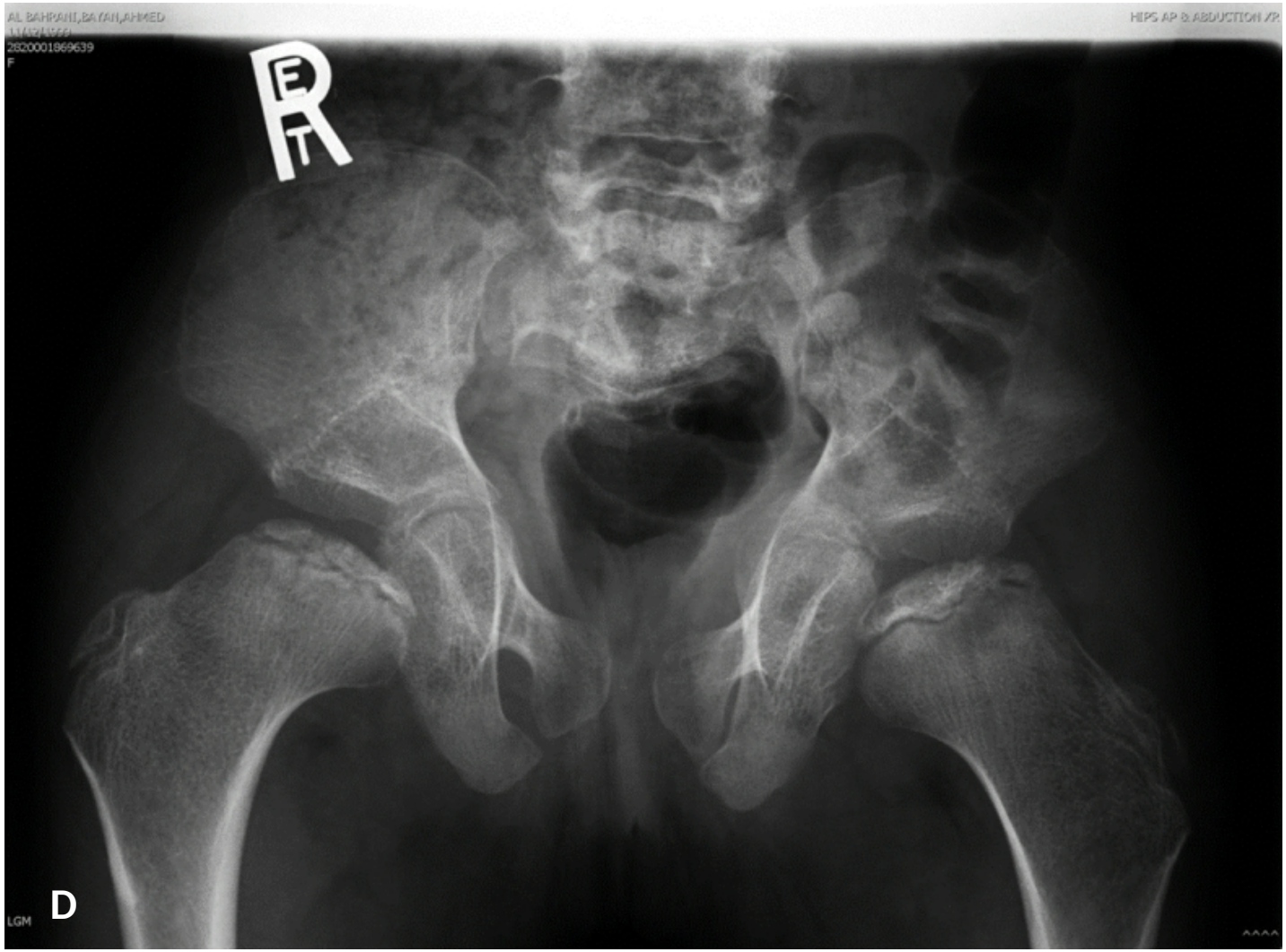

F

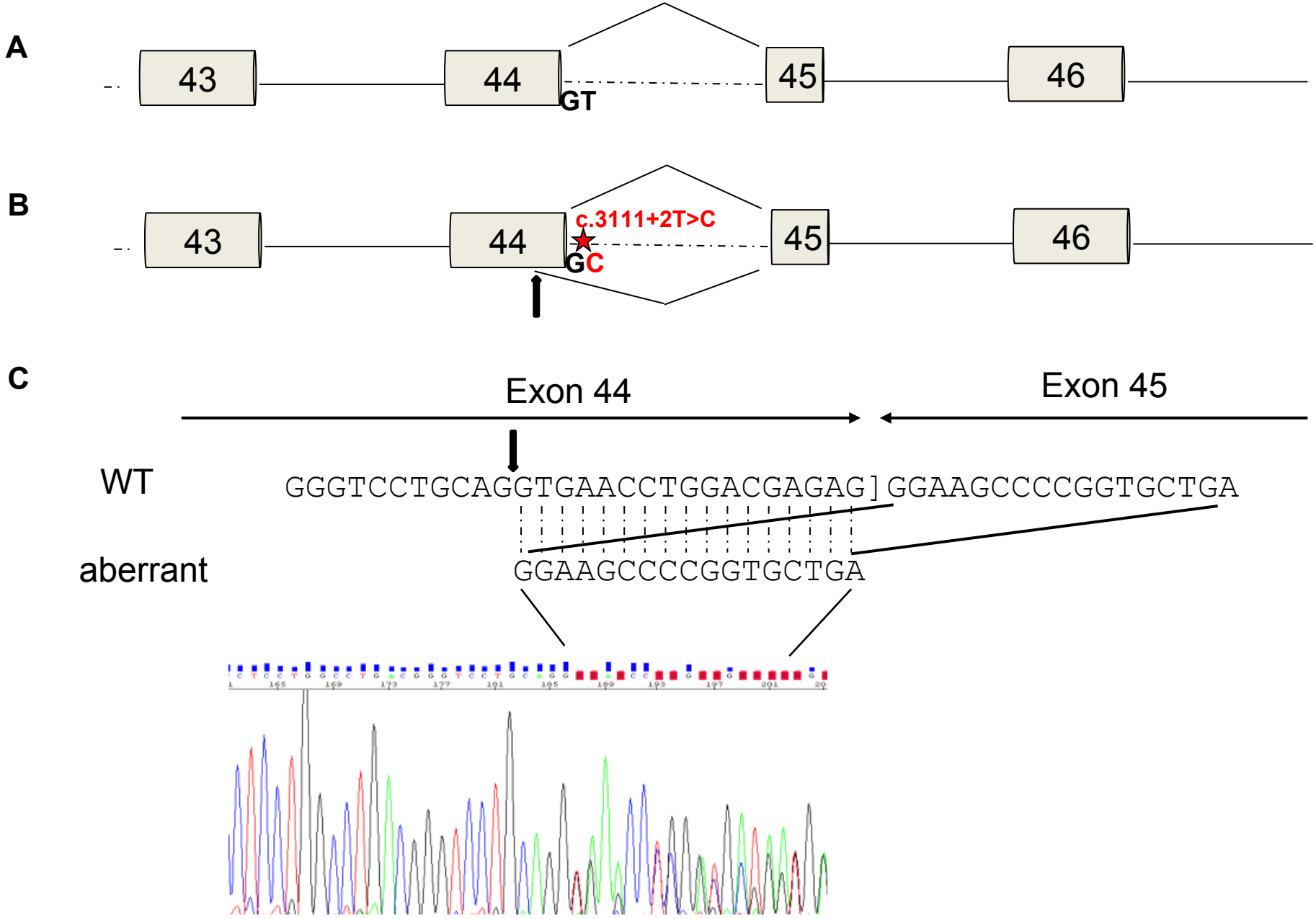

AL BAHRANI,ALI,AHMED
09/07/1990
M
R
HIPS AP & ABDUCTION ADULT XR
B
LGM


STANDING
A
L
STANDING


pa standing
C


LAT. STANDING
H


AL BAHRANI,BAYAN,AHMED
2820001869639
F
HIPS AP & ABDUCTION XR
R
LGM
D


A
43
44
45
46
GT
B
43
44
c.3111+2T>C
45
46
GC
C
Exon 44
Exon 45
WT
GGGTCCTGCAGGTGAACCTGGACGAGAG]GGAAGCCCCGGTGCTGA
aberrant
GGAAGCCCCGGTGCTGA

CRediT Author Statement

Author	contribution
N. A. Al-Sannaa	Writing initial draft, resources
K.P. Hoornaert	Investigation
L. Van Laer	Methodology
H. Y. Al-Abdulwahed	resources
G. Mortier	Writing, review, and editing

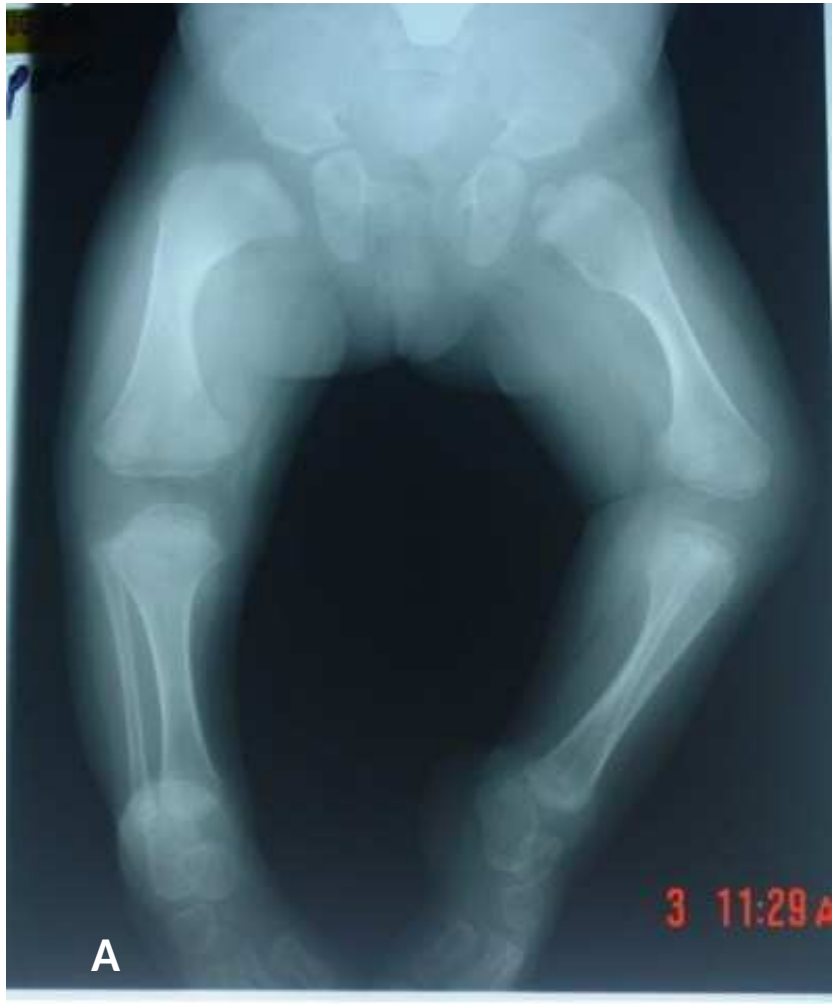
Statement to publish the patients photos without mask

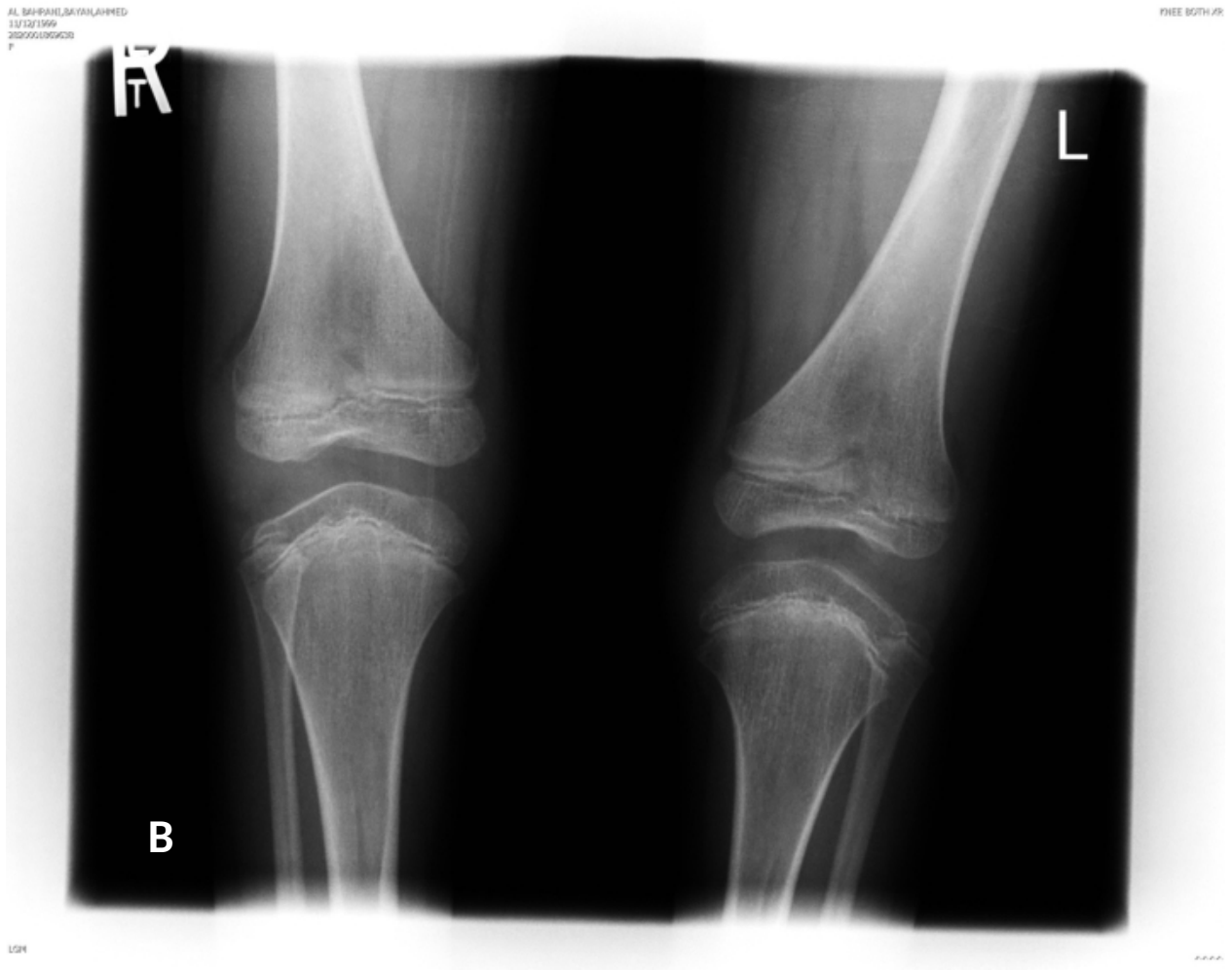
Here, I declare that a signed consent was obtained from the parents to get medical photos for sibling 1 & 2. The photos include the face, trunk, extremities and will be used for publication in a medical journal unmasked.

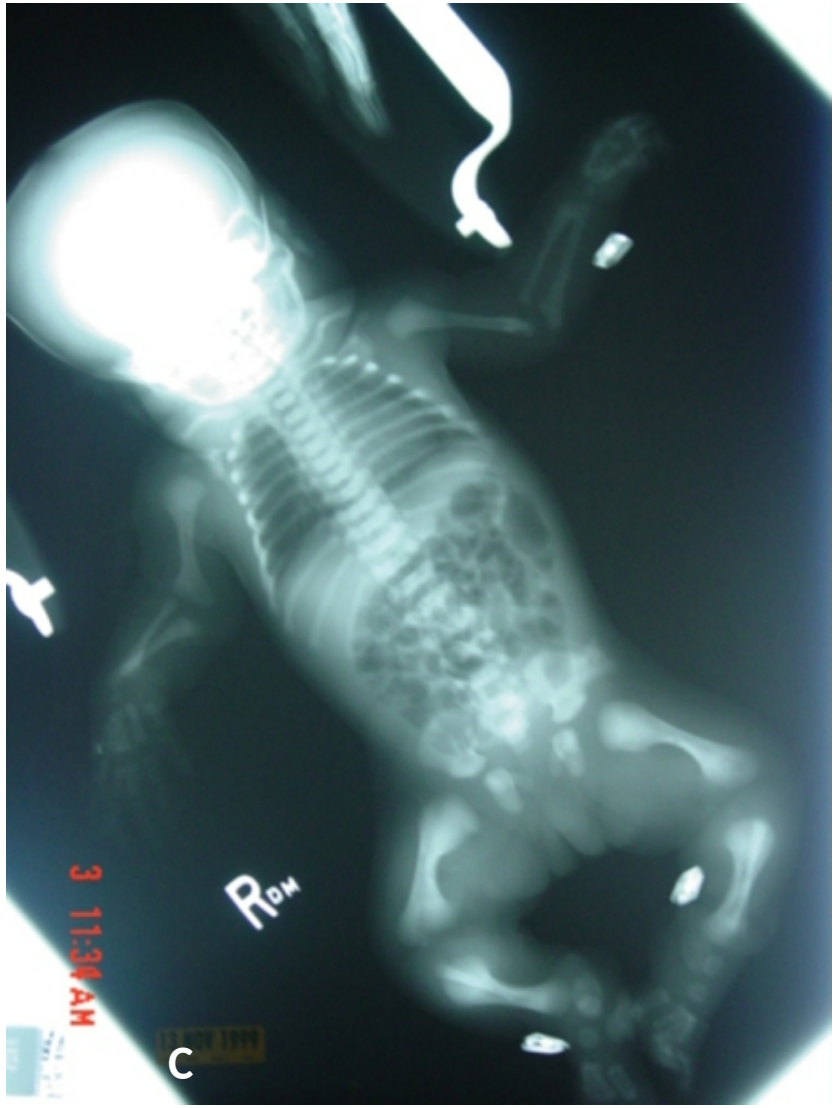
Corresponding/first author

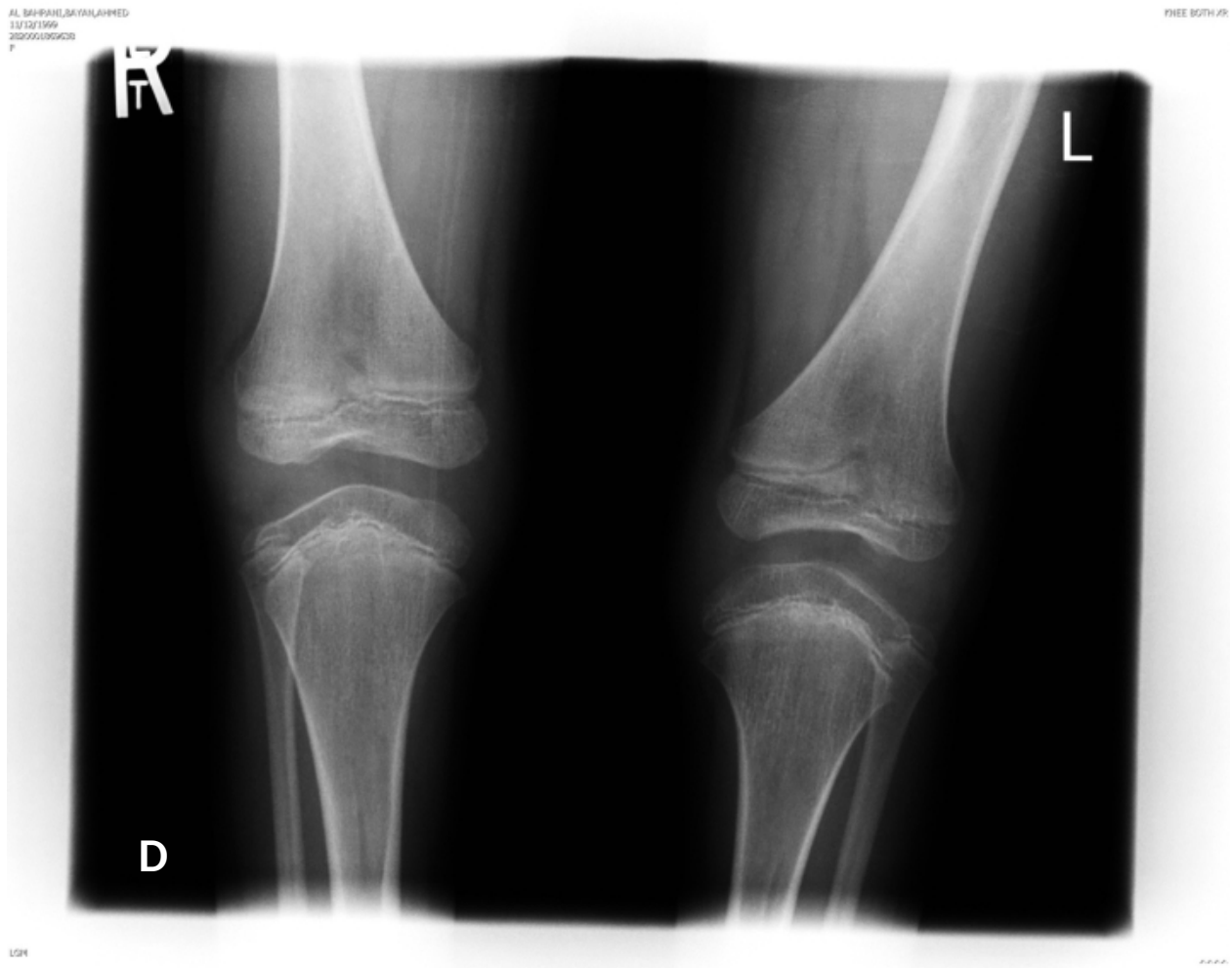
Nouriya A. Al-Sannaa

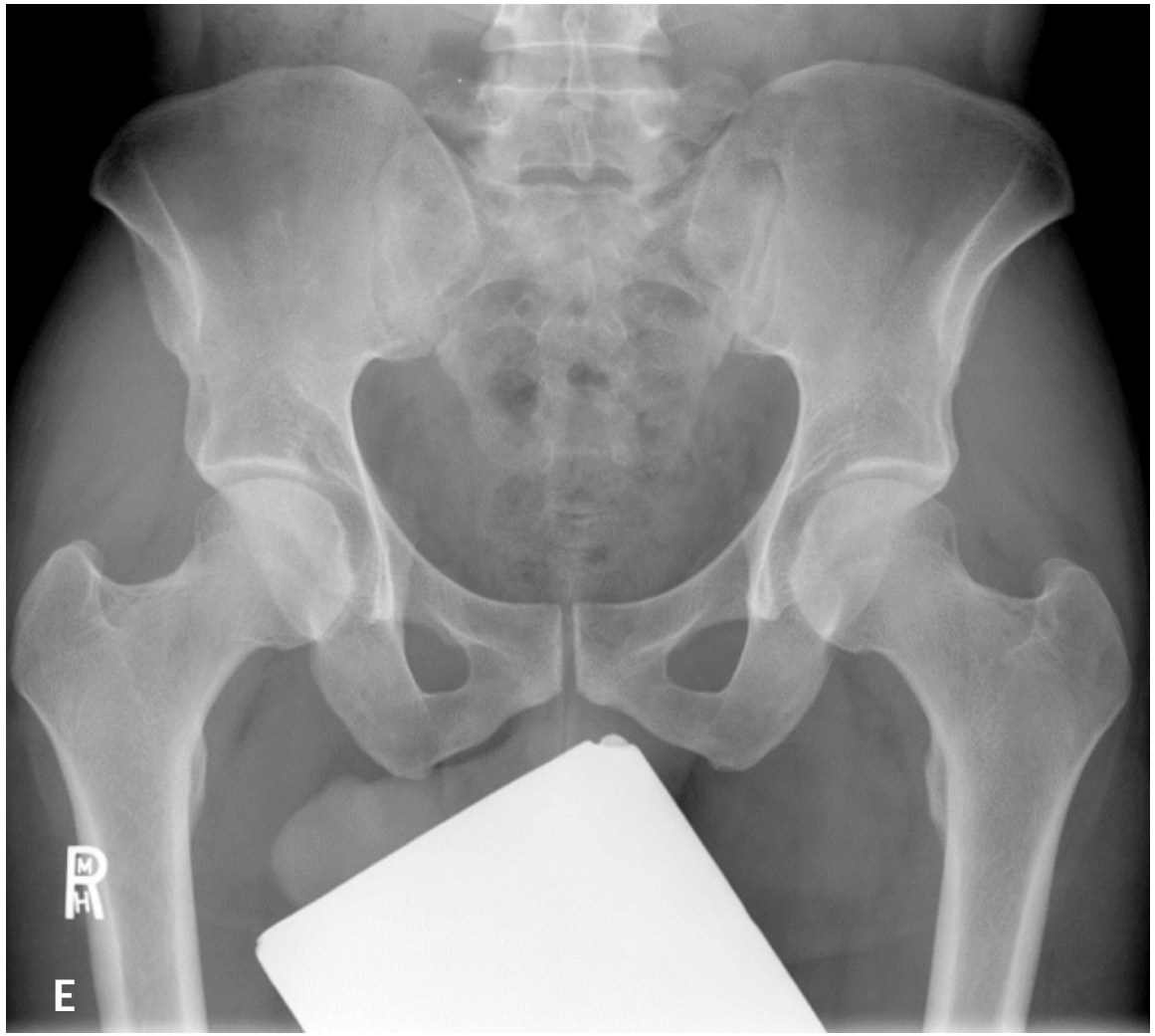
16/08/2020

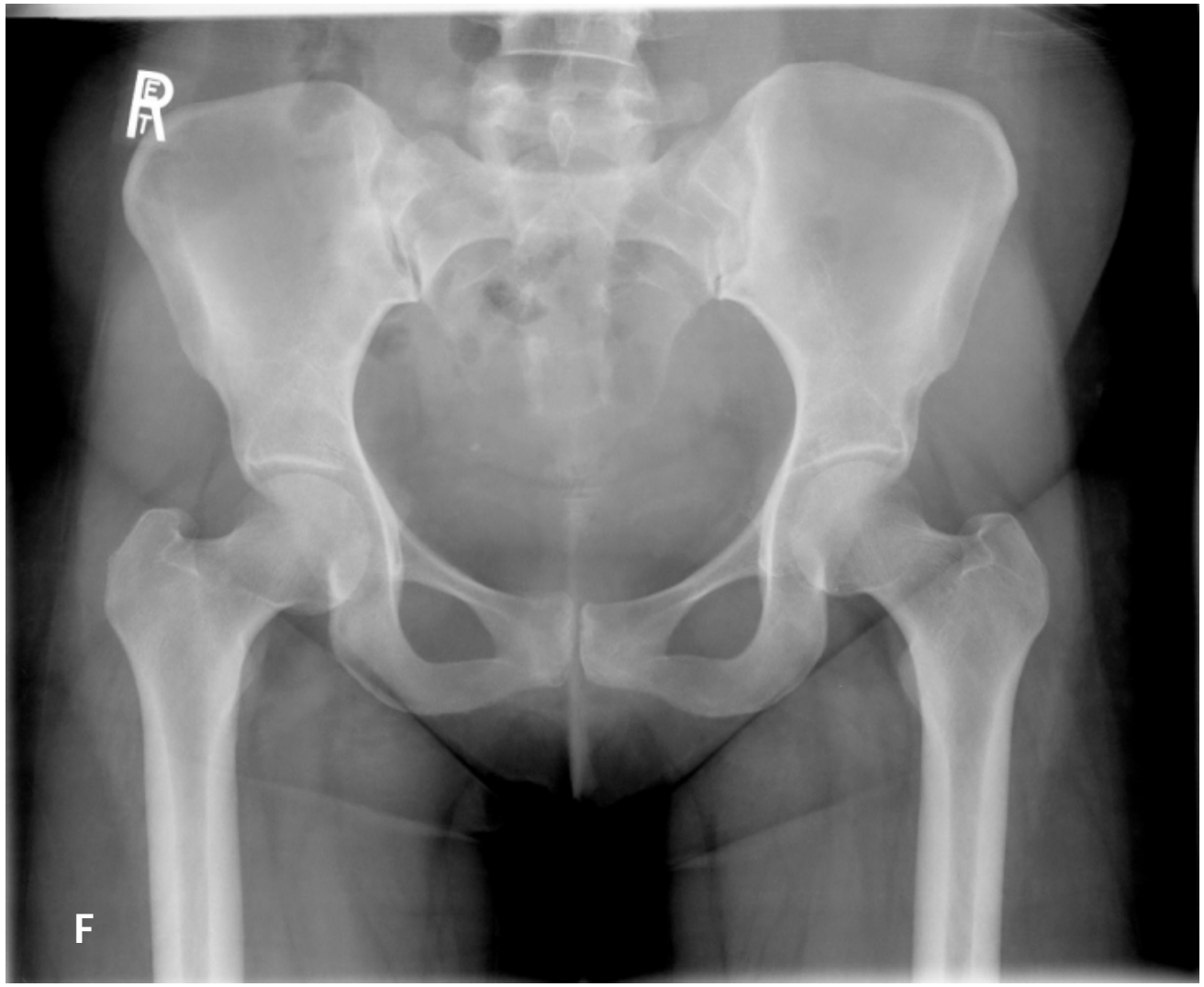

3 11:29
A


R
L
B


3 11:30 AM
RDM
C


AL BAHRANI,BAYAN,AHMED
11/12/1969
KNEE BOTH AP
R
L
D


R
E


R
F