CRTAP variants in early-onset osteoporosis and recurrent fractures

Alice Costantini[1], Ilkka Vuorimies[2,3], Riikka Mäkitie[2], Mervi K Mäyränpää[3], Jutta Becker[4], Minna Pekkinen[2,3], Helena Valta[3], Christian Netzer[4], Anders Kämpe[1], Fulya Taylan[1], Hong Jiao[5,6] and Outi Mäkitie[1,2,3,7]

[1] Department of Molecular Medicine and Surgery and Center for Molecular Medicine, Karolinska Institutet, Stockholm, Sweden, [2] Folkhälsan Institute of Genetics and University of Helsinki, Helsinki, Finland, [3] Children's Hospital, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [4] Institute of Human Genetics, University of Cologne, Cologne, Germany, [5] Department of Biosciences and Nutrition, Science for Life Laboratory, Karolinska Institutet, Stockholm, Sweden, [6] Clinical Research Centre, Karolinska University Hospital, Huddinge, Sweden, [7] Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden

Introduction

Early-onset osteoporosis presents as bone weakness and increased risk of fractures in children and young adults. The genetic causes and molecular mechanisms underlying this disease are poorly defined. However, the study of other rare bone diseases has recently led to the identification of new genes causing osteoporosis.

Aim of the project

To explore the role of variations in the cartilage-associated protein (*CRTAP*) gene in early-onset osteoporosis and/or recurrent fractures.

Patients

1) Eleven-year-old Iraqi girl with severe osteogenesis imperfecta (OI) (Fig. 1)
2) Osteoporosis group (30 patients). Inclusion criteria:
   - a BMD Z-score below -2.0 and/or
   - a history of increased bone fragility (at least three peripheral fractures and/or one or more vertebral compression fractures)
   - exclusion of secondary osteoporosis and
   - age below 30 years before the diagnosis of osteoporosis
3) Fracture-prone group (66 patients). Inclusion criteria:
   - age between 4-16 years
   - a history of at least two low-energy long bone fractures before age 10 years, three low-energy long bone fractures before age 16 years, and/or at least one low-energy vertebral compression fracture

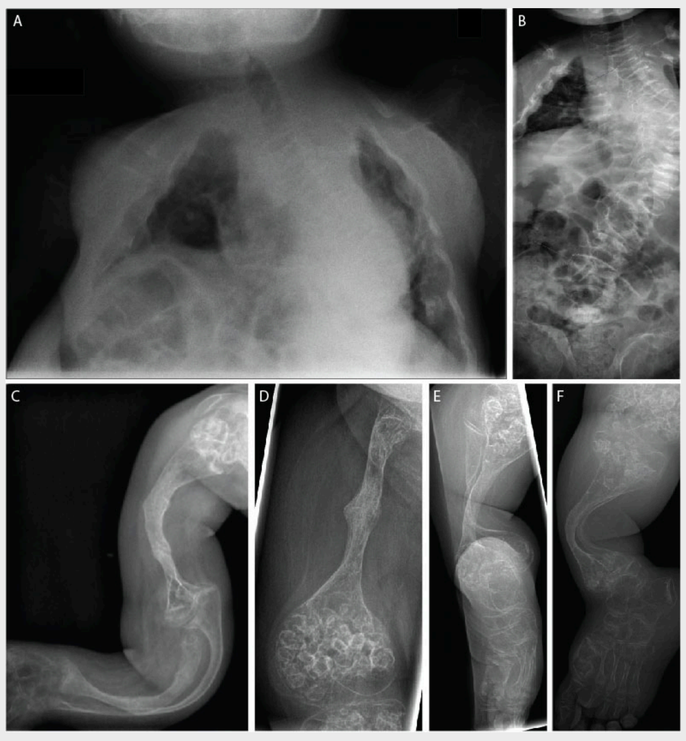

Fig. 1 Index patient's radiographs at 11 years before bisphosphonate treatment. She has small, narrow thorax (A) and severe kyphoscoliosis (B) with spinal compression fractures involving the whole spine (B). All long bones in arms (C) and legs (D-F) are short, deformed and osteoporotic. Metaphyseal regions are wide and show "pop-corn"-like irregular calcification (C-F)

Methods

- Homozygosity mapping with 1-2 STS-markers
- Sanger sequencing

Results

Index girl with OI

- Novel one-nucleotide frameshift duplication c.141dupC (p.Tyr48Leufs*113) (Fig. 2)

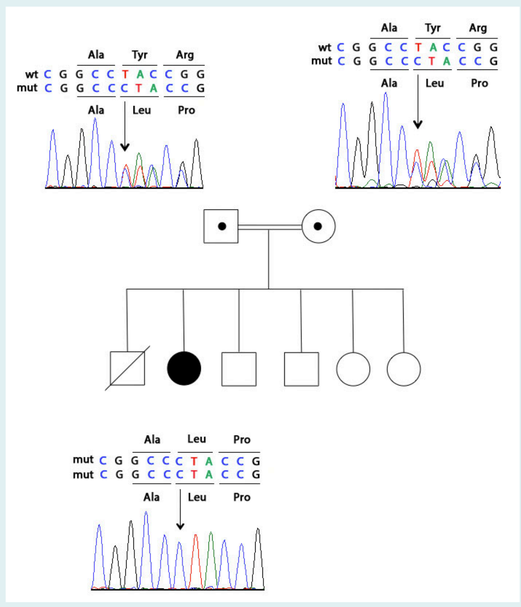

Fig. 2 Pedigree of the family and Sanger electropherograms of the frameshift mutation c.141dupC (p.Tyr48Leufs*113). The mutation is homozygous in the index girl whereas her consanguineous parents are healthy carriers. The index patient has two healthy sisters and two healthy brothers. The third brother died early after birth because of a similar skeletal phenotype. Abbreviations: wt=wild type, mut=mutated; Ala=Alanine; Tyr=Tyrosine; Arg=Arginine; Leu=Leucine; Pro=Proline

Osteoporosis group and fracture-prone group

- 5 synonymous single nucleotide polymorphisms (SNPs) (Table 1)

SNP	DNA *	Amino acid	Effect	MAF healthy population **	MAF osteoporosis group	MAF fracture-prone group	# patients osteoporosis group		# patients fracture-group	
rs11558338	c.213G>A	p.L71L	synonymous	22%	20%	8%	10		8	
							Het 8	Hom 2	Het 6	Hom 2
rs4076086	c.534C>T	p.D178D	synonymous	17%	22%	18%	11		21	
							Het 9	Hom 2	Het 18	Hom 3
rs35357409	c.558A>G	p.A186A	synonymous	1%	0%	1%	0		1	
							Het /	Hom /	Het 1	Hom /
rs1135127	c.1032T>G	p.T344T	synonymous	38%	38%	40%	20		42	
							Het 17	Hom 3	Het 31	Hom 11
rs1135128	c.1044G>A	p.S348S	synonymous	38%	38%	40%	20		42	
							Het 17	Hom 3	Het 31	Hom 11

* Reference sequence: NM_006371.4
** ExAC database; total allele frequency from all the populations

Table 1 Genetic variants identified in the *CRTAP* gene. The minor allele frequencies (MAFs) were comparable to healthy population (p>0.05)

Conclusions

- We identified a novel *CRTAP* homozygous mutation, c.141dupC, in a girl with severe OI (Fig. 3)
- We confirmed absence of carrier phenotype in her parents
- We excluded monoallelic variants in *CRTAP* as common risk factors for milder skeletal fragility

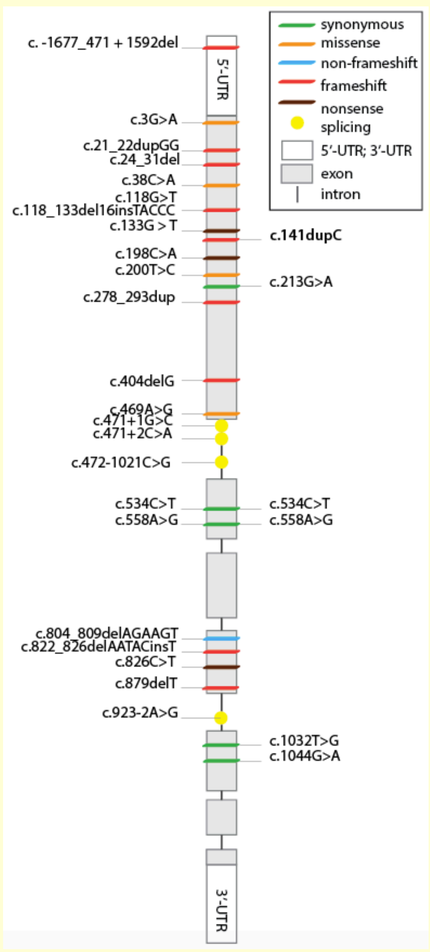

Fig. 3 A schematic representation of the *CRTAP* gene and location of all exonic and intronic pathogenic variants that have previously been reported in literature, marked on left side of the diagram. All the genetic variants found in our study, the c.141dupC and the 5 SNPs, are marked on the right side of the figure

Authors declare there is no conflict of interest

Alice Costantini, PhD student
Department of Molecular Medicine and Surgery
CMM L8:02, Karolinska University Hospital
17176 Stockholm, Sweden
E-mail: alice.costantini@ki.se

Karolinska Institutet