

Age Dependent Progression of Multiple Epiphyseal Dysplasia and Pseudoachondroplasia Due to Heterozygous Mutations in *COMP* Gene

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Abstract: Dominantly inherited mutations in *COMP* gene encoding cartilage oligomeric matrix protein may cause two dwarfing skeletal dysplasias, milder multiple epiphyseal dysplasia (MED) and more severe pseudoachondroplasia (PSACH). We studied the phenotype and X-rays of 11 patients from 5 unrelated families with different *COMP* mutations. Whole exome and/or Sangers sequencing were used for molecular analyses. Four to ten X-ray images of hands hips, knees or spine were available for each patient for retrospective analyses. Eight patients with MED have mutation c.1220G>A and 3 children with PSACH mutations c.1359C>A, c.1336G>A, or the novel mutation c.1126G>T in *COMP*. Progressive failure in growth developed in all patients from early childhood and resulted in short stature < 3rd percentile in 7 patients and very short stature < 1st percentile in four. Most patients had joint pain since childhood, severe stiffness in shoulders and elbows but

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increased mobility in wrists. Six children had bowlegs and two had knock knees. In all patients, X-rays of hands, hips and knees showed progressive, age-dependent skeletal involvement more pronounced in the epiphyses of long rather than short tubular bones. Anterior elongation and biconvex configuration of vertebral bodies were more conspicuous for kids. Six children had correction of knees and two adults had hip replacement. Skeletal and joint impairment in patients with MED and PSACH due to *COMP* mutation start in early childhood. Although the clinical severity is mutation and age dependent, many symptoms represent a continuous phenotypic spectrum between both diseases. Most patients may benefit from orthopaedic surgeries.

Introduction

Cartilage oligomeric matrix protein (*COMP*) encoded by *COMP* gene and synthesized by chondrocytes, is the multifunctional extracellular matrix calcium-binding glycoprotein involved in the enhancement of chondrocyte attachment, proliferation and cartilage production, maintenance, and homeostasis (Hecht et al., 2005; Merritt et al., 2006; Briggs et al., 2014; Posey et al., 2019). Mutations in *COMP* may cause two different types of severe short-limb dwarfism with autosomal dominant inheritance pattern affecting especially the epiphyses of long bones including multiple epiphyseal dysplasia (MED/EDM1, MIM132400) and pseudoachondroplasia (PSACH, MIM177170), but an overlap between both disorders was also described as a part of a continuous phenotypic spectrum (Jackson et al., 2012; Spranger et al., 2012).

In our study we analysed the clinical course of the disease and available X-rays in 11 patients from 5 unrelated families with MED/EDM1 or PSACH due to different heterozygous mutations in *COMP* gene including c.1220G>A, c.1359C>A, c.1336G>A, and a novel mutation c.1126G>T.

Material and Methods

The study involved 11 patients from 5 unrelated families. All of them developed progressive failure in their growth from early childhood starting between the age of 2 and 7 years. This growth failure resulted in short stature < 3rd percentile in 7 patients and short stature < 1st percentile in another four (Table 1). Most patients have stiff shoulders and elbows with severely restricted range of motion. Contrary, their wrists mobility is increased. Progressive and mostly painful gait disturbances due to legs deformities with bowlegs was present in 6 children and knock knees in two. Surgical correction of lower extremities was necessary in 6 patients during childhood and a hip replacement was required in the two oldest patients (Table 1). All patients have normal cognitive functions. Due to the lack of adequate mobility, four patients are overweight, and one is obese.

Genomic DNA from all of these patients and their parents was used for whole-exome sequencing (WES) in families A, C and E. Direct Sanger sequencing of *COMP* gene exons was performed in families B and D. Exome enrichment for WES was

Table 1 – The main clinical symptoms in 11 patients from 5 families with skeletal dysplasias due to mutations in the COMP gene for cartilage oligomeric matrix protein

	Family A P1–4	Family B P5–8	Family C P9	Family D P10	Family E P11
Mutation in COMP gene	c.1220G>A p.Cys407Tyr	c.1220G>A p.Cys407Tyr	c.1359C>A p.Asn453Lys	c.1336G>A p.Asp446Asn	c.1126G>T p.Asp376Tyr
Skeletal dysplasia	MED	MED	PSACH	PSACH	PSACH
Age (years)	14–47	16–48	2.5	12	13
Short stature (< 3 rd percentile)	4/4	4/4	+	+	+
Very short stature (< 1 st percentile)	1/4	1/4	–	+	+
Overweight	3/4	–	–	+	obesity
↑↑ stiff shoulders	4/4	4/4	–	+	+
↑↑ stiff elbows	4/4	4/4	–	+	+
↑ laxity in wrist	4/4	4/4	–	+	+
Bowlegs	4/4	–	–	+	+
Knock knees	–	2/4	–	+	–
Hip replacement	–	2/4	–	–	–
Knee surgery	2/4	2/4	–	+	+

MED/EDM1 – multiple epiphyseal dysplasia; PSACH – pseudoachondroplasia; overweight – BMI (kg/m²) > 90th percentiles for height; obesity – BMI (kg/m²) > 97th percentiles for height

performed on individually barcoded samples using SeqCap EZ MedExome Probes (Roche, USA) and sequenced using HiSeq 2500 (Illumina, USA). Reads were aligned to the hg19 reference genome using NovoalignCS version 3.1.11.08 (Novocraft, Malaysia) with default parameters. Sequence variants in analysed samples were identified using the GATK SAMtools package (version 3.1.10.1.8). High confidence variants list (SNP qual > 100 and Indel qual > 50) was annotated using Annovar

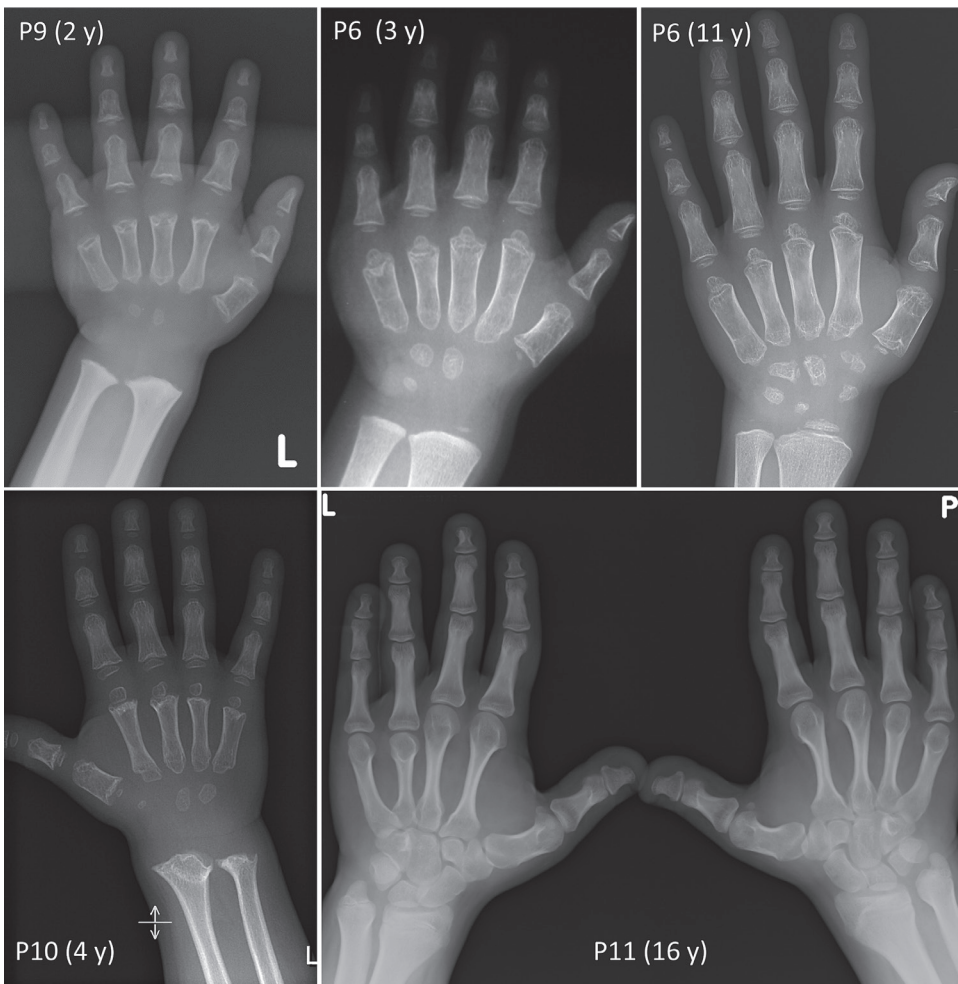


Figure 1 – X-ray survey of the hands and wrists since childhood to adulthood in patients P6, 9, 10 and 11 with multiple epiphyseal dysplasia or pseudoachondroplasia due to different mutations in *COMP* gene show deformity of the metaphyses of the shortened metacarpal bones with uneven surface. The shortening becomes more apparent after growth plate fusion. Mild shortening can be appreciated in the phalanges as well. The epiphyseal ossification centres of the metacarpal bones, distal radius and carpal bones are smaller and, in some patients, have irregular shape. The distal metaphyses of the radius and ulna have irregular contours and in adolescence show deformity that involves the radio-carpal and the distal radio-ulnar joint. The ossification is delayed.

(hg19). Due to an assumed autosomal dominant mode of inheritance, variants present in affected individuals and not present in healthy relatives were taken into consideration. Genetic background of the diagnosis of MED was reviewed and genes associated with this disorder were considered first. Presence of candidate variants was confirmed by Sanger sequencing and segregation of the variant in the whole family was performed. Four to ten X-ray images of various bones and joints from different orthopaedic departments were available for each patient.

The study was approved by the Ethics Committee of the General University Hospital in Prague and was conducted in agreement with institutional guidelines. Written informed consent for molecular analyses was obtained from all patients or their parents.

Results

Molecular analyses in 8 patients with MED/EDM1 from families A and B revealed a dominantly inherited mutation c.1220G>A (p.Cys407Tyr) in *COMP* gene. *De novo* mutations c.1359C>A (p.Asn453Lys), c.1336G>A (p.Asp446Asn) and a new mutation c.1126G>T (p.Asp376Tyr) were found in another three patients from families C, D and E (Table 1).

In all patients, X-rays of the hands, hips and knees showed age-dependent progression of epiphyseal changes, more pronounced in the epiphyses of long than short bones. Anterior elongation and biconvex configuration of vertebral bodies

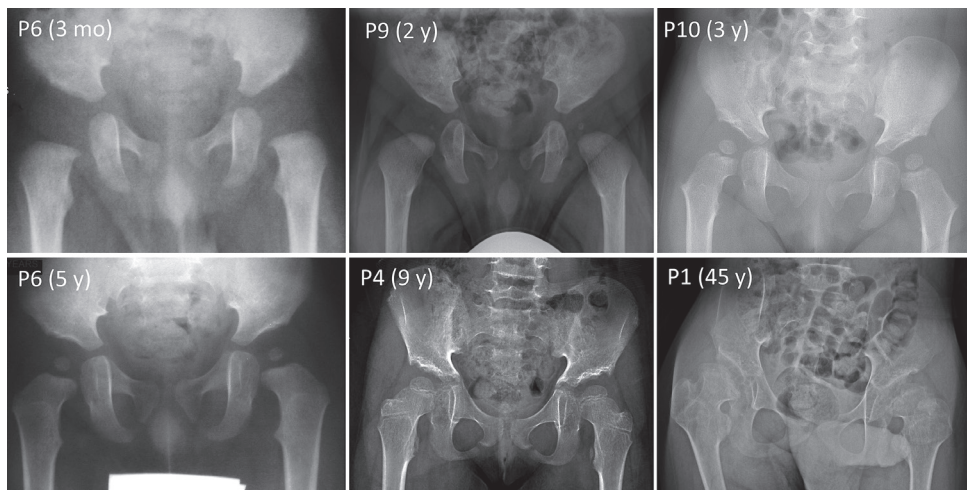


Figure 2 – X-ray survey of the hip joints since childhood to adulthood in patients P1, 4, 6, 9 and 10 with multiple epiphyseal dysplasia or pseudoachondroplasia due to different mutations in *COMP* gene show flattened, widened, robust femoral necks, decreased inclination of acetabular roofs that are shallow (dysplastic) with pointed lateral margins. The ossification centres of the femoral head are small. The iliac wings appear flared. Adult P1 developed early osteoarthritis in the setting of pre-existing severe deformity of the hip joint.

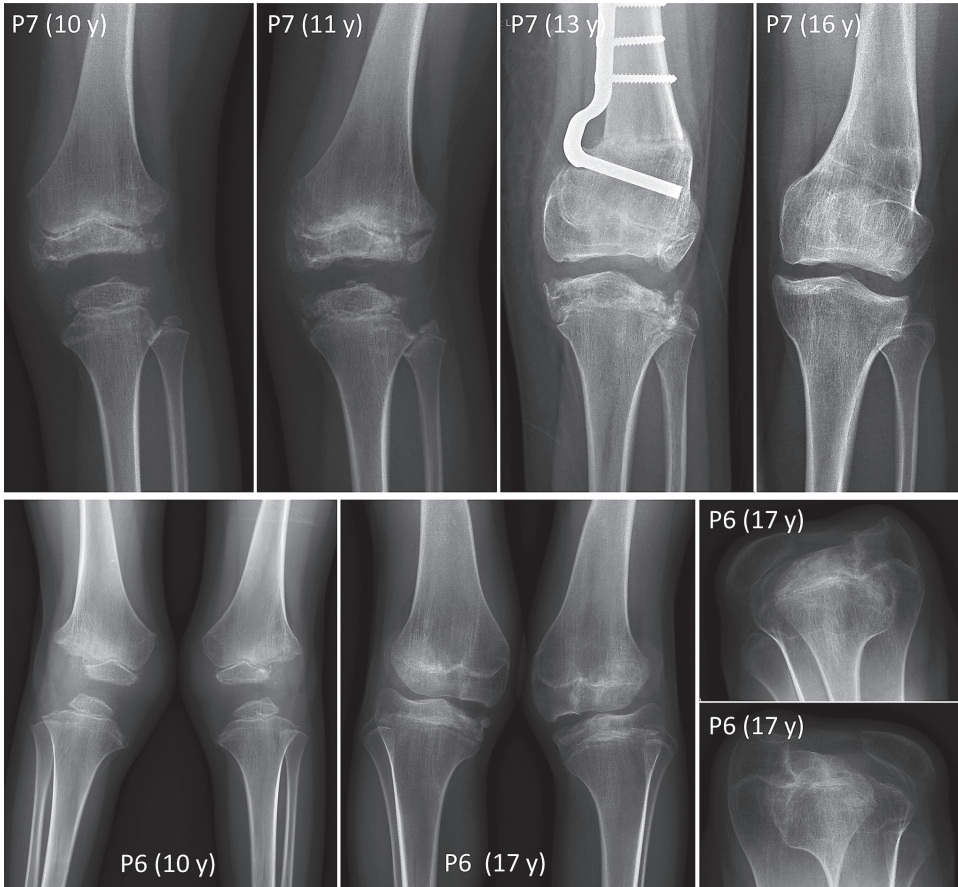


Figure 3 – X-ray survey of the knees (AP and axial views) of P6 and P7 with multiple epiphyseal dysplasia due to mutations *c.1220G>A* in *COMP* gene show progression of bone deformities during growth and ossification. The shortened metaphyses with irregular ossification progressed to deformity of their shape and axial deformity of the proximal tibia and distal femur (surgically corrected in P7), subluxation both in the femoro-tibial and femoro-patellar part of the joint in P7.

were more conspicuous in younger children. The selection of X-ray images of the wrists, pelvis, knees and spine in different age groups is shown in Figures 1–4.

Discussion

More than 300 mutations in *COMP* gene have already been identified in patients with two different dwarfing conditions including multiple epiphyseal dysplasia (MED/EDM1) and pseudoachondroplasia (PSACH) (Briggs et al., 2014; Chen et al., 2019). Although both disorders still represent individual diseases, an overlap between them was suggested on clinical and radiologic levels (Spranger et al., 2012). The first symptoms usually start in toddlers or preschool children with progression

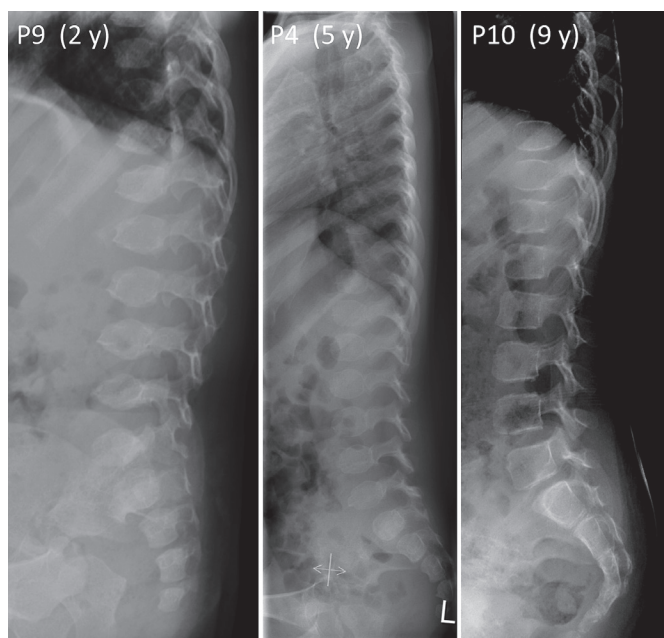


Figure 4 – X-ray survey of the lateral views of the spine of P4, 9 and P10 with multiple epiphyseal dysplasia or pseudoachondroplasia due to different mutations in *COMP* gene show anterior elongation and biconvex configuration of vertebral bodies that are more conspicuous in two younger probands.

of leg deformities and gait and growth disturbances resulting in a very short stature. Although X-ray images may help with the diagnosis of skeletal involvement, a molecular testing is usually necessary for definitive diagnostics (Anthony et al., 2015).

Most patients with PSACH have a mutation in *COMP* gene. The most common is mutation Asp469del, which impedes trafficking of *COMP* and type IX collagen in chondrocytes (Chen et al., 2008). Contrary to PSACH, multiple epiphyseal dysplasia may be except mutation in *COMP* gene also caused by mutations in other genes including *COL9A1* for collagen type IX α -1, *COL9A2* for collagen type IX α -2, *COL9A3* for collagen type IX α -3, and *MATN3* for matrilin-3 resulting in similarly disorganized epiphyseal ossification and destruction of the articular cartilage (Jackson et al., 2004; Hecht et al., 2005; Spranger et al., 2012). In addition, the autosomal recessive variant of MED is caused by a mutation of the sulphate transporter gene *SLC26A2* (Anthony et al., 2015). Therefore, MED belongs to the most genetically heterogeneous disorders between skeletal dysplasias (Unger and Hecht, 2001). Mutations in specific residues or regions of *COMP* are significantly associated with either PSACH or MED but other factors including genetic modifiers are likely to influence the disease severity, which has already been reported in MED caused by *MATN3* mutations (Briggs et al., 2014).

In our patients with heterozygous mutations in *COMP*, the most common variant was c.1220G>A identified in 8 patients with MED from two unrelated families. The variant is present in public Human Gene Mutation Database and according

to the American College of Medical Genetics and Genomics classification is likely pathogenic. It is located in a well-established functional domain (calcium binding type III repeats), where no benign variations are present. The mutation c.1220G>A was previously reported in a sporadic Chinese girl with PSACH (Cao et al., 2011). Her radiographs showed platyspondyly of the spine and anterior fractures of the vertebrae, significant epiphyseal and metaphyseal changes in the joints of the long and short tubular bones, short metacarpal bones and phalanges and small irregular carpal bones compatible with PSACH (Cao et al., 2011). Unlike the Chinese girl, our patients with the same mutation lack a severe impairment of the spine and their phenotypes are more compatible with MED. Our results suggest that not only different mutations, but also the same variant in *COMP* gene may cause either multiple epiphyseal dysplasia or pseudoachondroplasia. This is similar to other amino acid changes in the same protein position as p.Cys407Tyr resulting in MED/EDM1 (Kennedy et al., 2005) or PSACH (Chen et al., 2019). This is supported by experiments described in a *Coll1Tg^{co8}* mouse.

The induction of endoplasmic reticulum (ER) stress in the proliferative zone of chondrocytes produced decreased cell proliferation and bone growth and demonstrated the central role of classical ER stress in the MED and PSACH disease process. However, the induced ER stress was not sufficient enough to replicate all of the pathological features of MED and PSACH, suggesting that a combination of more factors including the presence of a defective cartilage extracellular matrix in addition to an increased ER stress is likely to contribute to the pathogenesis of MED and PSACH (Kung et al., 2015; Posey et al., 2019).

Mutations p.Asp376Tyr, p.Asn453Lys and p.Asp446Asn are also located within the type III repeat domain of *COMP*, which is the most common domain for both MED and PSACH. Mutation p.Asp376Tyr was published previously in a patient with PSACH, who accumulated *COMP* and type IX collagen in chondrocytes (Maddox et al., 1997). Mutation p.Asp446Asn has not been described yet, but 3 different substitutions of Asp446 were published in two patients with MED (Zankl et al., 2007; Kim et al., 2011) and one patient with PSACH (Kennedy et al., 2005).

The quality of life is strongly affected in patients with a mutation in *COMP* gene. Treatment is based on the severity of limb deformity, the level of joint destruction, age of the patient and his/her needs. In our group of patients, the most common orthopaedic surgeries during childhood were focused on bowlegs and knock knees. Especially children can benefit greatly from limb realignment procedures. Adults may also have good functional outcomes with joint arthroplasty (Anthony et al., 2015), but discussing the proper timing for the surgery may be of importance. Interestingly, adolescents with idiopathic scoliosis (AIS) have higher *COMP* promoter methylation and lower gene expression, which correlates with young age and high Cobb angle of the main curve. *COMP* promoter methylation may provide prognostic information in predicting the susceptibility and curve progression of AIS (Mao et al., 2018).

Conclusion

In both multiple epiphyseal dysplasia and pseudoachondroplasia, the skeletal and joint impairment due to mutation in *COMP* start in early childhood. Although the clinical severity is mutation and age dependent, many symptoms represent a continuous phenotypic spectrum between both diseases. Most patients may benefit from orthopaedic surgeries, but the disease still has severe negative impact on their quality of life. Early establishing of the diagnosis is important for genetic counselling and eventual pre-implantation genetic testing.

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