A Novel *COMP* Gene Mutation in a Korean Kindred with Multiple Epiphyseal Dysplasia

Jung Min Ko[1,2] · Kyu Sung Kwack[3] · Kum-Nyeo Baek[2] · Dae-Yeon Cho[4] · Hyon-Ju Kim[2]

[1]Department of Medical Genetics, [2]Center for Genetic Disease, Ajou University Hospital, Suwon, Korea
[3]Department of Radiology, Ajou University Hospital, Suwon, Korea
[4]Clinical Research Institute, Labgenomics Co., Ltd., Seoul, Korea

Multiple epiphyseal dysplasia (MED) is a clinically and genetically heterogeneous chondroplasia, characterized by delayed development of the ossification centers and, deformities of the extremities that involve only the epiphysis and result in mild short stature. Mutations in the cartilage oligomeric matrix protein (*COMP*) gene are most commonly found, and most of the mutations are located in the calmodulin-like repeats and the C-terminal domain. We report a Korean kindred of 12 family members with MED in four generations who were found to have a novel mutation in the *COMP* gene. A pedigree showed early onset osteoarthritis requiring arthroplasty that was an autosomal dominant inherited trait. Radiological examinations demonstrated the presence of osteochondral defects in the medial femoral condyles, and the knee and hip joints showed variable degrees of precocious degenerative changes. Mutation analysis of the *COMP* gene in the proband and five other affected family members identified a novel missense mutation, c.1280G>C (p.Gly427Ala) in exon 12, which was not found in three unaffected family members. Direct sequencing of the *COMP* gene may yield pathogenic mutations in dominantly inherited MED cases, and may provide opportunities of carrier detection among high-risk family members, leading to genetic counseling for early diagnosis and intervention before the onset of complications.

Key Words: Multiple epiphyseal dysplasia, Pseudoachondroplasia, Cartilage oligomeric matrix protein gene, Korean

Introduction

Multiple epiphyseal dysplasia (MED, OMIM #600969) is a clinically variable skeletal dysplasia characterized by irregular and delayed development of the ossification centers, early-onset osteoarthritis and a mild to moderate short stature. Usually the hips and knees show most pronounced radiological findings and there is little or no vertebral involvement. MED is a mainly autosomal dominant trait, and five genes have been identified as causative genes of dominant MED, including cartilage oligomeric matrix protein (*COMP*), *MATN3, COL9A1, COL9A2* and *COL9A3*. Although mutations remain undetected in approximately 10-20% of patients with dominant MED, the contribution of the *COMP* gene to overall dominant MED has been recently reported as high as 80%[1)].

접 수: 2009년 5월 19일
수정본접수: 2009년 5월 22일
게재승인일: 2009년 6월 16일
게 재 일: 2009년 6월 30일
Corresponding auther: Hyon-Ju Kim
Center for Genetic Disease, Ajou University Hospital, San 5, Wonchon-dong, Yeongtong-gu, Suwon, 443-721, Republic of Korea
Tel: +82-31-219-4522, Fax: +82-31-219-4521
E-mail: genetics@kornet.net

The *COMP* gene is located on chromosome 19p12-13.1 and the gene, consists of 19 exons and encodes the *COMP*. *COMP* is a 550 kDa homopentametric protein found in the extracellular matrix of cartilage, tendons and ligaments [2]. *COMP* contains an N-terminal pentameric domain, four epidermal growth factor (EGF)-like domains, eight calmodulin like repeats (CLR) and C-terminal globular domain. The CLRs and a C-terminal globular domain are highly conserved among members of the thrombospondin family. To date, about 100 mutations in the *COMP* gene have been reported, and most mutations are located in the exons that encode the CLRs and C-terminal domain[2, 3]. A recent study has demonstrated that approximately 70% of mutations reside in exons 10, 11 and 13 as mutation hot-spots [4].

In this report, we describe the identification of a novel *COMP* mutation outside of the hot-spots in a Korean kindred of 12 family members with MED in four generations.

Case report

The proband, a 23 year-old female, was referred for genetic counseling for a family history of early- onset osteoarthritis requiring arthroplasty of the hips. In the past medical history, the proband was born with a normal birth weight after full term of gestation and was the first child born to non-consanguineous parents. Joint pain in the knees and the hips developed since the age of seven years. However, the joint pain was considered as growing pain without a specific diagnosis. The joint pain and gait disturbance became aggravated and arthroscopic surgery of both knee joints and total hip arthroplasty of the left femur was performed under the diagnosis of osteoarthritis at the age of 21 years and 22 years respectively.

On a physical examination performed at the age of 23 years, patient height was 153.4 cm (3^{rd}-10^{th} percentile and −1.60 SD from the mean value for normal Korean females) and the limbs showed mild disproportionate shortening without bowing. The digits were short and stubby. However, the range of motion of all limb joints did not show any restriction, and the face and intelligence were normal. Radiological examinations demonstrated the presence of osteochondral defects in both medial femoral condyles (Fig. 1) and showed narrowing of the joint spaces, with incongruity of the articular surface in the knee and hip joints of variable degrees. The spine did not show any abnormal findings. Such precocious osteoarthropathies supported a diagnosis of MED.

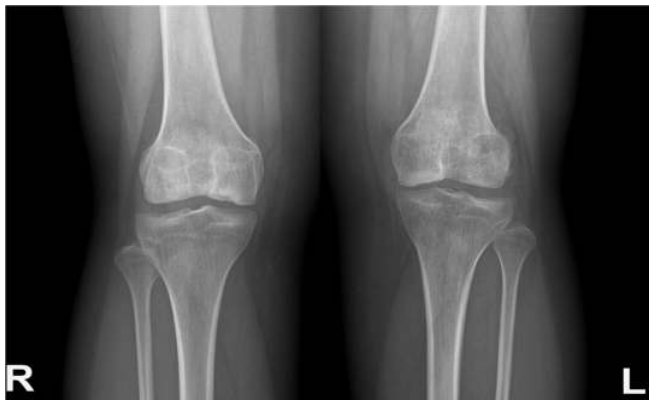

Fig. 1. The osteochondral defect and precocious degenerative changes in the medial femoral condyles of the proband are shown in this picture.

The family history of autosomal dominantly inherited, early-onset osteoarthritis was remarkable as seen on a pedigree shown in Fig. 2. The father of the proband (III:5, a 60 year-old) has a height of 150 cm (−4.46 SD) and had suffered from scoliosis of the thoracic spine and knee and hip joint osteoarthiritis that required wheelchair ambulation. One of the uncles of the proband (III:7, a 57 year-old; height, 164.7 cm, −1.66 SD) had undergone arthroplasty of both hip joints at the age of 56 years and his daughter (IV:7, a 15 year-old; height, 158 cm, −0.59 SD) also showed waddling gait and complained the knee joints pain during the ambulation. An aunt of the proband (III:10, a 54 year-old; height, 150 cm, −1.53 SD) and her son (IV:9, a 25 year-old; height, 165.2 cm, −1.56 SD) were diagnosed as having osteoarthiritis in both hip joints and surgical management was necessary

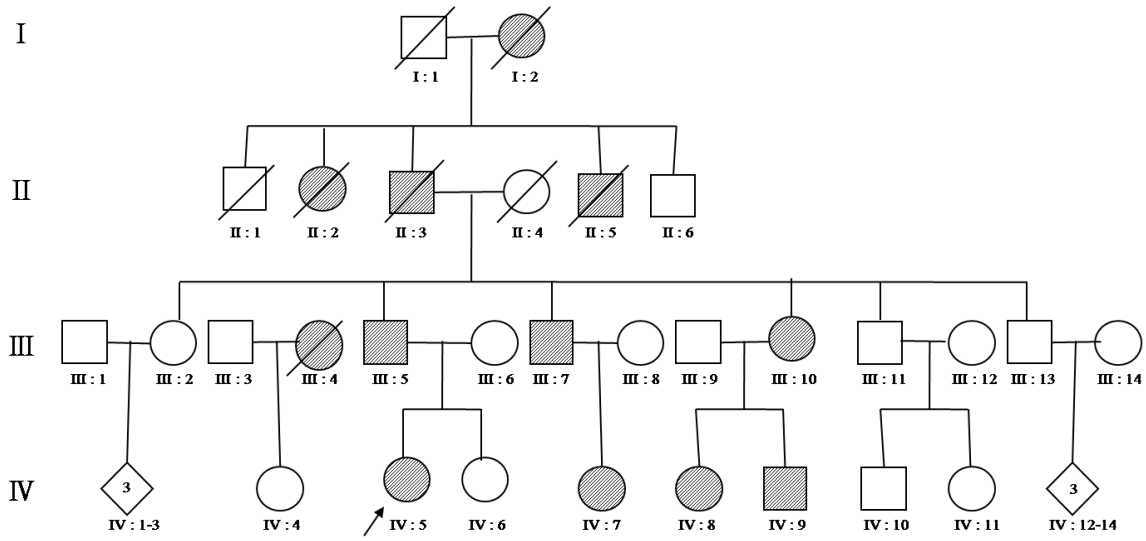

Fig. 2. A pedigree of the family spanning four generations shows the autosomal dominant trait. Generations are numbered with roman numerals. The relative position of each individual of the same generation is designated by the use of Arabic numbers. IV:5 is the proband.

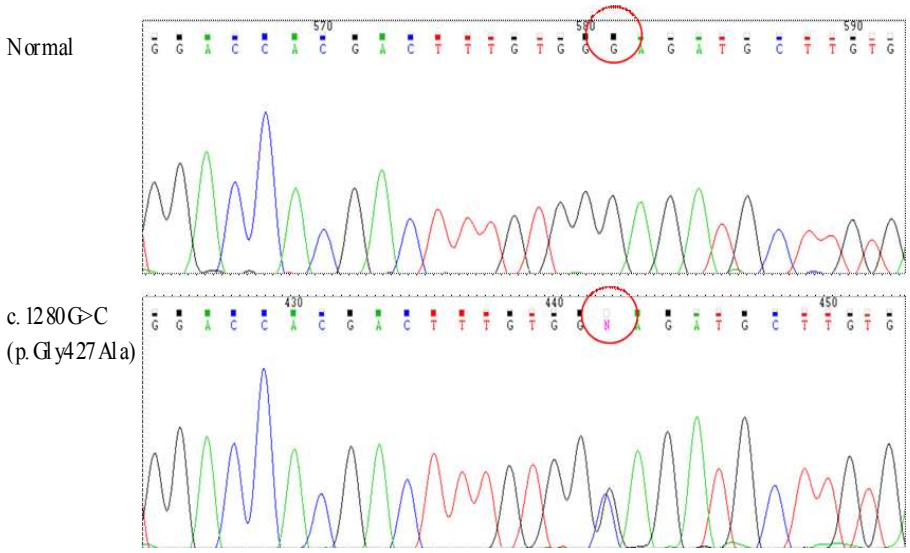

Fig. 3. A novel mutation, c.1280G>C (p.Gly427Ala) of the *COMP* gene was identified by direct DNA sequencing of the PCR product of the proband.

due to severe pain. For the family members with MED phenotypes, joint pain after exercise started at an approximate age of ten years and was aggravated thereafter. However, there was no other family members had shown any symptoms and signs of osteoarthiritis including the mother of the proband (III:6, a 55 year-old; height, 153 cm, −1.69 SD) and a sister (IV:6, a 22 year-old; height, 165 cm, 0.95 SD).

Mutation analysis and findings

Informed consent was obtained before the sampling of blood. Genomic DNA was isolated from peripheral blood lymphocytes. In the proband, exons 8-19 and the intronic flanking regions of the *COMP* gene were amplified by the use of PCR. Subsequently, PCR products were purified and DNA sequencing was performed.

Direct DNA sequencing of the product revealed that

the proband was heterozygous for a G-to-C transition at nucleotide 1280 (c.1280G>C, p.Gly427Ala) in exon 12 (Fig. 3). This was a novel missense sequence altration with as yet unverified clinical significance. DNA sequencing analysis was then performed sequence analysis for this lesion in eight other family members (III:5, III:6, III:7, III:10, III:11, IV:6, IV:7 and IV:9) to confirm this novel sequence alteration in *COMP* as a pathological mutation. The same missense sequence alteration was found in five (III:5, III:7, III:10, IV:7 and IV:9) of eight family members that showed MED phenotypes. However, no sequence alteration was found in three unaffected family members (III:6, III:11 and IV:6).

Discussion

Dominantly inherited MED affects 1 in 10,000 individuals, and shows relatively mild and clinically variable osteochondrodysplasia. The diagnosis is based on the clinical and radiographic presentations, which is supported by a positive family history of early onset osteoarthritis. Molecular genetic testing and mutation identification is confirmatory diagnostic testing and can be used for prenatal diagnosis.

COMP was the first identified gene among the six genes associated with MED in 1995[5]. Mutations in the *COMP* gene are known to be responsible for two skeletal dysplasias, pseudoachondroplasia (PSACH, OMIM #177170) and MED. These two diseases belong to the same diagnostic group and they comprise a clinical spectrum with phenotype overlap between MED and PSACH[6]. The diseases have been divided based on radiographic and clinical severity, but this is a somewhat arbitrary separation. Although PSACH results almost exclusively from mutations in the *COMP* gene, MED shows considerable genetic heterogeneity and *COMP* mutations had been identified in only 25-36% of MED patients[2, 4], prior to a recent report showing a mutation detection rate as high as 80%[1]. Mutations in the *COMP* gene cause a cellular phenotype characterized by large rough endoplasmic reticulum (rER) cisternae that are filled with lamellar depositions of mutant *COMP* protein and several other cartilage ectracellualr matrix proteins. Inhibition of *COMP* secretion and mutant *COMP* retention in the rER seems to compromise functions of chondrocytes[7, 8]. A recent report showed that circulating *COMP* protein levels could reflect genetic abnormalities in the *COMP* gene and might serve as a rapid and cost-efficient diagnostic method[9].

More than 100 mutations have been identified in the *COMP* gene, with the majority (85-96%) occurring in the CLRs with the remainder in the C-terminal domain (4-15%)[4, 10]. Approximately 70% of mutations reside in exons 10, 11 and 13 as mutation hot-spots, especially in exon 13[4]. We searched for a mutation first in exons 8-19, which encode all of the CLRs (exons 8-14) and the C-terminal domain (exons 15-19). In addition, a novel sequence alteration, p.Gly427Ala, was identified in exon 12 of the *COMP* gene. Although exon 12 is located in the sixth CLR, mutations have been infrequently found in exon 12.

Although this alteration has unverified clinical significance, it seems to be a strong candidate for a causal mutation of this familial disease for the following three reasons. First, this sequence alteration was located in the sixth CLR of calcium-binding pockets; this domain is highly conserved among members of the thrombospondin family and the domain has an important role in the function of *COMP*. To predict the functional impact of this amino acid change, we additionally assessed this novel sequence alteration by the use of two in silico prediction algorithms, PolyPhen (Polymorphism Phenotyping) and SIFT (Sorting Intolerant from Tolerance)[11, 12]. The PolyPhen and SIFT programs also showed that p.Gly427Ala was predicted to be a mutation with a probably damaging effect (PSIC score difference = 2.186) and to affect protein function (SIFT score = 0.03), respectively. Second, the family study showed that this alteration segregated with the MED phenotypes of only affected family members. Lastly, an alteration in

the same site (p.Gly427Glu) was already reported as a disease causing mutation in a patient showing a mild PSACH phenotype[13]. This mutation was also demonstrated as a pathological change by a study with the use of in vitro models[14]. It is known that mutations in the same site of *COMP* can show different clinical phenotypes (PSACH or MED)[15]. Moreover, patients with even the same mutation can have either PSACH or MED[4, 16]. The affected persons in our case also showed various skeletal deformities of different degrees. These findings suggest that genes other than *COMP* are significantly involved in cartilage matrix assembly[17].

In conclusion, we have described a Korean kindred of MED with a novel mutation of the *COMP* gene. Direct DNA sequencing of the *COMP* gene may yield pathogenic mutations in up to 80% of cases of dominantly inherited MED and may provide opportunities of carrier detection among high-risk family members, leading to genetic counseling for early diagnosis and intervention before the onset of complications.

국문초록

다발성 골단이형성증은 임상적 및 유전학적으로 이질적인 연골이형성증으로, 골화 중심의 발달 지연, 골단만을 침범하는 사지의 변형 및 경한 저신장을 보이는 질환이다. 원인으로는 *COMP* 유전자의 돌연변이가 가장 흔히 발견되고 있으며, 돌연변이의 대부분은 칼모듈린 유사 반복(calmodulin-like repeats)부분과 C-말단에 위치한다. 저자들은 4대에 걸쳐 12명의 가족구성원에서 다발성 골단이형성증을 보인 한 가계에서 *COMP* 유전자 분석으로 새로운 돌연변이를 발견하여 보고하는 바이다.

이 가계는 젊은 나이에 퇴행성 관절염이 발생하고, 추후 관절치환술이 요구되는 상염색체 우성 유전 방식을 보이는 질환을 가지고 있었다. 방사선학적 검사상 양측 무릎 관절의 내측 대퇴돌기에 골연골 결손을 보였으며, 양측 무릎과 엉덩이 관절은 다양한 정도의 퇴행성 변화가 관찰되었다. 계보발단자 및 5명의 다른 이환된 가족들에서 시행한 *COMP* 유전자 분석에서, 12번째 엑손에서 현재까지 보고되지 않은 새로운 돌연변이인 c.1280G>C (p.Gly427Ala)을 확인하였다. 이 돌연변이는 다른 3명의 이환되지 않은 가족들에서는 발견되지 않았다. 상염색체 우성 방식을 보이는 다발성 골단이형성증 환자들에서 *COMP* 유전자의 직접 염기서열 분석법은 질병의 원인이 되는 돌연변이를 찾는데 도움이 되며, 고위험 가족들에서 보인자를 찾아낼 수 있고, 질병의 합병증이 발생하기 이전에 조기 진단 및 중재를 가능케 하는 유전 상담의 기회를 제공해 줄 수 있다.

References

1) Zankl A, Jackson GC, Crettol LM, Taylor J, Elles R, Mortier GR, et al. Preselection of cases through expert clinical and radiological review significantly increases mutation detection rate in multiple epiphyseal dysplasia. Eur J Hum Genet 2007;15:150-4.
2) Briggs MD, Chapman KL. Pseudoachondroplasia and multiple epiphyseal dysplasia: mutation review, molecular interactions, and genotype to phenotype correlations. Hum Mutat 2002;19:465-78.
3) Unger S, Hecht JT. Pseudoachondroplasia and multiple epiphyseal dysplasia: New etiologic developments. Am J Med Genet 2001;106:244-50.
4) Kennedy J, Jackson G, Ramsden S, Taylor J, Newman W, Wright MJ, et al. COMP mutation screening as an aid for the clinical diagnosis and counselling of patients with a suspected diagnosis of pseudoachondroplasia or multiple epiphyseal dysplasia. Eur J Hum Genet 2005;13:547-55.
5) Briggs MD, Hoffman SM, King LM, Olsen AS, Mohrenweiser H, Leroy JG, et al. Pseudoachondroplasia and multiple epiphyseal dysplasia due to mutations in the cartilage oligomeric matrix protein gene. Nat Genet 1995;10:330-6.
6) Rimoin DL, Rasmussen IM, Briggs MD, Roughley PJ, Gruber HE, Warman ML, et al. A large family with features of pseudoachondroplasia and multiple epiphyseal dysplasia: exclusion of seven candidate gene loci that encode proteins of the cartilage extracellular matrix. Hum Genet 1994;93:236-42.
7) Hecht JT, Hayes E, Snuggs M, Decker G, Montufar-Solis D, Doege K, et al. Calreticulin, PDI, Grp94 and BiP chaperone proteins are associated with retained COMP in pseudoachondroplasia chondrocytes. Matrix Biol 2001;20:251-62.
8) Hecht JT, Makitie O, Hayes E, Haynes R, Susic M, Montufar-Solis D, et al. Chondrocyte cell death and intracellular distribution of COMP and type IX collagen in the pseudoachondroplasia growth plate. J Orthop Res 2004;22:759-67.

9) Mabuchi A, Momohara S, Ohashi H, Takatori Y, Haga N, Nishimura G, et al. Circulating COMP is decreased in pseudoachondroplasia and multiple epiphyseal dysplasia patients carrying COMP mutations. Am J Med Genet A 2004;129A:35-8.
10) Kennedy J, Jackson GC, Barker FS, Nundlall S, Bella J, Wright MJ, et al. Novel and recurrent mutations in the C-terminal domain of COMP cluster in two distinct regions and result in a spectrum of phenotypes within the pseudoachondroplasia--multiple epiphyseal dysplasia disease group. Hum Mutat 2005; 25:593-4.
11) Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res 2002;30:3894-900.
12) Ng PC, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. Nucleic Acids Res 2003;31:3812-4.
13) Deere M, Sanford T, Ferguson HL, Daniels K, Hecht JT. Identification of twelve mutations in cartilage oligomeric matrix protein (COMP) in patients with pseudoachondroplasia. Am J Med Genet 1998;80: 510-3.
14) Posey KL, Yang Y, Veerisetty AC, Sharan SK, Hecht JT. Model systems for studying skeletal dysplasias caused by TSP-5/COMP mutations. Cell Mol Life Sci 2008;65:687-99.
15) Briggs MD, Mortier GR, Cole WG, King LM, Golik SS, Bonaventure J, et al. Diverse mutations in the gene for cartilage oligomeric matrix protein in the pseudoachondroplasia-multiple epiphyseal dysplasia disease spectrum. Am J Hum Genet 1998;62:311-9.
16) Nakashima E, Mabuchi A, Kubota M, Ishikiriyama S, Ohashi H, Nishimura G, et al. Novel and recurrent exon 13 mutations of COMP in pseudoachondroplasia. Am J Med Genet A 2005;132A:108-9.
17) Holden P, Meadows RS, Chapman KL, Grant ME,

Kadler KE, Briggs MD. Cartilage oligomeric matrix protein interacts with type IX collagen, and disruptions to these interactions identify a pathogenetic mechanism in a bone dysplasia family. J Biol Chem 2001;276:6046-55.