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

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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 ossifica-
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. *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. A recent study has demonstrated that approximately 70% of mutations reside in exons 10, 11 and 13 as mutation hot-spots. 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 (3rd–10th 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 osteoarthopathies supported a diagnosis of MED.

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 osteoarthritis 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 osteoarthritis in both hip joints and surgical management was necessary.
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 member who had shown any symptoms and signs of osteoarthritis 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→C transition at nucleotide 1280 (c.1280G>C, p.Gly427Ala) in exon 12 (Fig. 3). This was a novel missense sequence alteration 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. Mutations in the COMP gene are known to be responsible for two skeletal dysplasias, pseudoachondrodysplasia (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. 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, prior to a recent report showing a mutation detection rate as high as 80%. 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 extracellular matrix proteins. Inhibition of COMP secretion and mutant COMP retention in the rER seems to compromise functions of chondrocytes. 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.

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%). Approximately 70% of mutations reside in exons 10, 11 and 13 as mutation hotspots, especially in exon 13. 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). 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...
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The same site (p.Gly427Glu) was already reported as a disease causing mutation in a patient showing a mild PSACH phenotype. This mutation was also demonstrated as a pathological change by a study with the use of in vitro models. It is known that mutations in the same site of COMP can show different clinical phenotypes (PSACH or MED). Moreover, patients with even the same mutation can have either PSACH or MED. 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.

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.

References


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