Concise Report

A large kindred of early-onset osteoarthritis of the knee and hip: excluding the link to COL2A1 gene

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Objectives. To characterize a large extended family with early-onset OA of the knee and investigate its associations with the COL2A1 gene.

Methods. Phenotype assessments were conducted in a six-generation family to identify individuals affected with OA. Short tandem repeat polymorphic (STRP) markers and DNA sequencing were performed to investigate the involvement of the COL2A1 gene in this family.

Results. The kindred affected with OA showed autosomal dominant inheritance. The mean age of onset was 37.3 ± 12.0 years for generations IV, V and VI, respectively, and 25 ± 16.1 years for males and 34.3 ± 15.5 years for females. The height of the affected males was shorter than the unaffected males (155.9 ± 11.4 vs 164.5 ± 16.0 cm, P = 0.010). Arm span in the affected males was also significantly shorter than the unaffected males (158.4 ± 12.5 vs 165.3 ± 16.7 cm, P = 0.027). However, both height and arm span were not reduced in the affected female OA patients. STRP markers surrounding COL2A1 locus did not show linkage of the COL2A1 locus with the OA. Sequencing of COL2A1 gene revealed three single nucleotide polymorphisms but no mutation was found in the affected patients.

Conclusions. The COL2A1 was not a susceptibility gene responsible for the OA phenotype in a large extended kindred with familial early-onset OA. The availability of DNA samples will allow genome-wide linkage study to identify the susceptibility locus.

Key words: Early-onset OA, COL2A1 gene, Short tandem repeat polymorphic markers, Phenotype, Genome-wide linkage.

Introduction

OA is a degenerative joint disease that is often accompanied by hypertrophic bone changes with osteophyte formation [1, 2]. Heritability in OA was first documented >60 years ago in a report that the female siblings with hand OA were three times more likely than the general population to exhibit nodal OA beginning in the fifth decade of life [3]. Subsequent studies showed that the frequency of generalized hand OA was nearly twice for familial OA than in the general population [4, 5]. Twin studies showed that the influence of genetic factor is between 39 and 65% in women with hand and knee OA, 60% in hip OA and 70% in spine OA [5]. Numerous past and recent studies reported mapping the susceptibility genes for OA [6-11].

The search for genetic bases of the rare familial form of OA has been more fruitful [12-16]. Although familial OA is rare, it could be affected by various factors [17].

Here, we reported the identification and clinical characteristics of a familial form of OA in a large extended six-generation family. We also investigated whether COL2A1 is a disease-causing gene because clinical phenotypes of this family resemble a previously reported early onset of primary generalized OA caused by COL2A1 mutation [12, 13].

Patients and methods

Patients

A six-generation OA kindred with autosomal dominant inheritance (Fig. 1) was identified at the National Taiwan University Hospital. This kindred consisted of 274 members from two pedigrees that shared common ancestor through the connection of proband’s great grandmother and her brother. We recruited 70 individuals from this family for this study.

Phenotype assessment

All individuals were examined by one physician to prevent inter-examiner variability (S.C.M.). Height, weight, BMI, arm span and blood pressure were recorded. Blood uric acid and RF were measured. All 25 but 2 (Nos IV: 18 and IV: 44) affected patients consented to the radiological evaluation. Anterior-posterior and lateral view of radiographs of the hands, spine, knees and hips were obtained from affected and clinically suspected cases. Radiographic hip OA was defined as a joint space of \( \leq 2.5 \text{ mm} \) [18, 19]. Knee, hand and spine OA were defined as the presence of Kellgren/Lawrence (KL) changes of Grade \( \geq 2 \). All radiographs were read by one radiologist (L.K.C.) for grading the severity of OA. The final diagnosis of OA is based on history, physical examination and/or radiographic finding.

The study was approved by the Institutional Review Board, and informed consent was obtained from all of the participants.

Short tandem repeat polymorphism linkage study

Genomic DNA from peripheral blood was isolated using PUREGENE DNA purification system (Gentra Systems, MN, USA) according to the manufacturer’s instructions. Four short tandem repeat (STRP) markers: D12S1663, D12S85, D12S368 and D12S83, on chromosome 12 q were used for linkage study. These markers flanked the candidate gene COL2A1 (NCBI build 33). Genotyping was performed using the ABI PRISM Linkage Mapping Set v 2.5 HD5 (Applied Biosystems, Foster City,
CA, USA). Allele size calling was determined using the Genemapper software program (Applied Biosystems).

DNA sequencing

COL2A1 DNA sequence variants of all 54 exons and exon-intron junctions were determined by direct sequencing using the Applied Biosystems 3730 DNA analyser. Primers were designed by Primer3 PCR primer picking program (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). The PCR reactions were performed in a final volume of 50 µl containing 0.2 µM each primer, 5 mM Tris–HCl (pH 8.3), 5 mM KCl, 1.5 mM MgCl2, 0.01 mM EDTA, 0.05 mM DTT, 200 µM dNTPs and 2.5 U TaqTM (Violet, Taiwan). Amplification conditions consisted of an initial denaturation of 3 min at 94°C, followed by 20 cycles of touchdown PCR in 30 s at 94°C, 30 s at 65°C (decrease 0.5°C per cycle), 40 s at 72°C and a final 20 cycles in 30 s at 94°C, 30 s at 55°C, 40 s at 72°C.

Results

Phenotype assessment

A total of 70 individuals were recruited for the study, among them 25 were diagnosed with OA based on history, physical examination and/or radiographic finding (Fig. 1). Because of age-dependent variation in the expression of OA, 15 subjects with the age <20 years and who had no symptoms were labelled as ‘unknown status’.

The proband (No. IV: 1) had problem running since childhood. At the age of 30 years, he started to feel soreness in the neck then progressed to the both knee and hip joints. He received arthroplasty for both hip joints and right knee joint at the age of 50 years. The proband’s son (No. V: 4) had early onset of OA at the age of 13 years in knee and hip joints, this gradually progressed to ankle joints and cervical spine at the age of 20 years. OA in this family spans at least three generations and affected patients suffered from stiffness and pain in the knee and hip joints, sometimes they also complained about back soreness. Most of the patients remembered that one of the first signs of illness was difficulty in performing squatting. The average ages of onset were 25 years for men and 34 years for women. When broken down by generation, there were 6, 16 and 3 affected cases and the average onset age was 37.3 ± 19.2, 29.8 ± 13.7 and 12.0 ± 7.2 years in the IV, V and VI generations, respectively (Table 1). All patients had knee and/or hip joints OA, in addition, seven patients had spine OA, two patients had ankle joint OA and two patients had hand OA (Table 1).

The body weight, BMI, blood pressure, and uric acid, were not significantly different between the affected and unaffected cases. Gout and pseudo-gout were not detected in this family. RF was normal except for one affected female (No. V: 9) who had elevated RF (36 IU/ml). Affected males, but not females, were shorter than the unaffected (155.9 ± 11.4 vs 164.5 ± 16.0 cm, P = 0.010). Arm span in the affected males, but not in the affected females, was also significantly shorter (158.4 ± 12.5 vs 165.3 ± 16.7 cm, P = 0.027). There was no reduced life span in the affected patients compared with the average life expectancy in the Taiwanese population. Furthermore, there was no mental retardation, CNS or peripheral nervous system disease, myopathy, muscle weakness, ocular disease, internal organ abnormality, spontaneous fractures, joint contractures or hypermobility in this family.

Radiographs

All 25 but 2 affected patients had radiological evaluation; the two (Nos IV: 18 and IV: 44 in generation IV) who did not have radiograph were due to advanced ages and inconvenient status.

Fig. 1. A six-generation with early-onset OA kindred. Those numbered were recruited for this study. Symbols with single diagonal lines represent deceased individuals. Arrow indicates the proband in generation IV. (●) Early-onset OA; (?) disease status unknown.
According to KL radiographic grading scale, 10 patients showed Grade 3 and 4 patients (Nos IV: 1; IV: 58; V: 60 and V: 4) had KL Grade 4 radiological findings in spine, hip or knee joint with changes consistent with advanced OA. The proband (No. IV: 1), at the age of 45 years, had sclerosis which was present over C1 and C2 (KL Grade 4) and C1–C7 showed osteophytes formation (KL Grade 3). The first radiographic signs were cysts in the femoral head and narrow joint space in a male patient (No. V: 4) at the age of 36 years. The decreased joint space and osteophyte formation were present in both knee joints of female patient in the study family (No. V: 62) at the age of 40 years.

In generations IV and V, the most involved joints were hip and knee and all three patients had knee OA in generation VI (Table 1).

Linkage analysis

The clinical presentations in these familial early-onset OA patients resemble those previously reported patients with COL2A1 gene mutation [12, 13]. STRP markers were used for linkage study as the first step to confirm or to exclude the involvement of the COL2A1 gene. Six individuals were chosen for analysis from the core family (Nos IV: 1; IV: 2; V: 3; V: 4; V: 5 and V: 6 in Fig. 1). Four markers flanking the COL2A1 gene were selected from ABI PRISM® system linkage mapping set. The COL2A1 gene is located 1.1 cM from marker D12S85 and 4.2 cM from marker D12S368. The result showed that the proband (No. IV: 1) who is heterozygous for the marker alleles passed the allele (size in base pair: 172, 108, 210, 105) to the affected son, but also passed the same allele to the unaffected sons. Thus, the suspicious region including the candidate gene COL2A1 could be ruled out by linkage analysis.

Sequencing the COL2A1 gene

Direct sequencing of the COL2A1 gene was then performed to confirm the results of linkage analysis. Sequencing all 54 exons and exon–intron junctions of the COL2A1 gene revealed one sequence variant in the proband. The variant was a non-synonymous variant in exon 1 (p. T95S, rs 3803183). This variant represented single nucleotide polymorphism (SNP) as it has been previously reported in normal individuals, thus unlikely to be pathogenic. Based on the result of COL2A1 gene sequencing and linkage data, we conclude that COL2A1 is not a susceptibility gene responsible for the phenotype in our OA family.

Discussion

We identified a family with an inherited joint disease that is indistinguishable from primary hip or knee OA by symptoms, physical and radiographic features in the involved joints and bones, except our family had a much earlier onset of joint disease than the primary OA. Radiographs of the involved joints in this family were similar to those observed in moderate and advanced idiopathic OA. By physical and laboratory findings, we have ruled out most of the secondary causes of OA, including congenital syndromes, metabolic and endocrine diseases.

In this kindred, the average age of onset in affected males was younger than in females (25 years for men and 34 years for women) and both height and arm span were reduced in male patients but not in females. These findings suggested that males were more severely affected than females. This is different from the common OA in which OA is more prevalent in women than in men [18, 19]. The reason for this gender difference in clinical severity in this family is not clear at this time; no history of occupational physical activity/stresses could be elicited to account for the gender difference in our family.

It is also interesting to observe that when broken down by generation, the age of onset of OA becomes progressively younger with each generation (37.3 ± 19.2, 29.8 ± 13.7 and 12.0 ± 7.2 years in the generations IV, V and VI, respectively). We attributed this to the early recognition and early diagnosis by increased awareness of the disease runs in the family. However, we were not able to exclude the possibility of the anticipation event due to the expansion of the number of tri-nucleotide repeats as seen in many of triplet repeat disorders [20]. Further study to identify the responsible OA gene for this family is needed.

Several investigations have reported association of OA with certain candidate gene loci and functional polymorphisms [8–12]. However, most of the pathogenic mutations identified in OA are in rare genetic syndromes in which other secondary features such as severe bony dysplasia and myopathy are prominent [14, 15]. The phenotypes of our affected patients do not exhibit these rare severe genetic syndromes. On the other hand, mutations in Type II procollagen (COL2A1) can cause hereditary form of joint disorder with a wide spectrum of phenotypes ranging from primary OA with mild chondrodysplasia, mild spondylo-epiphyseal dysplasia and osteonecrosis to severe generalized OA, including achondrogenesis and hypochondrogenesis [13]. Our study does not fully support that COL2A1 could be implicated in primary OA of other non-Asian ethnic groups since ethnic variability in gene susceptibility is very well documented. The clinical features of early-onset OA, mild clinical phenotypes and one patient (No. IV: 9) with osteonecrosis of the femoral head strongly suggested that COL2A1 may also be the underlying cause of OA in our family. Linkage analysis and direct sequencing of COL2A1, however, clearly rule out this possibility. Further study with genome-wide linkage will be needed to identify susceptibility locus.

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