Two Novel COL2A1 Mutations Associated with a Legg-Calvé-Perthes Disease-like Presentation

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Abstract

Background Abnormal development and growth of the capital femoral epiphysis and acetabulum are associated with a wide variety of underlying etiologies, one of which is Legg-Calvé-Perthes disease.

Case Description We report the cases of two children who presented with abnormal development of both hips and in whom novel mutations in the *COL2A1* gene were

found. These cases illustrate the importance of identifying individuals with a type II collagen abnormality, as it informs management, allows investigation for other complications, and provides the opportunity for accurate genetic counseling and consideration of other family members who might be at risk.

Literature Review The literature documents numerous private mutations in *COL2A1* associated with diverse clinical phenotypes including bilateral hip dysplasia and premature osteoarthritis. Some of these mutations are associated with a joint-specific phenotype but few other skeletal or extraskeletal manifestations. Only careful clinical examination of children presenting with hip anomalies therefore will reveal additional findings that warrant an evaluation by a clinical geneticist. DNA mutation analysis may be useful for making a specific diagnosis and identifying other at-risk family members.

Purposes and Clinical Relevance The purpose of our report is to alert clinicians to the possibility that children who present with bilateral Perthes-like disease of the hip might have an underlying mutation in the gene encoding type II collagen. It is important to consider this in the differential diagnosis and workup of such children as it has specific prognostic, clinical, genetic counseling, and reproductive sequelae.

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Each author certifies that his or her institution approved the reporting of this case report, that all investigations were conducted in conformity with ethical principles of research, and that informed consent for participation in the study was obtained.

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Introduction

Although bilateral degenerative hip disease in children is commonly recognized in Legg-Calvé-Perthes (LCP) disease, it is important to consider genetic conditions that cause hip dysplasia, such as type II collagen-related disorders and multiple epiphyseal dysplasia (MED). Type II collagen is the predominant collagen of hyaline and

articular cartilage, and the retinal vitreous humor. Abnormalities of type II collagen should be considered specifically in the differential diagnosis of individuals presenting with irregular vertebral end plates and long-bone epiphyseal dysplasia (spondyloepiphyseal dysplasia), especially when accompanied by ocular, orofacial, and auditory problems [8].

Type II collagen is a large, homotrimeric protein with a triple helical domain encoded by the triplet repeat motive Gly-X-Y in the gene *COL2A1*. Greater than 100 different *COL2A1* human mutations are described and listed in the Cardiff University human gene mutation database [15, 18] and a spectrum of disease severity exists. Missense mutations substituting glycine for bulkier amino acids generally disrupt the helical protein structure, resulting in severe short stature. Nonsense mutations lead to haploinsufficiency of type II collagen through nonsense-mediated decay, predominantly causing a milder phenotype, such as abnormalities of articular cartilage, as seen in Stickler syndrome [6] (hereditary arthroophthalmopathy, OMIM 108300) and premature-onset arthritis (OMIM 604864) [1]. The phenotypic effect of these mutations remains difficult to predict however, as it is dependent on several factors, including the quantity of type II collagen synthesized as a consequence of the mutation, the degree of difference of the amino acid substituting for glycine in the triple helical part of the molecule, and the disruption of protein-protein interactions caused as a result of the mutation.

We report two novel missense mutations in *COL2A1* in two unrelated children who presented with bilateral LCP-like disease, but whose disorders would be better described as lying within the spondyloepiphyseal spectrum. These cases highlight the importance of identifying individuals with bilateral hip dysplasia who might have an abnormality of type II collagen, because of their complex needs, different management, and the requirement for appropriate genetic input.

Methods

COL2A1 mutation analysis was performed prospectively in a cohort of patients who presented with bilateral hip disease with a LCP-like phenotype. Information regarding the clinical and radiologic phenotype was obtained from all participants.

After informed consent, genomic DNA was isolated from affected individuals using 700 µL of EDTA-treated blood with the BioRobot M48 Automated DNA extraction machine (Qiagen, Valencia, CA, USA).

Primers were designed using Primer 3 software [14] to span the entire coding region of the *COL2A1* gene (NM_001844.3) for each of the 54 exons covering

intron/exon boundaries. Primers were synthesized at Sigma-Aldrich (Sigma-Aldrich Canada Ltd, Oakville, ON, Canada). Primers all contain M13 tags.

Two PCR reactions for each fragment, using 25 µL reactions containing 50 ng genomic DNA, 0.1 µmol/L of each forward and reverse primer, 1× PCR Buffer II (Qiagen), 1 mmol/L MgCl₂, 0.2 mmol/L each of Na dATP, dCTP, dGTP, dTTP (Roche), and 0.5 U Hot Star Taq (Qiagen), were performed on the MJD Tetrad (Bio Rad, Hercules, CA, USA) for 35 cycles, with an initial denaturation at 95°C for 1 minute, followed by 95°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute. Final extension was at 72°C for 1 minute. PCR products were purified using ExoSAP-IT as described by the manufacturer (USB, Cleveland, OH, USA) and sequenced in forward and reverse directions, with M13 primers, using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). Sequenced PCR products were purified with a BD-Xterminator kit (Applied Biosystems), and sequenced with a 3730 DNA Analyzer (Applied Biosystems).

The DNA mutation results were analyzed using Mutation Surveyor version 3.01 software (Softgenetics, State College, PA, USA).

Case Reports

Patient 1

This male patient was the first child of a healthy nonconsanguineous couple. The pregnancy and delivery were uneventful. He walked after age 18 months, and presented at the age of 4 years 10 months with short stature. Measurements of his height at two different time points suggested that the rate of height increase was not abnormal when compared to other male children at the same age. He subsequently was assessed by the orthopaedic surgeons for an abnormal gait. Pelvic radiography at this time showed dysplastic capital femoral epiphyses and the possibility of bilateral LCP disease was raised. At the age of 5 years, myopia prompted further ophthalmic assessment, revealing mild degeneration and beading of the eye vitreous and peripheral cortical cataracts. In view of the associated ocular findings, the patient was referred to the genetics clinic. On examination, at the age of 5.5 years, his height was 97.2 cm (−4 SD) and his upper segment to lower segment ratio was 1.136 (within normal limits for his age). He had a flat face, mildly prominent eyes, a flat nasal bridge, mild anteversion of the nares, and a long, shallow philtrum. He had generalized joint hypermobility, flat feet, and mildly exaggerated lumbar lordosis. A skeletal survey was performed and the findings included abnormalities of

Fig. 1A–F (A) An AP radiograph shows the patient's pelvis and lower spine at age 5.5 years. Abnormalities of the proximal femoral epiphyses, which are more pronounced on the right than on the left are seen. (B) There was no evidence of platyspondyly. (C) The left proximal humeral epiphysis was flattened with an unusual convex appearance. (D) No abnormalities of the hand were seen on an AP radiograph. (E) An AP view of the left knee shows mild broadening of the distal femoral and proximal tibial metaphyses and broad, flattened epiphyses at the knees. (F) A left lateral knee radiograph shows delayed ossification of the left patella.

the proximal femoral epiphyses, more pronounced on the right than on the left, mild broadening of the distal femoral and proximal tibial metaphyses on the left, and broad, flattened epiphyses at the knees (Fig. 1). In addition, the left proximal humeral epiphysis was flattened with an unusual convex appearance and ossification of the left patella was delayed. There were no abnormalities of the spine. *COL2A1* analysis detected a c.2014G>T (G/C) mutation, not present in either parent, predicted to disrupt the collagen II helical structure. Subsequent imaging of the spine at the age of 6.5 years to investigate mild thoracolumbar scoliosis revealed mild platyspondyly. At age 8, he reported occasional clicking pains in his hips and knees. The physical examination showed full ROM in those joints, a negative Trendelenburg sign, and equal limb lengths. There was increasing lumbar lordosis and mild to moderate scoliosis. A repeat pelvic radiograph showed substantial flattening of the proximal femoral epiphyses but the femoral heads were not dislocated and the neck-shaft angles were within normal limits.

Patient 2

This female was the second child of a healthy nonconsanguineous Caucasian couple. Her mother was born with a

cleft palate and micrognathia, and had early-onset arthropathy. The pregnancy was complicated by minor placental abruption at 12 weeks and polyhydramnios developed in the second trimester. The patient was born by normal vaginal delivery at 38 weeks of gestation, following induction of labor, with a birth weight of 3.7 kg (> 75th percentile) and length of 55.0 cm (> 90th percentile). At birth, a cleft palate was detected and Pierre-Robin sequence diagnosed based on the presence of retrognathia and glossoptosis. An echocardiogram detected a patent ductus arteriosus, but no other structural cardiac abnormalities. Cytogenetic investigation revealed a normal female karyotype (46,XX) with no 22q12 microdeletion. At 2 years 9 months of age, she was noted to walk with a waddling gait and have prominent knees. A skeletal survey was performed and dysplastic hips were observed (Fig. 2). There was mild irregularity of the vertebral end plates, but no evidence of platyspondyly. The patient had mixed sensorineural and conductive hearing loss requiring hearing aids and also had myopia. Physical examination at 3 years of age revealed a height of 101.0 cm (> 97th percentile). She had midfacial hypoplasia, epicanthic folds, a broad nasal tip, and long tapering fingers. Her cognitive development was age-appropriate. In view of the constellation of problems, *COL2A1* mutation analysis was performed and disclosed a c.638G>A (G/A) mutation in the patient, which

Fig. 2A–B (A) An AP radiograph of the pelvis of Patient 2, aged 2 years 9 months, show bilateral dysplastic changes of the hips. (B) No evidence of platyspondyly is seen on this radiograph but the vertebral end plates were ragged.

was not present in either of her parents, raising the possibility of gonadal mosaicism in view of a consistent family history. Further ophthalmic evaluation performed in light of this result identified a membranous vitreopathy. Her hips have been relatively stable and her most recent pelvic radiograph reveals dysplastic femoral capital epiphyses and acetabula with lateral uncovering of the femoral heads. This has been stable for more than 6 years with no change or resolution.

Discussion

The differential diagnosis of abnormal hip development presenting in the first decade of life includes not only LCP disease, but also types of skeletal dysplasia affecting epiphyseal growth, such as multiple epiphyseal dysplasia (MED, OMIM 120210, 132400, 607078, 226900) and spondyloepiphyseal dysplasia, including Stickler syndrome [7]. Dysplasia of the capital femoral epiphyses and LCP can look similar radiographically as there is a spectrum of presentations with clinical and radiographic overlap. Also, there can be secondary avascular necrosis of the femoral head in patients with dysplastic hips (ie, in MED/type II collagen disorders). The key difference is that there are no healing and resolution in epiphyseal dysplasia versus natural history of LCP in most cases. Hip changes, similar to those of bilateral LCP disease, also are seen in many genetic syndromes with or without developmental delay, including the 6p25 chromosomal microdeletion syndrome, trichorhinophalangeal syndrome (TRP, OMIM 190350), angel-shaped phalangeal epiphyseal dysplasia (ASPED, OMIM 105835), and inborn errors of metabolism [4, 9]. Meyer dysplasia is a developmental disorder of the hip radiographically characterized by delayed ossification of

the femoral epiphyses followed by the appearance of either a single granular ossific focus or multiple centers of ossification. These gradually enlarge and eventually fuse at an average age of 5.5 years. The resulting epiphysis has normal density and texture but is slightly flattened. The majority of children have no symptoms, although a few may have mild inconsistent waddling gait. The disorder often is confused with LCP but the typical radiologic findings in LCP, namely the appearance of a subchondral fracture line, increased epiphyseal density, and lateral displacement of the femoral head, should differentiate the two conditions.

Originally, Patient 1 was thought to have bilateral LCP disease. Bilateral hip involvement, particularly if symmetrical and synchronous, is unusual in LCP and warrants further genetic evaluation. When a more comprehensive assessment was done, additional features similar to those for Patient 2 became apparent, suggestive of an abnormality of type II collagen. *COL2A1* molecular analysis showed that both had missense mutations, disrupting expression of the collagen II helical region through the substitution of glycine residues for the bulkier amino acids, cysteine and aspartine, respectively.

The *COL2A1* mutations identified in these two patients are novel, to the best of our knowledge. Although the disorders of these two children best fit into the Stickler syndrome phenotype because of the ocular and vertebral abnormalities, surprisingly their phenotype is not attributable to a truncating *COL2A1* mutation, but a missense change resulting in a glycine substitution [3].

The two cases presented here illustrate that there are other conditions that could be etiologic in bilateral hip dysplasia and which should be considered in the face of bilateral LCP. In particular, diagnosis of conditions characterized by abnormal type II collagen requires multisystem assessment, with suggestive features including joint hypermobility, round face with epicanthic folds, hearing loss, retinovitreal anomalies or severe myopia, and occasionally mild short stature. This also may be supported by a family history suggesting autosomal dominant transmission of related problems, such as premature onset of osteoarthritis. It is essential to detect individuals with bilateral hip dysplasia secondary to abnormal type II collagen because of the severe and preventable complications, including retinal detachment, and it also allows for nonoperative management to be implemented and accurate genetic counseling.

Three other *COL2A1* mutations resulting in helical glycine substitutions have been reported (Gly393Ser, Gly1170Ser and Gly717Ser) in association with LCP disease and avascular necrosis of the femoral head [8, 12, 13]. In these cases though, characteristic clinical features suggesting an underlying type II collagen disorder, such as a small jaw, cleft palate, flat midface, and visual or hearing

impairment, were not observed. They illustrate a specific phenotype that is isolated to hip development, a rare finding [16], as a study of 119 individuals presenting with LCP disease to a Tel Aviv medical center did not identify any individuals with either of these three mutations [10].

Mutations in *COL2A1* also have been associated with milder types of skeletal dysplasia, such as Czech dysplasia (OMIM 609192), a milder type of skeletal dysplasia affecting the vertebral bodies and metatarsals [2, 5, 11, 19] and premature-onset arthritis [1, 8] (OMIM604864). The Arg275Cys *COL2A1* mutations seen recurrently in Czech dysplasia do not disrupt the collagen II triple helix because of a glycine substitution, but rather through replacement of arginine with cysteine in the “Y” position. Affected individuals often have normal stature without ocular or orofacial abnormalities. This appears to have a specific effect on development of the third and fourth metatarsals [5]. Other *COL2A1* mutations have been described in association with bilateral hip disease and osteoarthritis in the second decade of life, but without ocular abnormalities or short stature [8].

The molecular effects of the *COL2A1* mutations on cartilage function remain speculative. Cartilage from the affected cases is currently unavailable and type II collagen is not otherwise obtainable from living individuals. Unlike glycine (molecular mass 75 g/mol), asparagine (molecular mass 105 g/mol) is a charged amino acid and alanine (molecular mass 89 g/mol) contains a methyl side group. Neither of these bulkier amino acids fits as neatly into the helical center as the smaller glycine residue and so is predicted to disrupt the structure and weaken the helix. Analysis of cartilage from an individual with a G1170S *COL2A1* mutation and hip-specific disease has been reported [17]. Alteration in the expression and distribution of type II collagen, in the arrangement of collagen fibers and in the structure of the cartilage was observed, as was dilatation of the rough endoplasmic reticulum (RER) in cartilage chondrocytes on electron microscopy. Dilated RER is suggestive of a reduction in the secretion of type II collagen, possibly attributable to an unfolded protein response.

The recognition that bilateral hip dysplasia can be attributable to causes other than isolated LCP disease, such as a *COL2A1* mutation, is clinically important to pediatricians and orthopaedic surgeons, as specialists to whom affected children may first present. The identification of a type II collagen-related condition is essential in providing anticipatory surveillance for associated complications and for accurate provision of genetic counseling to the wider family. These cases contribute further to current knowledge that missense and nonsense mutations may cause Stickler syndrome. Additional analysis is required to investigate the true predominance of this type of mutation in Stickler syndrome.

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