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Small Deletions in the Type II Collagen Triple Helix Produce Kniest Dysplasia

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Kniest dysplasia is a moderately severe type II collagenopathy, characterized by short trunk and limbs, kyphoscoliosis, midface hypoplasia, severe myopia, and hearing loss. Mutations in the gene that encodes type II collagen (COL2A1), the predominant protein of cartilage, have been identified in a number of individuals with Kniest dysplasia. All but two of these previously described mutations cause in-frame deletions in type II collagen, either by small deletions in the gene or splice site alterations. Furthermore, all but one of these mutations is located between exons 12 and 24 in the COL2A1 gene. We used heteroduplex analysis to identify sequence anomalies in five individuals with Kniest dysplasia. Sequencing of the index patients' genomic DNA identified four new dominant mutations in COL2A1 that result in Kniest dysplasia: a 21-bp deletion in exon 16, an 18-bp deletion in exon 19, and 4-bp deletions in the splice donor sites of introns 14 and 20. A previously described 28-bp deletion at the COL2A1 exon 12–intron 12 junction, deleting the splice donor site, was identified in the fifth case. The latter three mutations are predicted to result in exon skipping in the mRNA encoded from the mutant allele. These data suggest that Kniest dysplasia results from shorter type II collagen monomers, and support the hypothesis that alteration of a specific COL2A1 domain, which may span from exons 12 to 24, leads to the Kniest dysplasia phenotype. Am. J Med. Genet. 85:105–112, 1999.

KEY WORDS: Kniest dysplasia; type II collagen; COL2A1; mutation

INTRODUCTION

Kniest dysplasia is a moderately severe autosomal dominant type II collagen disorder. It lies in the middle of a phenotypic spectrum of disease, with the lethal achondrogenesis type II/hypochondrogenesis at the severe end, the spondyloepi(meta)physeal dysplasias (SED/SEMD) also in the middle, and the genetically heterogeneous Stickler syndrome and familial osteoarthritis at the milder end [Spranger et al., 1994].

Manifestations of Kniest dysplasia include both skeletal and craniofacial characteristics. Skeletal anomalies include disproportionate dwarfism, a short trunk and small pelvis, kyphoscoliosis, short limbs, and prominent joints and premature osteoarthritis that restrict movement [Rimoin and Lachman, 1990]. Characteristic craniofacial manifestations of Kniest dysplasia include midface hypoplasia, cleft palate, early onset myopia, retinal detachment, and hearing loss [Maumenee and Traboulsi, 1985; Rimoin and Lachman, 1990]. Kniest dysplasia, like SED congenita, can be phenotypically variable. Individuals can die of respiratory distress shortly after birth, or can lead a relatively normal life, with mild disproportionate short stature, kyphoscoliosis, and/or craniofacial manifestations. Distinct radiographic changes distinguish Kniest

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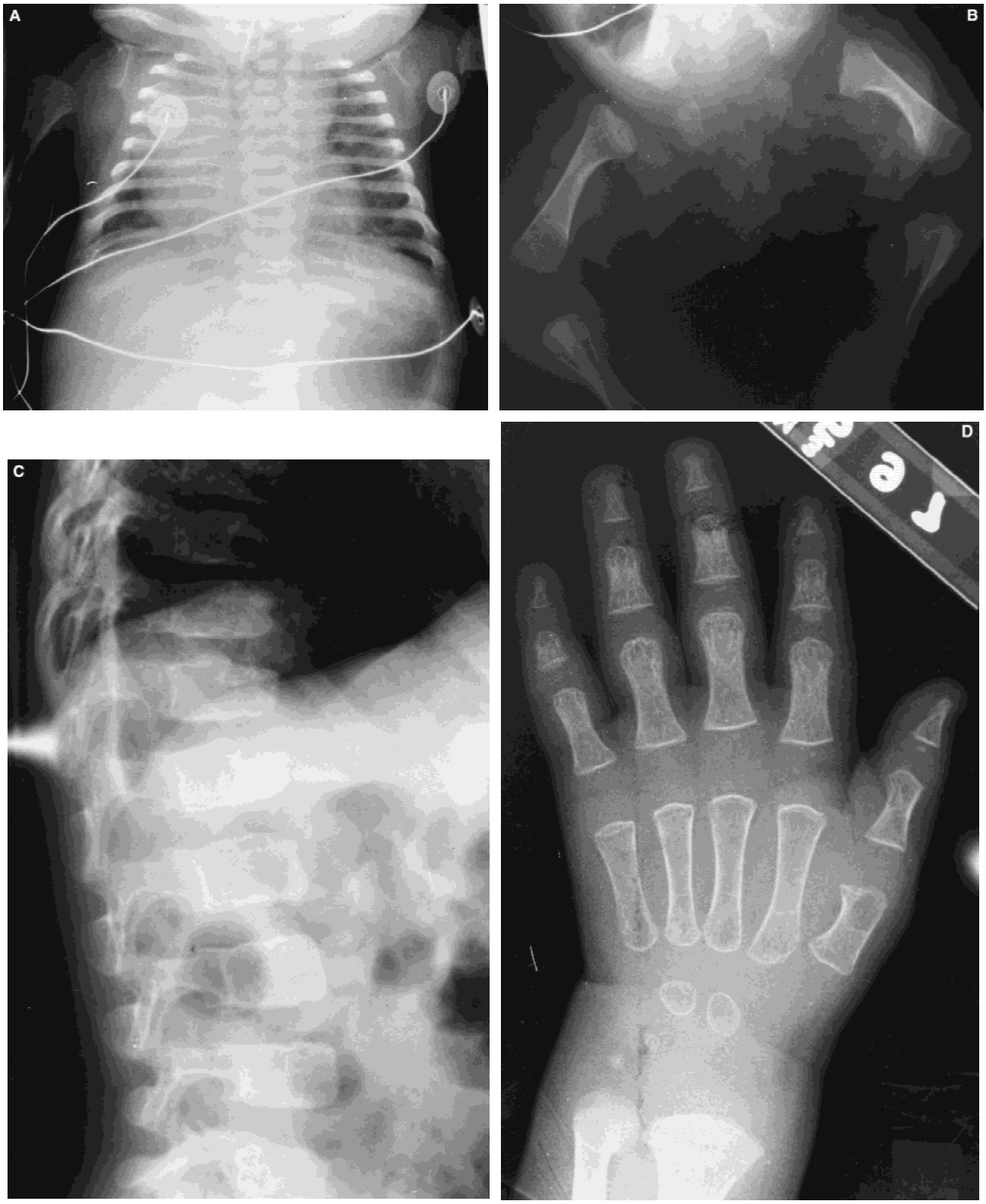

Fig. 1. Radiographs demonstrating the Kniest dysplasia phenotype in a newborn **(A,B)** and young child **(C–E). A:** Patient 3. Newborn. Radiograph of chest showing a small thorax and a dumbbell shape of the proximal humerus on the right side. **B:** Patient 3. Radiograph of the pelvis and lower extremities in the perinatal period showing absence of pubic bone ossification, widening vertical ischia, dumbbell-shaped femora, and generalized rhizomelia and mesomelia. **C:** Patient 1. 17 months. Lateral thoraco-lumbar spine showing residual of coronal clefting in several of the thoraco-lumbar vertebral bodies, with mild platyspondyly and upper end-plate irregularity. **D:** Patient 1. 17 months. Hand radiograph showing typical metaphyseal flaring, especially in the metacarpals, and proximal and middle phalanges with significantly delayed epiphyseal ossification. Also note the shortening of the proximal middle and distal phalanges with widening in the interphalangeal joint space regions of the soft tissue.

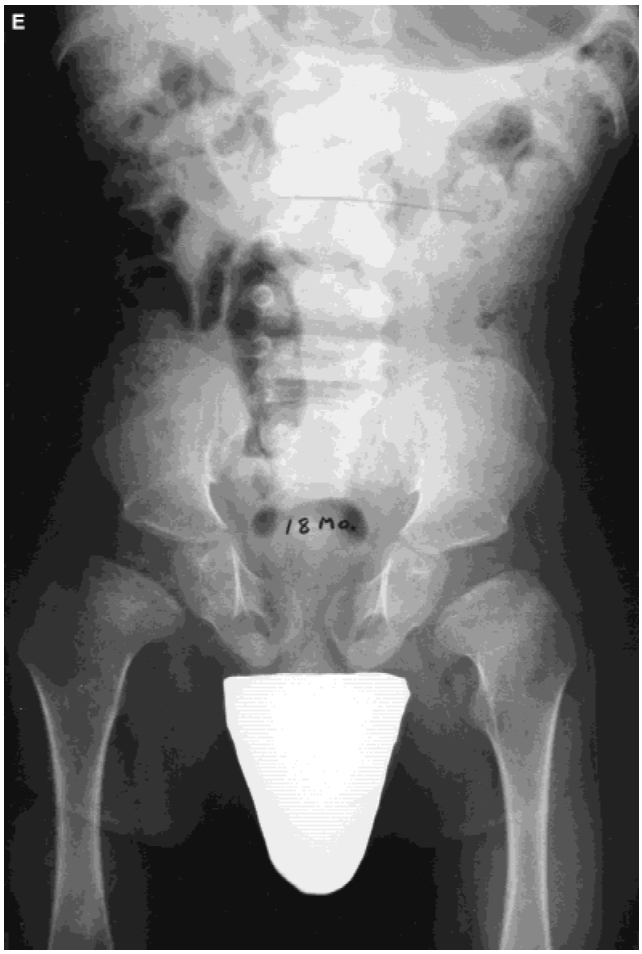

Fig. 1 (Continued) **E:** Patient 2. 18 months. Radiograph of the pelvis and proximal femora showing marked ossification delay of the capital femoral epiphyses, flattened acetabular roofs, and enlarged ischia and pubic bones.

dysplasia from other type II collagenopathies and can often be identified at birth. Coronal clefts of the vertebrae and dumbbell-shaped femora are characteristic of the disorder, and dysplastic epiphyses and metaphyses, narrowed joint spaces, platyspondyly, and short tubular bones are also observed [Taybi and Lachman, 1996]. The chondroosseous morphology in Kniest dysplasia is pathognomonic with perilacunar "foaminess" and sparse, aggregated collagen fibrils resulting in an interterritorial matrix with a "Swiss-cheese" appearance [Horton and Rimoin, 1970; Rimoin et al., 1973]. The chondrocytes contain inclusion bodies that stain with antibody to type II collagen [Horton and Rimoin, 1970; Rimoin et al., 1973]. Together these changes suggested that Kniest dysplasia was likely to result from mutations in COL2A1, the gene that encodes type II collagen, the predominant protein of cartilage.

Recently, nine mutations in COL2A1 were identified in individuals with Kniest dysplasia [Winterpacht et al., 1993, 1994, 1996; Wilkin et al., 1994a; Bogaert et al., 1994; Chen et al., 1996; Spranger et al., 1997; Weis et al., 1998; Fernandes et al., 1998]. All but two of these mutations are small deletions in COL2A1, or point mutations that disrupt normal splicing of the type II collagen mRNA. The splicing mutations result in exon skipping or other small in-frame deletions in the protein. The missense mutations in Kniest dysplasia are substitutions of aspartic acid for glycine 103 and glycine 127 in the collagen triple helix [Wilkin et al., 1994a; Weis et al., 1998]. Small deletions that include glycine 103 have been found in six other individuals with Kniest dysplasia [Winterpacht et al., 1993; Bogaert et al., 1994; Chen et al., 1996].

We have studied patients with Kniest dysplasia and found four new dominant deletions in COL2A1: a 21-bp deletion within exon 16, an 18-bp deletion within exon 19, and 4-bp deletions in the splice donor sites of introns 14 and 20. In DNA from a fifth patient we have identified a 28-bp deletion at the exon 12–intron 12 junction, deleting the donor splice site. This mutation was previously identified in an unrelated patient with Kniest dysplasia [Winterpacht et al., 1993]. The latter three mutations are predicted to result in exon skipping. These data demonstrate that Kniest dysplasia results from small deletions in type II collagen, and further add to the hypothesis of a functional domain of type II collagen that, when altered, leads to the Kniest dysplasia phenotype.

SUBJECTS AND METHODS

Clinical Summary

Patient 1. The male patient was the product of an uncomplicated term pregnancy. The mother was 29 and the father 28. Initial ascertainment was at 16 months of age due to strabismus and severe myopia. On physical examination at 17 months of age, the patient was noted to have a flat face, mild rhizomelic shortening of the limbs, prominent knees, and normal hands and palate. The patient was referred to two of us (D.L.R., R.S.L.) for diagnosis. Radiographs (Fig. 1) confirmed the diagnosis of Kniest dysplasia. The spine exhibited generalized platyspondyly, particularly in the thoracic spine, and odontoid hypoplasia. The long bones were all short, with dumbbell-shaped femurs and hypoplasia of the capital femoral epiphyses. There was hypoplasia of the acetabular roof with slightly hypoplastic vertical ischia.

Patient 2. The male patient was ascertained due to short stature noted by the clinically normal parents. He was of normal length at birth (50th centile) but by 2.5 years of age was at the 5th centile. Physical examination was significant for mild short stature and flexion contractures at the knees. There was midface hypoplasia, but neither micrognathia nor a cleft palate. The patient was referred to two of us (D.L.R., R.S.L.) for diagnosis. Radiographic examination was consistent with the diagnosis of Kniest dysplasia (Fig. 1). The spine showed mild platyspondyly and odontoid hypoplasia. Shortening of the long bones with dumbbell-shaped femora, hypoplastic capital femoral epiphyses, and metaphyseal widening was noted. The pelvis showed narrow sacrosciatic notches and a flattened acetabular roof.

Patient 3. This female patient was born at 37 5/7 weeks gestation to a 26-year-old mother via C-section

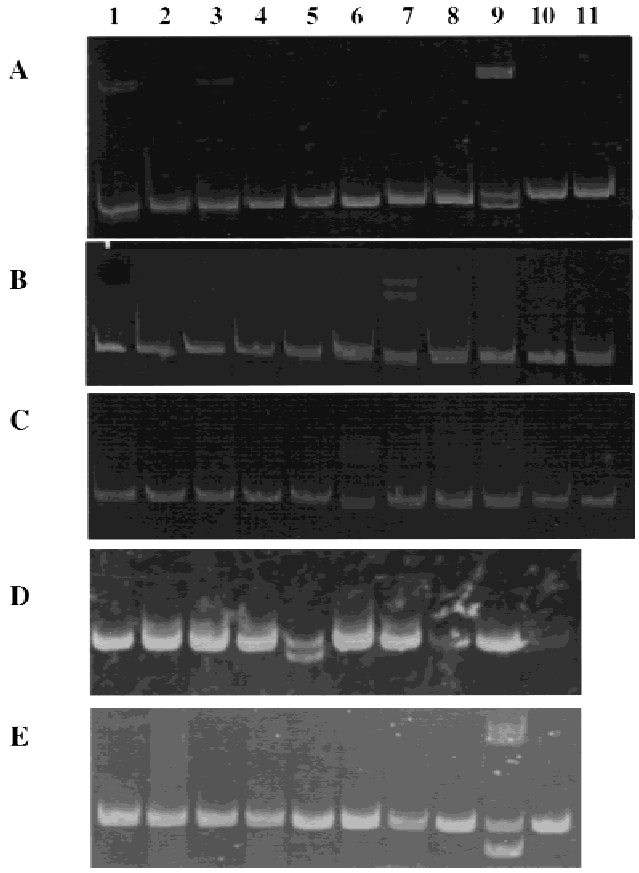

Fig. 2. Polyacrylamide gel electrophoresis (PAGE) of PCR products. A combination of 17 total individuals with Kniest dysplasia and related type II collagenopathies was analyzed by PCR and PAGE. PCR products contained exons and the flanking splice donor and acceptor sites. **A:** Exons 18 and 19. **B:** Exon 14. **C:** Exon 20. **D:** Exons 11 and 12. **E:** Exon 16. Patient 1: panel A, lane 9; patient 2: panel B, lane 7; patient 3: panel C, lane 6; patient 4: panel D, lane 5, and patient 5: panel E, lane 9. The sizes of the PCR products are **(A)** 307 bp, **(B)** 125 bp, **(C)** 133 bp, **(D)** 385 bp, and **(E)** 132 bp.

for failure to progress. The pregnancy was complicated by polyhydramnios and a prenatal diagnosis of achondroplasia was made. At birth the patient was noted to have dwarfism, including short femora and humeri, as well as a small posteriorly located tongue and posterior cleft palate. The patient was referred to one of us (D.L.R.) for diagnosis. Review of radiographs established the diagnosis of Kniest dysplasia. Radiographs showed a short barrel-shaped chest with mild rib shortness, platyspondyly, small vertebral bodies, and absent pubic bone ossification. Rhizomelia and mesomelia of the long bones with flared metaphyses and shortened diaphyses, including dumbbell shaped femora, were also present (Fig. 1). Midface hypoplasia and a hypoplastic mandible were noted. The patient died at 3 months due to respiratory distress.

Patient 4. This female patient was previously reported on by Siggers [1974], Siggers et al. [1974], and Maumenee and Traboulsi [1985]. At birth the patient had bilateral dislocation of the hips and scoliosis. This patient sat at 18 months, stood with assistance at 24 months, stood without assistance at 2 1/2 years of age, and walked by 3 years. She was never able to walk normally.

At age 10 1/2, she was referred to one of us (V.A.M.) for evaluation, when a diagnosis of Kniest dysplasia was made. At the time of evaluation, her height was 110.5 cm (height age 4 10/12 years). She had a flat and round face, prominent eyes with high myopia, and flat bridge of her nose, with broad and prominent forehead. She also had a cleft uvula. Radiographs demonstrated severe platyspondyly, with greatest involvement of the dorsal spine. The superior and inferior end plates of the vertebral bodies were quite irregular, with spotted mineralization. There was considerable middorsal kyphosis and lumbar lordosis, as well as moderate scoliosis. The anterior-posterior diameters of the vertebral bodies appeared relatively wide, as did the interpedicular spaces. The limbs and hands had short bones, with shafts of normal to slightly diminished diameter, and greatly flared metaphysis and epiphyses. Ossification of the epiphyses was irregular and spotty, with some of the cartilaginous epiphyseal plates relatively wide, particularly at the distal radius and ulna.

Patient 5. The male patient was the product of an uncomplicated term pregnancy. The diagnosis of a skeletal dysplasia was made by ultrasound a few days prior to birth. At birth the patient was noted to have dwarfism, including short femora and humeri, and a short barrel-shaped chest with mild pectus deformity. This patient was referred to two of us (C.S., C.A.F.) for diagnosis. Craniofacial manifestations in this patient included a small midface with frontal bossing, a flat facial profile, micrognathia, bulbous nose with anteverted nostrils, high-arched palate with a small cleft in the soft palate, and an apparently low-set and posteriorly angulated right ear. The patient required corrective lenses at 2 months for severe myopia. Radiographically, there were dumbbell-shaped femora, tibiae, and coronal clefts of the vertebrae with platyspondyly, characteristic of Kniest dysplasia. The patient died at 13 months of age, due to aspiration and respiratory

TABLE I. Sequences of the COL2A1 Mutations in Patients 1–5*

Patient 1	Intron 18/exon 19	5′-cag GGT GCT CCT GGC ATT GCT GGT GCT CCT GGC TTC CCT-3′
Patient 2	Exon 14/intron 14	5′-GCT GCG gtga gtaatt-3′
Patient 3	Exon 20/intron 20	5′-GAA CCT gtga gtatct-3′
Patient 4	Exon 12/intron 12	5′-AAG G GA GAG GCG GGT GCT CCT GGT GTG AAG gt gagaggccagaaa-3′
Patient 5	Intron 15/exon 16	5′-ctctctccag GGT CCT GTC GGT CCT GCT GGT GGT CCT G GC TTC CCT-3′

*The relative position of the mutations in the COL2A1 gene are indicated. Deletions are boxed. Repeated sequences are underlined. Intron sequences are shown in lowercase letters. Exon sequences are shown in uppercase letters.

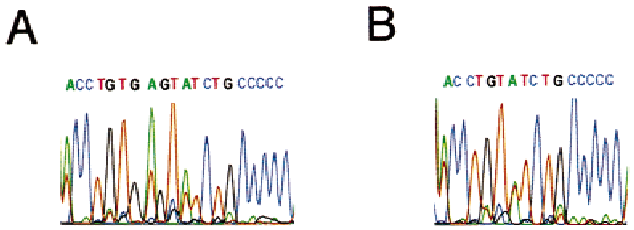

Fig. 3. Partial sequence of COL2A1 exon 20–intron 20 junction from patient 3. **A:** Normal allele. **B:** Mutant allele showing 4-bp deletion.

arrest. Cartilage was obtained at autopsy for histological studies.

COL2A1 Analysis

Genomic DNA was isolated from blood or lymphoblastoid cell lines by standard procedures. Primers were synthesized and used in the polymerase chain reaction (PCR) to amplify genome DNA fragments. Oligonucleotide primer sequences are available upon request. PCR amplifications were done in 25-μl reactions with 1.5 mM $MgCl_2$, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 200 mM each dNTP, 1.25 pmol forward and reverse primers, 50 ng genomic DNA, and 1U Taq DNA polymerase (Perkin-Elmer, Foster City, CA). Amplification conditions consisted of an initial 2 min at 94°C, followed by 35 cycles of 94°C for 1 min, 58–60°C for 1 min, and 72°C for 1 min, with the final elongation step extended to 10 min. PCR products were analyzed by 6% polyacrylamide gel electrophoresis and ethidium bromide staining. PCR products demonstrating heteroduplex formation were purified using the Qiaquick spin purification kit (Qiagen, Valencia, CA), and either sequenced directly or cloned into a plasmid vector using the TA cloning kit (Invitrogen, Carlsbad, CA). Cloned plasmid DNA was purified using the Qiaprep spin plasmid kit (Qiagen). DNA sequence analysis of cloned DNA was done with Sequenase Ver 2.0 (US Biochemicals, Cleveland, OH), and analyzed by denaturing 8% polyacrylamide gel electrophoresis and autoradiography, or analyzed on an ABI 377 automated sequencer using dye-terminator chemistry (Applied Biosystems, Perkin-Elmer).

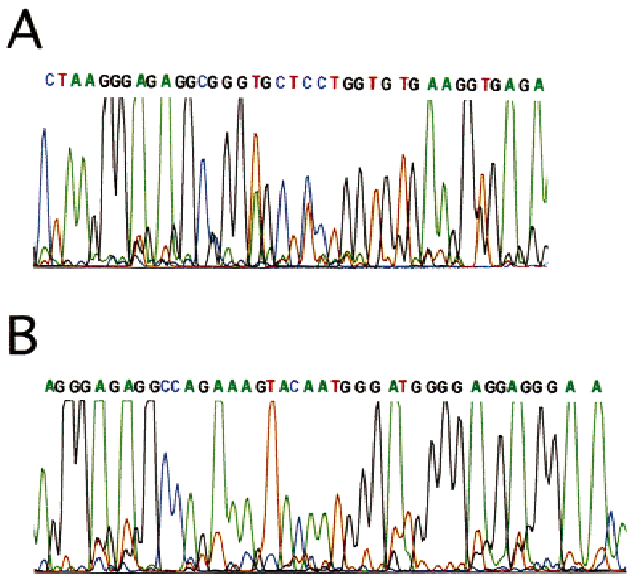

Fig. 4. Partial sequence of COL2A1 exon 12–intron 12 junction from patient 4. **A:** Normal allele. **B:** Mutant allele showing 28-bp deletion.

Histology

Cartilage from patients 2, 3, and 5 was prepared for light and electron microscopy as previously described [Bogaert et al. 1992].

RESULTS

COL2A1 Analysis

As previous mutations in COL2A1 in Kniest dysplasia have been identified in exons 12–24, we examined the exons and adjacent splice junctions for mutations in individuals with this disorder. COL2A1 exons 11 to 24 were analyzed either individually or in pairs. PCR primers were designed to include the consensus splice sequences in the amplified products. Allelic heterozygosity, identified by either the appearance of multiple bands and/or heteroduplexes in the PCR products, suggested mutations in the corresponding portion of the gene for five different patients (Fig. 2). Multiple PCR products were observed for patient 1 in the PCR product containing COL2A1 exons 18 and 19 (Fig. 2A, lane

TABLE II. Previously Identified COL2A1 Mutations Resulting in Kniest Dysplasia*

COL2A1 Mutation	Effect	Reference
Base substitution: splice donor site: intron 24	Skipping of 18 residues encoded by exon 24: amino acids 361–378	A
Base substitution exon 12	Creation of a cryptic splice site: deletion of the last seven residues encoded by exon 12: amino acids 102–108 observed in four unrelated individuals	B, C
28-bp Deletion exon 12/intron 12	Skipping of 18 residues encoded by exon 12: amino acids 91–108	D
Base substitution: splice acceptor site: intron 15	Skipping of 15 residues encoded by exon 15: amino acids 142–156	E
Base substitution: splice donor site: intron 20	Alternative splicing: skipping of six residues encoded by exon 21: amino acids 274–279	F
Single base deletion: first base intron 18	Skipping of 15 residues encoded by exon 18: amino acids 208–222	G
Deletion in exon 49	Deletion of amino acids 1007–1012 encoded by exon 49	H
Base substitution: exon 12	Gly103Asp	I
Base substitution: exon 14	Gly127Asp	A, E

*Listed are the mutation and the resulting effect. A, Weis et al., 1998; B, Bogaert et al., 1994; C, Chen et al., 1996; D, Winterpacht et al., 1993; E, Fernandes et al., 1988; F, Winterpacht et al., 1994; G, Spranger et al., 1997; H, Winterpacht et al., 1996; I, Wilkin et al., 1994.

9), patient 4 in the PCR product containing exons 11 and 12 (Fig. 2D, lane 5), and patient 5 in the PCR product containing exon 16 (Fig. 2E, lane 9). A heteroduplex was observed for patient 2 in the PCR product containing exon 14 (Fig. 2B, lane 7), and in an amplified DNA fragment from patient 3 that included exon 20 (Fig. 2C, lane 6).

PCR products demonstrating allelic heterogeneity were further characterized by sequencing. Sequence analysis of genomic DNA identified four novel dominant COL2A1 mutations. Patient 1 was heterozygous for an 18-bp deletion in exon 19, corresponding to six consecutive amino acids between residues 223 and 232 (Table I). Neither of the unaffected parents of patient 1 carried the mutation (data not shown). Patient 2 was heterozygous for a 4-bp deletion at the exon 14–intron 14 splice donor site, resulting in "gtaatt" as the 5′ sequence of intron 14 (Table I). Patient 3 was heterozygous for a 4-bp deletion in the splice donor site at the exon 20–intron 20 junction, resulting in "gtatct" as the 5′ sequence of intron 20 (Table I; Fig. 3). Patient 4 was heterozygous for the same 28-bp deletion described by Winterpacht et al. [1993], spanning the 3′ end of exon 12 and 5′ end of intron 12, removing the splice donor site of intron 12 (Fig. 4). Patient 5 was heterozygous for a 21-bp deletion within exon 16 (Table I).

Histology

Cartilage from patients 2, 3, and 5 was examined by light and electron microscopy. As seen in other individuals with Kniest dysplasia [Bogaert et al., 1994; Weis et al., 1998], a perilacunar foamy appearance was observed in the matrix surrounding the chondrocytes. Electron micrographs demonstrated sparse, thin collagen fibrils immediately surrounding the cells with thickened and aggregated collagen fibrils in the periphery. Chondrocytes contained inclusion bodies filled with a granular material (data not shown).

DISCUSSION

Prior to this study, 10 of 12 dominant mutations in the COL2A1 gene characterized in patients with Kniest dysplasia caused small deletions in the type II collagen molecule (Table II). Deletion of exons 12 [Winterpacht et al., 1993], 15 [Fernandes et al., 1998], 18 [Winterpacht et al., 1994], or 24 [Weis et al., 1998], deletions of six residues in exons 21 or 49 [Winterpacht et al., 1994, 1996], and deletion of the last 7 residues of exon 12, which has been identified in four unrelated individuals [Bogaert et al., 1994; Chen et al., 1996], led to the hypothesis that the Kniest dysplasia phenotype results predominantly from small deletions in the type II collagen chain [Winterpacht et al., 1996; Mortier et al., 1995b; Wilkin et al., 1996]. Two missense mutations have been identified that result in Kniest dysplasia: a Gly103Asp amino acid substitution in exon 12 [Wilkin et al., 1994a], altering a residue deleted in two of the Kniest dysplasia deletion mutations mentioned above, and a Gly127Asp substitution in exon 14 [Weis et al., 1998]. We have identified four new mutations that result in Kniest dysplasia, and one mutation previously identified in an unrelated individual, all causing small deletions in the type II collagen molecule. All but one of the Kniest dysplasia mutations are clustered between COL2A1 exons 12 and 24, suggesting the presence of a functional domain within the type II collagen molecule, which when altered, results in the Kniest dysplasia phenotype [Wilkin et al., 1994a, 1994b; Fernandes et al., 1998].

All five patients were heterozygous for a dominant mutation predicted to cause a deletion in type II collagen (Table III). The mutations in patients 2, 3, and 4 each disrupt a splice donor sequence. By comparison to other known COL2A1 exon-skip mutations that result in Kniest dysplasia (Table II) [Winterpacht et al., 1993; Bogaert et al., 1994; Spranger et al., 1997; Weis et al., 1998; Fernandes et al., 1998], it is likely that these mutations will also result in exon skipping. The splice donor site mutations in patients 2 and 3 disrupt the mammalian splice donor consensus sequence, and the mutation in patient 4 deletes the splice donor site of intron 12.

The common phenotypic manifestations of the type II collagen disorders including high myopia, sensorineural hearing loss, cleft palate, and short trunked dwarfism, may be due to changes in the molecule as a result of any mutation in COL2A1. However, the Kniest phenotype is distinct both radiographically and histologically when compared to other type II collagen disorders. Radiographically, dumbbell-shaped femora and coronal clefts are characteristic of this particular phenotype, as is the "Swiss-cheese" appearance of the cartilage matrix. A specific type of mutation and/or position of mutation within the collagen chain may explain the unique features of Kniest dysplasia among the type II collagenopathies.

The mechanism by which small deletions in type II collagen produce the Kniest phenotype is likely to be complex. It is known that virtually all structural defects in type II procollagen result in the intracellular retention of a proportion of the abnormal molecules inside the chondrocytes [Poole et al., 1988], suggesting that both decreased amounts of type II collagen in the matrix and presence of abnormal molecules in cartilage are most likely major factors in determining the Kniest dysplasia phenotype, as well as the phenotypes of other type II collagen disorders. However, the role these mutant chains play in the establishment of "Swiss-cheese" cartilage and the unusual features of Kniest dysplasia compared to other type II collagen disorders remain to

TABLE III. COL2A1 Mutations of Patients 1–5*

Patient	COL2A1 Mutation	Effect
1	18-bp deletion exon 19	Deletion of six residues encoded by exon 19
2	4-bp Deletion splice donor site: intron 14	Skipping 18 residues encoded by exon 14
3	4-bp Deletion splice donor site: intron 20	Skipping 18 residues encoded by exon 20
4	28-bp Deletion exon 12/intron 12	Skipping 18 residues encoded by exon 12
5	21-bp Deletion exon 16	Deletion of seven residues encoded by exon 16

*The consequences of the mutations are listed.

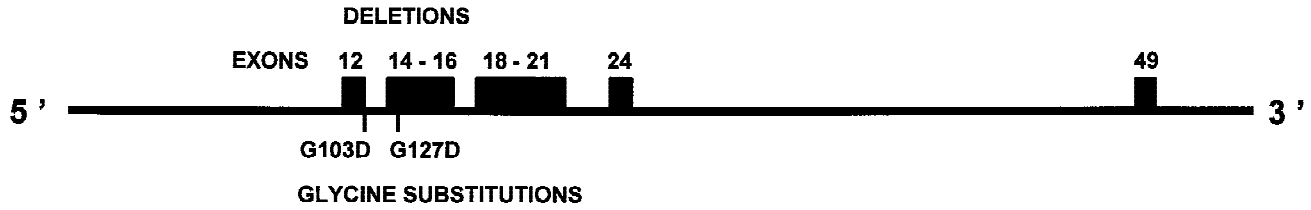

Fig. 5. Clustering of Kniest dysplasia mutations in COL2A1. Shown is the COL2A1cDNA with the locations of COL2A1 mutations which result in Kniest dysplasia. Boxes represent exons in which either complete exon deletions (exon-skip mutations) or partial deletions (see text) have been identified. The exon numbers are identified. Exons with full exon deletions are as follows (the amino acid numbers for the deleted residues are shown in parenthesis): exon 12 (91–108), exon 14 (124–141), exon 15 (142–156), exon 18 (208–222), exon 20 (256–273), and exon 24 (361–378). Exons with partial deletions are: exon 12 (102–108), exon 16 (157–163), exon 19 (223–228), exon 21 (274–309), and exon 49 (1007–1012). The two glycine substitutions are shown below the gene.

be elucidated. For Kniest dysplasia in particular we have shown that Kniest cartilage has a significant amount of the abnormal chains in the matrix [Bogaert et al., 1994; Fernandes et al., 1998; Weis et al., 1998]. This, together with the restricted domain within which the mutations occur, suggest a relationship between molecular defect and molecular pathology. The other type II collagen disorders have mutations in different locations along the helix and for most part result from glycine substitutions [Spranger et al., 1994]. There is a relationship between the amount of type II collagen in the matrix and phenotypic severity, with less type II collagen in cartilage in the more severe disorders, such as achondrogenesis type II [Mortier et al., 1995a]. Thus there is a quantitative component that, in addition to the qualitative effect, is likely to modulate phenotypic expression.

We have proposed a model to explain how the deleted type II collagen chains interact with normal length chains [Weis et al., 1998]. In the model, mutant and normal chains maintain an in-register triple helix adjacent to the mutation site, accommodated by a looping out of the normal α-chain sequence. This model is based on a COL2A1 mutation that results in the skipping of exon 24, and caused Kniest dysplasia [Weis et al., 1998]. Looping out of the normal α-chain sequence would result in shorter collagen molecules with a non-triple helical bulge. This model suggests that the shorter molecules are one of the keys in the development of the Kniest phenotype.

While there are insufficient data available at this time to correlate genotype with phenotype in all of the type II collagen disorders, the accumulating evidence suggests there is a "Kniest dysplasia domain" in COL2A1. If so, the unidentified Kniest dysplasia mutations may also be in the "domain" encoded by COL2A1 exons 12–24 (Fig. 5). These mutations may be point mutations, especially at splice junctions, or other aspartic acid substitutions, which may be markedly disruptive to the collagen triple helix. However, it is likely that both the type of mutation and its location within the molecule will be important in determining the clinical outcome of each mutation. As has been seen for the type I collagen gene mutations that produce osteogenesis imperfecta [Byers, 1990], the relationship between mutation and phenotype is unlikely to be simple, and may emerge only after many more mutations have been characterized.

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