ORIGINAL ARTICLE

The phenotypic spectrum in patients with arginine to cysteine mutations in the COL2A1 gene

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Background: The majority of COL2A1 missense mutations are substitutions of obligatory glycine residues in the triple helical domain. Only a few non-glycine missense mutations have been reported and among these, the arginine to cysteine substitutions predominate.

Objective: To investigate in more detail the phenotype resulting from arginine to cysteine mutations in the COL2A1 gene.

Methods: The clinical and radiographic phenotype of all patients in whom an arginine to cysteine mutation in the COL2A1 gene was identified in our laboratory, was studied and correlated with the abnormal genotype. The COL2A1 genotyping involved DHPLC analysis with subsequent sequencing of the abnormal fragments.

Results: Six different mutations (R75C, R365C, R519C, R704C, R789C, R1076C) were found in 11 unrelated probands. Each mutation resulted in a rather constant and site-specific phenotype, but a perinatally lethal disorder was never observed. Spondyloarthropathy with normal stature and no ocular involvement were features of patients with the R75C, R519C, or R1076C mutation. Short third and/or fourth toes was a distinguishing feature of the R75C mutation and brachydactyly with enlarged finger joints a key feature of the R1076C substitution. Stickler dysplasia with brachydactyly was observed in patients with the R704C mutation. The R365C and R789C mutations resulted in classic Stickler dysplasia and spondyloepiphyseal dysplasia congenita (SEDC), respectively.

Conclusions: Arginine to cysteine mutations are rather infrequent COL2A1 mutations which cause a spectrum of phenotypes including classic SEDC and Stickler dysplasia, but also some unusual entities that have not yet been recognised and described as type II collagenopathies.

Heterozygous mutations in the type II collagen gene (COL2A1) cause a spectrum of phenotypes known as the type II collagenopathies.¹ These disorders range from the lethal achondrogenesis type II/hypochondrogenesis (OMIM 200610) and platyspondylic lethal skeletal dysplasia Torrance type (OMIM 151210), through several forms of short trunk dwarfism including spondyloepiphyseal dysplasia congenita (SEDC; OMIM 183900) and Kniest dysplasia (OMIM 156550), to disorders with normal stature but with premature osteoarthritis, such as Stickler dysplasia (OMIM 108300) and some forms of spondyloarthropathy (OMIM 165720).^{1, 2}

The majority of COL2A1 missense mutations are single nucleotide substitutions that change codons for obligatory glycine residues in the Gly-X-Y triplet repeats (characteristic for the triple helical domain) to codons for other, bulkier amino acids. In frame duplications and deletions, and splice site mutations leading to exon skipping, have also been identified. All these mutations usually result in short stature. These mutations most likely affect endochondral ossification and linear bone growth by a dominant negative mechanism through the presence of structurally abnormal type II collagen in the extracellular matrix. However, nonsense or frameshift mutations are usually identified in patients with normal stature but with precocious osteoarthritis such as in Stickler dysplasia. These mutations probably result in haploinsufficiency either through nonsense mediated decay or by generating truncated proteins lacking the carboxypropeptide necessary for chain association and incorporation into the type II collagen homotrimer.^{1, 3} Haploinsufficiency for

type II collagen seems to impair the integrity of articular cartilage more than it interferes with the linear growth of tubular bones.

Only a few non-glycine missense mutations in the repeating Gly-X-Y sequence have been reported and among these, the arginine to cysteine substitutions predominate.⁴ Cysteine residues are normally not found in the triple helical domain of fibrillar collagen genes.⁵

The aim of this study was to investigate in more detail the phenotype resulting from these arginine to cysteine mutations. We report 11 new probands and discuss the genotype-phenotype correlations for this specific group of missense mutations.

METHODS

Patients

All patients were evaluated by a clinical geneticist at the referring centre. A blood or DNA sample was sent for mutation analysis of the COL2A1 gene to confirm the diagnosis of a type II collagen disorder. Written informed consent was obtained for all patients. After identification of the mutation, further clinical data were collected and available radiographs evaluated.

Molecular analysis

Genomic DNA was extracted from blood samples by standard procedures, followed by touchdown PCR amplification of the

Abbreviations: MED, multiple epiphyseal dysplasia; SEDC, spondyloepiphyseal dysplasia congenita

Figure 1 Schematic representation of the type II collagen trimer with localisation of the arginine to cysteine mutations and their corresponding phenotypes.

54 COL2A1 exons using forward and reverse primers located in the flanking introns (primer sequences available upon request). The PCR products were analysed by gel electrophoresis and visualised by ethidium bromide staining on 2% agarose gels. DHPLC analysis was carried out using the WAVE DNA fragment analysis system (Transgenomic, Cheshire, UK). Oven temperatures were selected based on recommendations of the WAVEMAKER software program (Transgenomic). All fragments showing an aberrant pattern were directly sequenced on the ABI PRISM 3100 automated sequencer (Applied Biosystems, Foster City, CA) using BigDye terminator cycle sequencing chemistry. These sequences were compared to the wild type sequence as submitted under GenBank accession number NM 033150. The nucleotides were numbered starting from the first base of the start codon (ATG) of the cDNA reference sequence. Amino acid residues were numbered from the first glycine residue of the main triple helical domain of the $\alpha 1(\text{II})$ collagen chain.

RESULTS

Molecular analysis of the COL2A1 gene revealed six different arginine to cysteine mutations in 11 unrelated probands: R75C, R365C, R519C, R704C, R789C, and R1076C. For each familial case, the mutation was confirmed in other affected relatives. The mutations were not found in unaffected relatives or in a panel of 100 control individuals. The clinical and radiographic features of the affected individuals are summarised in tables 1 and 2. The mutations and corresponding phenotypes are shown in fig 1.

R75C mutation

The R75C mutation was identified in one patient (AT-3844). The parents of this boy noted uneven walking and dislike of sports in childhood. The boy started to complain of joint pain when he was about 13 years old. He was referred with a diagnosis of either progressive pseudorheumatoid dysplasia or multiple epiphyseal dysplasia (MED). Physical examination revealed normal height, but flat face, low nasal bridge, and short third and fourth toes. He had no other ocular abnormalities besides hypermetropia. Radiographic evaluation showed flattened vertebral bodies with increased anteroposterior diameter (most accentuated in the thoracic spine) and mild epiphyseal dysplasia of hips and knees (fig 2).

Figure 2 Patient AT-3844 at 19 years of age. (A) The patient has normal (rather tall) stature. (B) Radiograph of the thoracic spine shows mild platyspondyly with increased anteroposterior diameter. (C) Note the short third and fourth toes on the right foot and short fourth toe on the left foot. (Informed consent was obtained for the publication of these images.)

R365C mutation

The R365C mutation was found in three unrelated probands (DBA-2204, GR-2914, and AK-3319).

Patient DBA-2204 was diagnosed with Stickler syndrome because of severe myopia ($-16/-17$), unilateral retinal detachment, and sensorineural hearing loss. His mother also had a history of retinal detachments. The boy had normal stature and showed mild flattening of the lumbar vertebral bodies on radiographs.

Patient GR-2914 presented in infancy with severe myopia and a type 1 vitreous anomaly. Cataracts were diagnosed at 16 years of age. Ophthalmological evaluation of his affected brother also revealed myopia, retinal perivascular degeneration, and a unilateral retinal tear. Their mother and maternal aunt had a history of bilateral retinal detachments and the maternal aunt was also diagnosed with mixed hearing loss. They had all suffered from arthropathy since childhood. Clinical evaluation of these four affected relatives revealed normal stature, flat face, and low nasal bridge with radiographs only showing signs of premature osteoarthritis.

Patient AK-3319 presented with severe myopia in his first year of life. At the age of 3 years he started complaining of pain in his knees. Sensorineural hearing loss, most pronounced at high frequencies, was diagnosed. Physical examination of the proband revealed normal height, flat face, micrognathia, and low nasal bridge. Myopia was also present in his sister, brother, and mother who had in addition a history of retinal detachments.

R519C mutation

The R519C mutation was found in patient MO-3589. She was born with a cleft palate and started complaining of joint pain in childhood. Her affected mother had bilateral hip replacement at 23 years of age. Ophthalmological evaluation only revealed hypermetropia in the proband. Both mother and daughter had normal stature and a flat face with depressed nasal bridge. Radiographs of the proband at the age of 8 years only showed irregular vertebral endplates without significant platyspondyly; her hip and knee epiphyses were normal.

R704C mutation

The R704C mutation was found in two unrelated patients (SL-1650 and LD-3683).

Patient SL-1650 was short at birth with a length of 45 cm at 39 weeks of gestation. During childhood severe myopia ($-16.5/-18$) and bilateral sensorineural hearing loss were diagnosed. Physical evaluation at 8 years of age revealed normal stature with brachydactyly, flat face, and prominent joints. Radiographic examination showed marked platyspondyly with anterior tonguing and indentations of the midportions of upper and lower endplates. The pelvis was abnormal with small but squared iliac wings, horizontal acetabular roofs, mild flattening of femoral epiphyses, and rather broad femoral necks. The epiphyses at the knees and ankles were slightly enlarged. The hands showed brachydactyly (fig 3).

Patient LD-3683 also presented with severe myopia ($-15/-15$) and sensorineural hearing loss. He developed a retinal detachment in childhood. His height at 5 years of age was 2 SD below the mean and physical examination revealed brachydactyly, flat face, and prominent, hypermobile joints. In particular, the hand radiographs showed striking similarities with patient SL-1650 (fig 4).

R789C mutation

The R789C mutation was identified in three unrelated patients (MA-2337, JY-1916, and VDH-1162).

Figure 3 Radiographs of patient SL-1650 at the age of 7 years. (A) Radiograph of the left hand shows short metacarpals and phalanges with slightly flattened epiphyses. The distal ends of metacarpals and proximal phalanges are broad. (B) Marked platyspondyly is visible on the lateral view of the lumbar spine. The vertebral bodies have an increased anteroposterior diameter and show anterior tonguing (due to delayed ossification of the apophyses) with indentations of the midportions of the endplates. (C) The pelvis is abnormal with small and squared iliac wings, horizontal acetabular roofs, mild flattening of the capital femoral epiphyses, and broad femoral necks. (D) The knee epiphyses are slightly enlarged for age.³⁰

Patient MA-2337 was born with bowing of the lower limbs. The diagnosis of SEDC was made at 18 months after radiographic evaluation of the skeleton (fig 5). Clinical evaluation at 6 years of age showed short trunk dwarfism with lumbar hyperlordosis, flat face with low nasal bridge, and pes planus.

Patient JY-1916 was small at birth. During his childhood he developed coxa vara, hyperlordosis, and pectus carinatum. Radiographs at 5 years of age were diagnostic for SEDC (fig 5). At the age of 6 years and 4 months, his height was 91 cm (-6 SD). Midface hypoplasia and a bifid uvula were present.

Patient VDH-1162 and her two affected sons had SEDC (fig 5). Her adult height was 112 cm (-10 SD). One boy was 40 cm at birth and had a height of 84.5 cm at the age of 6 years 10 months (-8.5 SD). The other boy was 42 cm at birth and had a height of 77.6 cm at the age of 3.5 years (-6.4 SD).

R1076C mutation

The R1076C mutation was identified in patient DA-3473. He presented with pain in his hands, hips, and feet. Over the years he had been given several diagnoses including chondrodysplasia punctata, SED, and MED. Physical examination revealed normal adult height (175 cm) with normal body proportions and mild kyphoscoliosis. He had normal vision and a prominent nose. He had a bilateral metatarsus adductus and his fingers were expanded at the interphalangeal joints which had decreased flexion and resembled those

Figure 4 Patient LD-3683 at the age of 5 years. (A) Note the flat face with rather prominent eyes, low nasal bridge, and small chin. (B) Radiographs of the knees show small and flattened epiphyses with metaphyseal flaring. (C) Lateral view of the spine shows mild platyspondyly with indentations of the endplates. (D) Radiograph of the left hand shows short metacarpals and phalanges with broadened metaphyses (not all distal phalanges are shown). Epiphyseal ossification is delayed. (Informed consent was obtained for the publication of these images.)

of Kniest dysplasia. His adult radiographs showed thoracic platyspondyly with irregular endplates and narrow disc spaces and small deformed proximal femoral epiphyses. Prepubertal x rays showed fragmentation of the distal ends of the phalanges as seen in Kniest dysplasia (fig 6).

DISCUSSION

We report 11 new unrelated patients with a type II collagen disorder who are heterozygous for a missense mutation in the COL2A1 gene. Each mutation results in the substitution of an arginine to cysteine residue at one of the following codons: R75C, R365C, R519C, R704C, R789C, and R1076C. This group of arginine to cysteine mutations represents about 8% of the COL2A1 mutations that have been identified in our laboratory. The phenotype of the 11 patients is variable but never perinatally lethal and ranges from SEDC to Stickler syndrome or even mild spondyloarthropathy without ocular involvement. Interestingly, each mutation gives a rather uniform phenotype when comparing the patients in this series with those reported in the literature (tables 1 and 2).

The R75C substitution causes spondyloarthropathy with normal stature and no ocular involvement. It is notable that

Figure 5 Radiographs of SEDC patients with the R789C mutation. (A) Platyspondyly is visible on the lateral view of the thoracolumbar spine in patient MA-2337 (6.5 years of age). (B) Radiograph of the pelvis in patient MA-2337 (6.5 years of age). The pelvis is abnormal with small iliac bones, coxa vara, very short femoral necks, and small, fragmented ossification of the capital femoral epiphyses. (C) The pelvis of patient JY-1916 at 5 years of age shows similar findings including short iliac bones, horizontal acetabula, coxa vara with absent ossification of the femoral necks, and capital epiphyses except for a small medial ossification centre. (D) The pelvis of the affected son (32 months of age) of patient VDH-1162 shows short and broad iliac wings, horizontal acetabular roofs, and severe hypoplastic ossification of femoral necks and epiphyses.

short third and fourth metatarsals were found in our patient as well as in the four families previously reported with this mutation.⁴⁻⁹ All patients have osteoarthritis which started between 5 and 10 years of age; signs of osteoarthritis with mild spondyloepiphyseal involvement are usually present on radiographs.⁴⁻⁹ Sensorineural hearing loss seems to be an inconstant feature.⁶⁻⁷

Ocular involvement reminiscent of Stickler syndrome is characteristic for the R365C substitution. Affected individuals have normal stature but the facial features of Stickler syndrome. Cleft palate and hearing loss can be present.¹⁰ Myopia, retinal detachment, and a type 1 vitreous anomaly have been described in the two unrelated patients previously reported with this mutation.¹⁰

Spondyloarthropathy with normal stature and no ocular involvement are also features of the R519C substitution. Cleft palate can be present but hearing loss was not found in our family or previously reported patients.¹¹⁻¹⁶ Complaints of joint pain can start in childhood or early adulthood and mild spondyloepiphyseal involvement is usually found.¹³⁻¹⁶ Osteochondritis dissecans was observed in three families.¹⁴⁻¹⁶⁻¹⁷ In four families at least one patient with Perthes disease was diagnosed.¹³⁻¹⁵⁻¹⁶ There is evidence for a founder effect in these reported families.¹⁸

Mild short stature with brachydactyly seems to be specific for the R704C substitution. Affected individuals have features of Stickler syndrome including severe myopia and sensorineural hearing loss. Radiographs show mild platyspondyly and shortening of the tubular hand bones. Only one family with this mutation and a similar phenotype has been reported.¹⁹

Patients with the R789C substitution are much shorter and have clinical and radiographic features of SEDC. The clinical and radiographic features of the two families reported with this mutation have not been described in detail.²⁰⁻²¹

Figure 6 Radiographs of patient DA-3473 (in adulthood). (A) Radiograph of the right hand reveals short phalanges with broadened and irregular articular surfaces. (B) Osteoarthritis of the hips with flattened femoral heads. (C) The thoracic vertebrae are flattened with irregular endplates and narrow disc spaces.

Only in one patient was the R1076C substitution identified. No other patients with this mutation have been reported so far. Our patient has spondyloarthropathy with normal stature and no ocular involvement. Because of the enlarged finger joints and fragmentation of the distal ends of the phalanges on prepubertal radiographs, the diagnosis of mild Kniest dysplasia was initially considered.

The site-specific phenotype of arginine to cysteine substitutions in the COL2A1 gene is an intriguing observation. In some instances, the phenotype is difficult to classify within the pre-existing and well defined subgroups of the type II collagenopathies. Patients with normal stature and normal ocular findings but with osteoarthritis and significant spondyloepiphyseal involvement on radiographs are said to have spondyloarthropathy. They cannot be considered to have Stickler syndrome because of the lack of ocular

involvement and the presence of significant platyspondyly, nor SEDC because of normal stature. Brachydactyly, previously considered as being specific for mutations in the carboxypropeptide, is also observed in patients with the R75C or R704C substitution although both are located not in the carboxypropeptide but in the triple helical domain of the type II collagen molecule (fig 1).²

The position of the substituted arginine residue within the repeating Gly-X-Y triplet seems to influence the ultimate phenotype. Substitution of an arginine residue in the X position (R365C, R704C) results in a collagenopathy with ocular involvement. Substitutions that insert the arginine in the Y position (R75C, R519C, R789C) cause a disorder without ocular anomalies (tables 1 and 2). It is also interesting to note that the most carboxyl-terminal located substitution in the triple helical domain (R789C) causes the

Table 1 Clinical and radiographic findings in patients with arginine to cysteine substitutions						
	p.Arg75Cys c.616C>T		p.Arg365Cys c.1486C>T			
	This study	Literature ⁴⁻⁹	This study	Literature ¹⁰		
Proband	AT-3844	n = 4	DBA-2204	GR-2914	AK-3319	n = 2
Other affected relatives	None		1	3	3	
Sex	Male		Male	Male	Male	
Clinical						
Height	Normal (181 cm at 19 years)	Normal (4/4)	Normal (150 cm at 12 years 8 months)	Normal (4/4)	Normal (4/4)	Normal (2/2)
Flat face	Present	NR	Present	Present (4/4)	Present (2/4)	Present (2/2)
Low nasal bridge	Present	NR	Present	Present (4/4)	Present (2/4)	Present (2/2)
Ocular features	Hypermetropia	Absent (4/4)	Severe myopia	Severe myopia (4/4)	Severe myopia (4/4)	Myopia (2/2)
			Retinal detachment	Retinal detachment (3/4)	Retinal detachment (1/4)	Type 1 vitreous (2/2)
				Bilateral cataract (3/4)		Retinal detachment (1/2)
				Optically empty vitreous (1/4)		Cataract (1/2)
Cleft palate	Absent	Absent (4/4)	Absent	Absent (4/4)	Absent (4/4)	Present (1/2)
Hearing loss	Absent	Present (2/4)	Sensorineural	Mixed (1/4)	Sensorineural (1/4)	Sensorineural-mixed
Arthropathy	Present	Present (4/4)	Absent	Present (4/4)	Present (1/4)	Present (1/2)
Radiographical						
Hands/feet	Short 3–4 metatarsals	Short 3–5 metatarsals	Normal	Normal	Delayed bone age	NR
Spine	Mild platyspondyly	Platyspondyly	Mild lumbar flattening	NT	NT	Premature osteoarthritis
	Vertebrae with increased AP diameter	Irregular endplates				
Hips	Mild epiphyseal flattening	Hip replacement (3/4)	Normal	Premature osteoarthritis	Normal at age 4 years	Premature osteoarthritis
Knees	Mild epiphyseal flattening	NR	Normal	Premature osteoarthritis	Normal at age 4 years	Premature osteoarthritis
Phenotype	Spondyloarthropathy with short third and fourth toes		Stickler syndrome			

Table 2 Further clinical and radiographic findings in patients with arginine to cysteine substitutions

	p.Arg519Cys c.1948C>T		p.Arg704Cys c.2503C>T		
	This study	Literature ¹⁷⁻¹⁸	This study	Literature ¹⁹	
Proband	MO-3589	n = 6	SL-1650	LD-3683	n = 1
Other affected relatives	1	59	None	None	
Sex	Female		Male	Male	
Clinical					
Height	Normal (2/2)	Normal (6/6)	-1 SD (118 cm at age 7 years)	-2 SD (101 cm at age 5 years)	-2 SD (4/4)
Flat face	Present (2/2)	NR	Present	Present	Present (4/4)
Low nasal bridge	Present (2/2)	NR	Present	Present	NR
Ocular features	Hypermetropia (1/2)	NR	Severe myopia	Severe myopia Retinal detachment	Myopia (4/4) Cataract (4/4) Asteroïd hyalosis (4/4) Retinal thinning (4/4)
Cleft palate	Present (1/2)	NR	Absent	Absent	Absent (4/4)
Hearing loss	Absent (2/2)	NR	Sensorineural	Sensorineural	Present (4/4)
Arthropathy	Present (2/2)	Present (5/6)	Present	Absent	NR
Radiographical					
Hands/feet	Normal	NR	Brachydactyly	Brachydactyly	Brachydactyly
Spine	Irregular endplates	Platyspondyly (6/6) Irregular endplates (6/6)	Marked platyspondyly Vertebrae with increased AP diameter	Platyspondyly Indentation endplates	Mild platyspondyly Indentation endplates
Hips	Normal (age 8 years)	Perthes (4/6)	Small iliac wings	Normal	Widened femoral necks
Knees	Normal (age 8 years)	Osteochondritis dissecans (2/6)	Broad femoral necks Slightly enlarged epiphyses	Flattened epiphyses	Coxa valga Flattened epiphyses
Phenotype	Spondyloarthropathy		Stickler syndrome with brachydactyly		
	p.Arg789Cys c.2758C>T		p.Arg1076Cys c.3619C>T		
	This study	Literature ²⁰⁻²¹	This study	Literature ²⁰⁻²¹	
Proband	MA-2337	JY-1916	VDH-1162	n = 2	DA-3473
Other affected relatives	None	None	2		None
Sex	Female	Male	Female		Male
Clinical					
Height	Short trunk dwarfism (-7 SD)	Short trunk dwarfism (-6 SD)	Short trunk dwarfism (-10 SD)	NR	Normal (adult height: 175 cm)
Flat face	Present	Present	Present	NR	Absent
Low nasal bridge	Present	Absent	Absent (3/3)	NR	Absent
Ocular features	Absent	Absent	Absent (2/3)	NR	Absent
Cleft palate	Absent	Present	Absent (3/3)	NR	Absent
Hearing loss	Absent	Absent	Absent (3/3)	NR	Absent
Arthropathy	Present	Present	Present (3/3)	NR	Present
Radiographical					
Hands/feet	NT	Normal	Normal (3/3)	NR	Brachydactyly
Spine	Platyspondyly	Platyspondyly	Platyspondyly (3/3)	NR	Thoracic platyspondyly with irregular end plates
Hips	SEDC changes	SEDC changes	SEDC changes (3/3)	NR	Coxarthrosis
Knees	SEDC changes	NT	SEDC changes (3/3)	NR	Flattened femoral heads
Phenotype	SEDC				NT
					Spondyloarthropathy with brachydactyly

NR, not reported; NT, not taken.

most severe disorder in terms of stature (classic SEDC). It has been suggested that mutations (especially glycine substitutions) residing toward the carboxyl terminus of the triple helix tend to produce more severe manifestations than those that map toward the amino terminus of the helix. However, there are many exceptions to this rule which is why this statement has not been generally accepted.²²

The effects of these mutations on in vitro fibril formation have been studied in several reports using recombinant type II collagen.¹²⁻²³⁻²⁵ Collagen molecules with the R75C substitution have a normal structure and are able to form normal fibrils. Collagen homotrimers with the R519C mutation also have a normal structure but form abnormal fibrils when mixed with wild type collagen. In contrast to the R75C and R519C collagens, the R789C collagen homotrimers are

characterised by an abnormal structure with a kink at the site of the mutation.²⁵ Fibrils formed in a mixture of wild type and mutant collagens are poorly organised and have a filamentous structure with an increased number of non-assembled microfibrils in the background. Unlike the R75C and R519C mutations, the R789C substitution also seems to change the thermostability of the collagen triple helix. These observations may explain the more severe phenotype associated with the R789C mutation in comparison with the other more amino-terminal located mutations.

Arginine to cysteine substitutions not only affect the supramolecular organisation of the collagen fibrils in the cartilage extracellular matrix (dominant negative effect) but also the transport and secretion of the mutant molecules.²⁵ Biochemical studies on cartilage from a patient with the

R519C mutation have shown the presence of mutant collagen in the cartilage matrix. However, the content of mutant chains in the extracellular type II collagen was about 25% and thus lower than the predicted 50%, suggesting that some mutant chains were selectively degraded at or soon after synthesis.²⁶ Similarly, in the cartilage of a patient with the R789C substitution, one third rather than the expected half of the mutant collagen chains were found.²⁰ Moreover, dilated Golgi and RER cisternae have been observed in transgenic mice with the R789C or R519C mutation.^{27, 28}

The R1076C mutation in the carboxypropeptide of the procollagen $\alpha 1$ (II) chain has not been reported before. The carboxypropeptide contains two cysteine residues that form interchain disulphide bridges important for proper chain alignment and assembly. The introduction of an additional cysteine residue may impair proper trimer formation through chain misalignment.²⁹

We conclude that arginine to cysteine mutations in COL2A1 represent a minority of missense mutations within the type II collagen gene. It is of diagnostic relevance that some of these mutations seem to cause unusual phenotypes (spondyloarthropathy with short toes, spondyloarthropathy with brachydactyly, and Stickler syndrome with brachydactyly) within the spectrum of type II collagenopathies. Recognition of these phenotypes should prompt the molecular geneticist to look first for the corresponding arginine to cysteine substitution before screening the whole COL2A1 gene. Whereas loss of function mutations in COL2A1 usually cause a type II collagenopathy with normal stature (Stickler syndrome) and the other mutations including glycine substitutions, in frame deletions/duplications, and splice site mutations, result in a short trunk dwarfism, these arginine to cysteine mutations seem to produce a broader spectrum of phenotypes with either normal or short stature but never a severe, perinatally lethal condition. The way in which mutations in type II collagen alter hyaline cartilage is complex and can not be reduced to one simple pathway. They can disturb the thermostability of the collagen triple helix or alter its correct folding and secretion from cells. In addition, they can change the shape of the mutated molecule and in that way either affect the extracellular assembly into fibrils or the proper interaction with other (cysteine containing) proteins of the cartilage matrix. Those arginine to cysteine substitutions that prevent secretion of the mutated molecules into the cartilage matrix may mimic a loss of function mutation as is seen in Stickler syndrome and therefore result in a type II collagen disorder with normal stature but precocious osteoarthritis. Alternatively, those mutations that result in the secretion and incorporation of mutant collagen into the extracellular fibril may interfere in a dominant negative manner with endochondral ossification and linear bone growth and therefore result in short trunk dwarfism.

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