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

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Abstract

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 more in 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 lab, 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 respectively in classic Stickler dysplasia and SEDC.

Conclusions
Arginine-to-cysteine mutations are rather infrequent COL2A1 mutations that cause a spectrum of phenotypes including classic SEDC and Stickler dysplasia, but also some unusual entities that have not yet been recognized and described as type II collagenopathies.
INTRODUCTION
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. On the other hand, 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 carboxy-propeptide 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 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 more in 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.

MATERIALS AND METHODS
Patients
All patients were evaluated by a clinical geneticist at the referring center. A blood or DNA sample was sent for mutation analysis of the COL2A1 gene to confirm the diagnosis of a type II collagen disorder. For all patients, a written informed consent was obtained. 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 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 visualized 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, Cheshire, UK). All fragments showing an aberrant pattern were directly sequenced on the ABI PRISM 3100 automated sequencer (Applied Biosystems, Foster City, CA) using the BigDye terminator cycle sequencing chemistry. These sequences were compared to the wild-type sequence as submitted to 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 α1(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 and in a panel of 100 control individuals. The clinical and radiographic features of the affected individuals are summarized in Table 1. 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).

R365C mutation:
The R365C mutation was found in three unrelated probands (DBA-2204; GR-2914; AK-3319). Patient DBA-2204 was diagnosed with Stickler syndrome because of severe myopia (-16/-17), unilateral retinal detachment and sensorineural hearing loss. There was also a history of retinal detachments in the mother. The boy had a normal stature and showed on radiographs mild flattening of the lumbar vertebral bodies. Patient GR-2914 presented in infancy with severe myopia and a type 1 vitreous anomaly. Cataracts were diagnosed at the age of 16 years. 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, in addition, the maternal aunt was diagnosed with mixed hearing loss. They all suffered from arthropathy since their childhood. Clinical evaluation of these four affected relatives revealed normal stature, flat face and low nasal bridge with radiographs only showing signs of premature osteoarthrosis.

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. A sensorineural hearing loss, most pronounced at the 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 the age of 23. 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, also 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 high 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
tongueing 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 the age of 5 years was 2 sd
described above and physical exam revealed brachydactyly, flat face and prominent,
hypermobile joints. Especially 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; VDH-
1162).
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
age 6 years 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. At the age of 6 years and 4 months, his height was 91 cm
(-6sd). Midface hypoplasia and a bifid uvula were present. Radiographs at 5 years of age were
diagnostic for SEDC (Fig 5).
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 age 6 10/12 years (-8.5 sd).
The other boy was 42 cm at birth and had a height of 77.6 cm at age 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 exam revealed normal adult height (175
cm) with normal body proportions and mild kyphoscoliosis. He had normal vision and a
prominent nose. Furthermore, he had a bilateral metatarsus adductus and his fingers were
expanded at the interphalangeal joints which had a decreased flexion and resembled those 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 6 different codons: R75C, R365C, R519C,
R704C, R789C, R1076C. This group of arginine-to-cysteine mutations represents about 8 %
of the COL2A1 mutations that have been identified in our lab. 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 (Table 1).
<table>
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<td></td>
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* NT: not taken; NR: not reported
Table 1 Continued

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* NT: not taken; NR: not reported
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The **R75C substitution** causes a spondyloarthropathy with normal stature and no ocular involvement. Notable is the finding of short third and fourth metatarsals in our patient as well as in the four families previously reported with this mutation.\[4\] \[6\] \[7\] \[8\] \[9\] All patients suffer from osteoarthrosis that has started between the age of 5 years and the second decade. Sensorineural hearing loss seems to be an inconstant feature.\[6\] \[7\] On radiographs signs of osteoarthrosis with mild spondyloepiphyseal involvement are usually present.\[4\] \[6\] \[7\] \[8\] \[9\] 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 and previously reported patients.\[11\] \[12\] \[13\] \[14\] \[15\] \[16\] Complaints of joint pain can start in childhood or early adulthood and mild spondyloepiphyseal involvement is usually found.\[13\] \[16\] In three families osteochondritis dissecans was observed.\[14\] \[16\] \[17\] 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.\[20\] \[21\]

Only in one patient the **R1076C substitution** was 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. Entities with normal stature and normal ocular findings but with osteoarthrosis and significant spondyloepiphyseal involvement on radiographs are coined “spondyloarthropathy”. They cannot be considered as Stickler syndrome because of the lack of ocular involvement and presence of significant platyspondyly, and not as SEDC because of the 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 they both are not located 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 (Table 1). It is also interesting to note that the most carboxyterminal located substitution in the triple helical domain (R789C) is causing the 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 the reason why this statement has not been generally accepted.[22]

In several reports using recombinant type II collagen, the effects of these mutations on in vitro fibril formation have been studied.[12] [23][24][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 in mixture with wild type collagen these mutant molecules form abnormal fibrils. In contrast to the R75C and R519C collagens, the R789C collagen homotrimers are characterized 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 organized 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 aminoterminal located mutations.

Arginine-to-cysteine substitutions not only affect the supramolecular organization 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 one-quarter and thus lower than the predicted amount of 50 %, suggesting that some mutant molecules 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 α1 (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 by chain misalignment.[29]

We conclude that arginine-to-cysteine mutations in COL2A1 represent a minority of missense mutations within the type II collagen gene. Of diagnostic relevance is the fact that some of these mutations seem to cause unusual phenotypes (spondyloarthropathy with short toes; spondyloarthropathy with brachydactyly; 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 result in a type II collagenopathy with normal stature (Stickler syndrome) and the other mutations including glycine substitutions, in frame deletions/duplications and splice site mutations give a short-trunk dwarfism, these arginine-to-cysteine mutations seem to cause 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 mimick 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 osteoarthrosis. 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.
Acknowledgments

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Legends for the figures
Fig 1: Schematic representation of the type II collagen trimer with localisation of the arginine-to-cysteine mutations and their corresponding phenotypes

Fig 2: Patient AT-3844 at the age of 19 years. (a) The boy has a normal (rather tall) stature. (b) Note the short third and fourth toes on the right foot and short fourth toe on the left foot. (c) Radiograph of the thoracic spine shows mild platyspondyly with increased anteroposterior diameter. [Informed consent was obtained for publication of the clinical photographs]

Fig 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 “tongueing” (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]

Fig 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) On radiograph of the left hand shortening of metacarpals and phalanges is visible. The metacarpals and phalanges are stubby with broadened metaphyses. There is delay of the epiphyseal ossification. Not all distal phalanges are shown. [Informed consent was obtained for publication of the clinical photographs]

Fig 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 (age of 6.5 years). (b) Radiograph of the pelvis in patient MA-2337 (age of 6.5 years). 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 the age of 5 years 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 center. (d) The pelvis of the affected son (age 32 months) of patient VDH-1162 shows short and broad iliac wings, horizontal acetabular roofs and severe hypoplastic ossification of femoral necks and epiphyses

Fig 6: Radiographs of patient DA-3473 (adult age). (a) Radiograph of the right hand reveals short phalanges with broadened and irregular articular surfaces. (b) Osteoarthrosis of the hips with flattened femoral heads. (c) The thoracic vertebrae are flattened with irregular endplates and narrow disc spaces.
References


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