REVIEW

COL2A1 Gene Mutations: Mechanisms of Spondyloepiphyseal Dysplasia Congenita

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Abstract: The *COL2A1* gene consists of 54 exons spanning over 31.5 kb and encodes for type II collagen. Type II collagen is the main component of hyaline cartilage extracellular matrix, nucleus pulposus of intervertebral discus, vitreous humor of the eye and inner ear structure. Molecular defects in *COL2A1* gene cause a wide variety of rare autosomal-dominant conditions known as type II collagenopathies. A clear genotype–phenotype relationship is not yet known. However, some correlations are described. Spondyloephyseal dysplasia congenita was suggested for a short-trunk dwarfing condition affecting primarily the vertebrae and the proximal epiphyses of the long bones.
Keywords: *COL2A1* gene, type II collagen, spondyloepiphyseal dysplasia congenita

COL2A1 gene (12q13.1-q13.2) consists of 54 exons spanning over 31.5 kb and encodes for type II collagen, a 1487-amino acid (134.4 kDa) protein.[1]

Type II collagen, a large homotrimeric protein, is the main component of hyaline cartilage extracellular matrix (95% of the collagens and approximately 60% of dry weight in adults), nucleus pulposus of intervertebral discus, vitreous humor of the eye and inner ear structure. It plays a fundamental role on the endochondral bone formation and growth. In the cartilage growth plate, it is synthesized by proliferating chondrocytes until they differentiate on hypertrophic chondrocytes.

Type II collagen acts as an autocrine factor of proliferation and differentiation via multiple downstream effectors and a potent suppressor for chondrocyte hypertrophy and apoptosis through the negative regulation of SMAD1 activity.[2]

Type II collagen molecules have three identical α1-polypeptide chains of 1060 amino acid residues each, with a large uninterrupted triple-helical region and relatively short, non-helical telopeptides (19 amino acid residues in the N-telopeptide and 27 amino acid residues in the C-telopeptide) that do not possess the Gly-X-Y-repeating primary structure found in the triple-helical region. "X" and "Y" positions are frequently occupied by proline and hydroxyproline residues, respectively. N- and C-telopeptide regions allow the initiation of the triple-helical configuration.

Type II collagen molecules spontaneously self-assemble into fibrils, which, together with other macromolecules, form the extracellular scaffold critical for the integrity and biomechanical functions of cartilage.[3] The fibrils are cross-linked to form mature type II collagen fibers.

In vivo, the polymerization of collagen molecules into fibrils involves cellular and specific extracellular matrix interactions. Proteoglycans, such as decorin, fibromodulin,

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and biglycan, bind type II collagen fibrils to stabilize the larger fibril bundles (fibers composed of multiple fibrils).

Molecular defects in COL2A1 gene cause a wide variety of rare autosomal-dominant conditions known as type II collagenopathies.[4] So far more than 400 mutations have been described in public databases and previous literature (329 pathogenic variants, 153 variants of uncertain significance). Molecular spectrum of alterations includes point mutations (missense, nonsense, deletion, insertion, insertion-deletion and frame-shift mutation) and complex rearrangements[5–7] (Table 1). No mutational hot spots within COL2A1 gene have been identified and the severity of the phenotype can be explained by the nature of mutation and the localization in the protein. We used the reference sequence NM 001844.4 combined with mutalyzer release 2.0.6 (https://mutalyzer.nl/), which is a program suite that examines the sequence variant nomenclature according to HGVS guidelines.

A clear genotype–phenotype relationship is not yet known. However, some correlations are described. Type II collagenopathies dominant inheritance basically depends on two molecular mechanisms: dominant-negative mutants and haploinsufficiency. The most common mutation (over 70%, Table 1) is missense mutation, some of which lead to substitution of glycine residue in the Gly-X-Y repeat, presenting as dominant-negative effect,[8] generally seen in the more severe collagen type II disorders. A few truncating and some exon-skipping mutations have been reported to cause more severe type II collagenopathies.[9] Missense mutations leading to other amino acids than glycine substitution causes generally milder phenotype due to impairment in protein stability, and subsequent damage in helical structure and proper function of type II collagen.

Haploinsufficiency is a mechanism due to non-sense substitutions or out-of-frame deletions, resulting in premature stop codons which cause reduced synthesis of normal collagen. These mutations are associated with milder phenotypes.

Furthermore, phenotypic variation is likely caused by environmental factors and the polymorphisms in disease-modifying genes and/or regulatory elements.

Type II collagenopathies clinical features show a wide range of severity and complexity.

Moreover, several type II collagenopathies clinical features are shared by other syndromes due to defects in other components of cartilage (eg, otospondylomegaepiphyseal dysplasia caused by COL11A2 mutation, multiple epiphyseal dysplasia principally caused by COMP mutation).[10,11]

Phenotypic overlap in COL2A1-related disorders and wide inter- and intra-familiar phenotypic variation have been commonly reported.

At one end of the spectrum, achondrogenesis type II (ACG2)/hypochondrogenesis and platyspondylic lethal skeletal dysplasia, Torrance type (PLSDT) are perinatally lethal conditions. They are characterized by micromelia, narrow chest with pulmonary hypoplasia, absent ossification of vertebras bodies and sacrum, Pierre Robin sequence and several visceral anomalies. At the other end of the spectrum are listed some conditions typical of adolescent or adult age: avascular necrosis of the femoral head (ANFH), Legg-Calvè-Perthes disease, early-onset osteoarthritis (OA), Strickler syndrome type1 (STL1), vitreoretinopathy with phalangeal epiphyseal dysplasia (VPED). These conditions are characterized by normal stature and early development of arthrosis or ocular defects. A third group of type II collagenopathies shows at birth or during childhood clinical features of spondyloepiphyseal dysplasia with wide variety of severity. Flat midface and anomalies of eye and inner ear are also common in this group.

Table 1 Summary of the Variants Reported in UniProt, ClinVar & VarSome User Entries for a Given Gene (Version: 18_oct_2019)

Coding Impact	Pathogenic	Likely Pathogenic	Uncertain Significance	Likely Benign	Benign	Total
Synonymous	0	0	34	31	15	80
Missense	199	44	93	2	19	357
Nonsense	27	2	1	0	0	30
Frameshift	29	2	0	0	0	31
Inframe Indel	1	3	0	0	0	4
Splice junction loss	1	1	0	0	0	2
Non-coding	10	10	25	46	101	192
Total	267	62	153	79	135	696

Note: This summary is useful in order to obtain some high-level insights into the modes of pathogenicity for that gene, for example whether synonymous variants mostly benign, or whether the majority of frame-shift variants are pathogenic.

Kniest dysplasia, spondyloepimetaphyseal dysplasia (SEMD) Strudwick type and Algerian type, and spondyloepiphyseal dysplasia congenita (SEDC) are characterized by dwarfism caused by delayed ossification of the vertebrae and pubic bones. Frequently odontoid hypoplasia coexists. Long bones are short and kyphoscoliosis develops in childhood.

On the contrary, in spondyloperipheral dysplasia (SPPD), patients only show short stature associated with important lumbar lordosis, in Czech dysplasia (CD) instead hallmarks are broad or prominent knees and wide metatarsals and phalanges in the third or fourth toe.

In 1966, the spondyloephyseal dysplasia congenita was suggested by Spranger and Wiedemann[12] for a short-trunk dwarfing condition affecting primarily the vertebrae and the proximal epiphyses of the long bones.

SEDC is a rare disease with a prevalence of 3.4/ 1,000,000. At birth patients with SEDC are short (mean length 45 cm at term) with flattened vertebras; ossification is absent in pubic bones and distal femoral epiphyses, absent or reduced in cervical and sacral vertebras. The iliac bones are short in length, with acetabular roof more horizontal than normal and without normal flaring of iliac wings. Infants with severe SEDC are often stillborn or premature and die shortly after birth because of hypoventilation. The developing respiratory failure can be temporarily overcome only by delivering intensive ventilator support. Recently[13] we describe a case of a child that exceptionally survived to 13.5 years with the placement of airway stenting. The characteristics of the child (small rib cage, severe tracheo-bronchomalacia, hypo-expanded lungs, respiratory muscle failure and marked abdominal distention) in fact, led the child to suffer from a chronic severe obstructive respiratory disease that were constantly monitored and treated by endoscopic surgical procedures.

Frequently patients suffer from atlantoaxial instability due to odontoid hypoplasia that can cause cervical cord compression, especially when repairing during intubation or surgery.[14,15]

Shortly after birth, platispondyly of the lower thoracic and lumbar vertebral bodies develops. This ultimately leads to wedge-shaped thoracic vertebrae and severe kyphoscoliosis with lumbar lordosis. Tubular bones, except hands, are short with delayed and dysplastic epiphyseal ossification. From this it follows short-trunk dwarfism (mean adult height is 140 cm). Adult patients also show flat face for skeletal hypoplasia with prominent eyes and cleft palate, barrel-chest and pectus carinatum.

Their hands are normal but coxa vara, dislocated hips, talipes equinovarus, clubfeet and waddling gate are reported. Hypoplastic abdomen, mitral prolapse have also been reported.

Adult patients suffer from sensorineural (25% to 30% of reported cases) or less frequently mixed hearing loss. Ocular complications such as myopia have been reported in 45% of patients but retinal detachment is less frequent (12%) than in type 1 Strickler syndrome.[16]

In spondyloephyseal dysplasia congenita over 100 COL2A1 mutations have been described. Most common are in the triple helix (74% glycine substitutions and 10% Arg-to-Cys changes) and are dominantly inherited while only few mutations have been found involving C-peptide region. Furthermore, recently also a recessive pattern has been demonstrated.[5]

Differently from osteogenesis imperfecta,[15] in SEDC patients with glycine substitutions in the triple helical domain, it is not reported any amino-to-carboxyterminal gradient in the radiological phenotype severity.[17]

Pathophysiological mechanisms and relationship between gene mutations and cartilage/bone defects are largely speculative. Only few data result from studies in mice carrying spontaneous missense mutations in COL2A1 gene[18] or in transgenic mice harboring human gene COL2A1 mutations. In transgenic mice mutations with dominant-negative effect[19] and mutations causing haploinsufficiency have been generated.[20] In these models, the delay of ossification was observed very precociously in fetus development. The grow plates show severe alterations. Proliferative and hypertrophic zones of cartilage were shorter or indistinguishable and deposition of cartilage matrix is notably impaired, collagen fibrils were fewer and less elaborate.

Mutant type II collagen molecules show altered electrophoretic mobility, relatively low thermostability and slow rates of secretion into the extracellular space. They self-assemble in abnormal fibrils which are not able to properly interact with other elements of the extracellular matrix. Proper fibrillar architecture and mechanical characteristics of the interterritorial and pericellular collagenous matrix are critical for a correct columnar arrangement of chondrocytes at the growth plate.[21] In transgenic mice, moreover, chondrocytes show greatly extended cisternae of rough endoplasmic reticulum with a retention of procollagen and other molecules (eg, fibronectin). This retention hence causes endoplasmic reticulum stress sufficient to reduce proliferation rate at the growth plates.[22,23] Absence or marked reduction in the mRNA expression

of chondrocyte markers, including Cdkn1a, Ihh, Fgfr3, COL10A1, and Runx2, has also been reported.

The abnormal chondrocyte differentiation negatively affects linear bone growth altering the normal cells relationships and provision of growth factors during endochondral ossification.

Disclosure

The authors report no conflicts of interest in this work.

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