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Review article

Collagens, the basic proteins of the human body

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Abstract. Collagens are structural elements of many tissues in the human body. The family of collagens can be divided into fibrillar and non-fibrillar collagens. The criterion of the classification is the structure of these proteins. Mutations in the genes encoding collagens cause a variety of human diseases that include osteogenesis imperfecta, some forms of osteoporosis, chondrodysplasias, some types of Ehlers-Danlos syndrome, arterial and intracranial aneurysms, epidermolysis bullosa and the renal disease known as Alport syndrome. The detection of mutations is important both scientifically and clinically. Defining the molecular defects underlying a disorder helps in the understanding of not only the properties of the mutated protein but also the function of the normal protein. Even though many mutations in the genes encoding collagens have been described, the pathogenic consequences of some of the mutations are not fully understood. The important rationale for mutation detection is the clinical use of molecular diagnostics in genetic counselling and differential diagnosis.

Key words: fibrillar collagens, collagen genes, mutations, non-fibrillar collagens.

Introduction

Collagens are the most common proteins in the human body. Until now, 19 collagen types, encoded by more than 30 genes, have been discovered. They constitute a family of related proteins in the extracellular matrix, where they are assembled into a variety of supramolecular aggregates. The basic element of the collagenic structure is at least one triple helical domain containing three α-chains with a repeated -Gly-X-Y- motif. The X- and Y-positions are frequently occupied by proline and 4-hydroxyproline. The Gly-X-Y repeat allows the polypeptide to form

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a left-handed α-helix, which interacts with two identical (homotrimer) or dissimilar (heterotrimer) α-helices. Three α-helices form a right-handed super-helical molecule. Collagens are classified on the basis of their structure into two main groups: fibrillar and non-fibrillar collagens. These groups are divided into several subgroups according to the functional properties of collagens (KIELTY et al. 1993, PROCKOP, KIVIRIKKO 1995).

Classification of the human collagens

Fibrillar collagens

Collagens type I, II and III are classified as the **major fibrillar collagens**. The **minor fibrillar collagens** are types V and XI. Both subgroups form the structural framework for various tissues. They aggregate into rod-like fibrils in a quarter-staggered array. The fibrillar collagens are synthesized as procollagens with N- and C-terminal propeptides and a large, central, triple-helical domain, which is flanked at both ends, by short non-helical, telopeptide sequences. The central triple-helical domain consists of about 1000 amino acids or about 330 -Gly-X-Y- repeats in each α-chain (CHU, PROCKOP 1993). The analyses of the genes encoding fibrillar collagens suggest that they arose from a duplication of a primitive 54 bp gene (YAMADA et al. 1980). Therefore, the exons in the highly conserved sequences coding for triple-helices are 54 bp in size.

Collagen type I is the most abundant protein in the human body, and the structural component of many tissues, such as bones, tendons, ligaments, skin, teeth and fasciae. This protein is a heterotrimer consisting of two α1(I) chains and one α2(I) chain, which are encoded by two different genes, *COL1A1* (17q21.3-q22) and *COL1A2* (7q21.3-q22). The human *COL1A1* gene is about 18kb in size and contains 51 exons. The human *COL1A2* gene is about 38 kb in size and consists of 52 exons (HUERRE et al. 1984). The central, triple-helical domain of the α1(I) and α2(I) chains contains $(\text{-Gly-X-Y-})_{338}$ repeats.

Collagen type II constitutes 80-85% of the total collagen content of the cartilage (Figure 1). It is also the major collagen component of the vitreous body, nucleus pulposus and inner ear (TILLER et al. 1990). The *COL2A1* gene (12q13.11-q13.12) is about 31 kb in size, consists of 54 exons and encodes α chains, which form a homotrimer (ALA-KOKKO et al.1995). The first intron contains an alternatively spliced exon 2A. The collagen fibrils of the cartilage, the major component of which is collagen type II, are thinner than bone collagen fibrils, consisting mostly of type I collagen.

Collagen type III is the basis of the structure of elastic tissues, e.g. skin, blood vessels, gut and lung. This protein is a homotrimer and consists of three α1(III) chains encoded by the *COL3A1* gene (2q24.3-q31), which is about 44 kb in size and contains 52 exons (SOLOMON et al. 1985, CHU, PROCKOP 1993). The central

triple-helical domain of collagen type III reveals the presence of disulphide bonds, which is a unique feature among fibrillar collagens.

The **minor fibrillar collagens** show many similarities to major fibrillar collagens. The sizes and the number of exons in the *COL5A1* and *COL11A2* genes, that code for minor fibrillar collagens type V and XI, are different from the genes for major fibrillar collagens (VUORISTO et al. 1995). In the major fibrillar collagens, the entire propeptides are cleaved during a post-translational modification. The N-propeptides of collagens type V and XI are partially retained in the mature peptide. Collagens type V and XI form heterotypic fibrils with collagens I and II, respectively, and they are located inside the collagen I and II fibrils (MENDLER et al. 1989). Their function is probably related to the regulation of fibril formation.

Collagen type V is composed of three different α-chains, α1(V), α2(V) and α3(V), encoded by the *COL5A1*, *COL5A2* and *COL5A3* genes. This type of collagen forms fibrils with collagen type I.

Collagen type XI is a heterotrimer encoded by two genes, *COL11A1* and *COL11A2*. The fibrils of collagen type XI usually contain α1(XI), α2(XI) and α3(XI) chains, coded by the *COL11A1, COL11A2* and *COL2A1* genes. The α1(XI) and α2(XI) chains are products of the *COL11A1* and *COL11A2* genes, respectively, while the α3(XI) chain is an overglycosylated form of the IIB splicing variant product of the *COL2A1* gene. The *COL11A1* is located in the p21 region of chromosome 1. Only a part of the sequence of the *COL11A1* gene has been identified so far (LI et al. 1995).

The genomic organization of the *COL11A2* (6p21.2) is precisely described. The *COL11A2* gene is 28 kb in size and consists of 66 exons. Collagen type XI is expressed in the same tissues as collagen type II (Figure 1). The central triple-helical domain contains about 1000 amino acids, and that is flanked by a non-collagenous, C-terminal region as well as an N-terminal region that consists of a minor, triple-helical region with two non-collagenous domains.

Collagens type V and XI closely resemble each other in their structural and biological features. The vitreous body contains the α1(XI) and α2(V) chains, but no α2(XI) chain (MAYNE et al. 1993), because the chains of collagen type V and type XI can substitute each other in some of the tissues.

Non-fibrillar collagens

Up to now, we have got to know that fourteen types of collagens do not form fibrils, so they are classified as non-fibrillar collagens. The non-fibrillar collagens are divided into network-forming collagens, fibril-associated collagens with interrupted triple helix (FACIT), beaded filament-forming collagens, anchoring fibril-forming collagens, transmembrane collagens, and collagens with multiple triple helix domains and interruptions (MULTIPLEXINs). The common feature of the non-fibrillar collagens is the presence of one or more non-collagenous interruptions within the collagenous sequences (PROCKOP, KIVIRIKKO 1995).

Table 1. Expression of collagen genes and effects of their mutations in the human body

Protein	Gene	Location of the gene	Expression in tissues	Manifestation
Collagen type I	*COL1A1* *COL1A2*	17q21.3-q22 7q21.3-q22	Bone, tendon, ligament, skin, teeth, fasciae	Osteogenesis imperfecta, osteoporosis, Ehlers–Danlos syndrome type VIIA and VIIB
Collagen type II	*COL2A1*	12q13.11-q13.12	Cartilage, vitreous body, nucleus pulposus, inner ear	Chondrodysplasias
Collagen type III	*COL3A1*	2q24.3-q31	Skin, blood vessels, gut and lung	Ehlers-Danlos syndrome type IV, aortic aneurysms, intracranial aneurysms
Collagen type IV	*COL4A1,* *COL4A2,* *COL4A3,* *COL4A4,* *COL4A5,* *COL4A6*	13q34 13q34 2q36-q37 2q36-q37 Xq22.3 Xq22.3	Basement membranes	 Alport syndrome Alport syndrome Alport syndrome Alport syndrome
Collagen type V	*COL5A1,* *COL5A2,* *COL5A3*	9q34.2-q34.3 2q24.3-q31	Bone, tendon, ligament, skin, teeth, fasciae, vitreous body (only α2 chain)	Ehlers-Danlos syndrome type I and II
Collagen type VI	*COL6A1,* *COL6A2,* *COL6A3*	21q22.3 21q22.3 2q37	Most connective tissues	Bethlem myopathy
Collagen type VII	*COL7A1*	3p21.3	Skin, oral mucosa, cervix	Epidermolysis bullosa dystrophica, epidermolysis bullosa pruriginosa and epidermolysis bullosa, pretibial
Collagen type VIII	*COL8A1* *COL8A2*	3q12-q13.1 1p34.3-p.32.3	Many tissues, especially Descemet's membrane	
Collagen type IX	*COL9A1* *COL9A2* *COL9A3*	6q12-q14 1p32.2-p33 20q13.3	Cartilage, vitreous body, intervertebral disc	Multiple epiphyseal dysplasia, intervertebral disc disease
Collagen type X	*COL10A1*	6q21-q22.3	Hypertrophic chondrocytes localized in calcifying cartilage	Schmidt metaphyseal chondrodysplasia

Collagen type XI	*COL11A1* *COL11A2*	1p21 6p21.2	Cartilage, vitreous body (only α1 chain), nucleus pulposus, inner ear	Marshall syndrome, Stickler syndrome type I Stickler syndrome type II, primary osteoarthritis
Collagen type XII	*COL12A1*		Tissues containing collagen type I	
Collagen type XIII	COL13A1	10q22	Many tissues	
Collagen type XIV	*COL14A1*	8q23	Tissues containing collagen type I	
Collagen type XV	*COL15A1*	9q21-q22	Many tissues, mainly: adrenal gland, kidney, pancreas and lung	
Collagen type XVI	*COL16A1*	1p34	Connective tissues	
Collagen type XVII	*COL17A1*	10q24.3	Skin hemidesmosomes	Epidermolysis bullosa, generalized atrophic benign, epidermolysis bullosa, junctional localisata variant
Collagen type XVIII	*COL18A1*	21q22.3	Many tissues, mainly: liver, kidney and placenta	
Collagen type XIX	*COL19A1*	6q12-q14	Brain, eye, testis	

Network-like structures are formed by collagens type IV, VIII and X. **Collagen type IV**, a large and flexible molecule, provides structural integrity for basement membranes (KIELTY et al. 1993). **Collagen type VIII** is the major structural protein of Descemet's membrane, which is the basement membrane synthesized by corneal epithelial cells. **Collagen type X** is expressed in the hypertrophic chondrocytes limited to the zones of the cartilage undergoing enchondral ossification (PIHLAJANIEMI, REHN 1995).

Collagen types IX, XII, XIV, and XIX belong to the FACIT subfamily. They do not form fibrils, but are associated with collagen fibrils, and serve as molecular bridges in the interaction of the fibrils with each other and with other matrix components. The FACIT collagens stabilize the organization of the extracellular matrix. They have more than one triple-helical domain interrupted by short non-collagenous sequences.

Collagen type IX is a structural component of the cartilage, the vitreous body of the eye and the intervertebral disc (Figure 1). This molecule is covalently cross-linked to the surface of the collagen type II fibrils. Collagen type IX is a heterotrimer with three distinct α chains, α1(IX), α2 (IX) and α3(IX), encoded

by the *COL9A1, COL9A2* and *COL9A3* genes, respectively. The complete genomic structure of the human *COL9A1* and *COL9A2* genes has been reported recently (PIHLAJAMA et al. 1998). The *COL9A1* gene contains 38 exons and is about 90 kb in size. The *COL9A2* gene (1p32.3-p33), spanning 15 kb, has 32 exons. The *COL9A3* gene is located on chromosome 20q13.3. The sequencing of the *COL9A3* gene revealed that the gene is 23 kb in size and contains 32 exons (BREWTON et al. 1995, PASSILTA et al. 1999).

Collagen type XII, the member of the FACIT subgroup, contains two triple-helical domains, and three non-triple-helical domains. So far, two forms of the type XII collagen have been characterized. Form XIIA is longer than form XIIB and the size is a consequence of alternative splicing (KEENE et al. 1991). Types XIIB and XIIA are preferentially expressed in the tissues where collagen type I is presented. Probably the function of collagen type XII, located on the surface of collagen type I fibrils, is similar to the function of type IX collagen in the type II collagen fibrils.

Collagen type XIV shows similarities in structure and distribution to collagen type XII, consisting of a triple helical tail and three finger-like regions attached to the central globule (SHAW 1991).

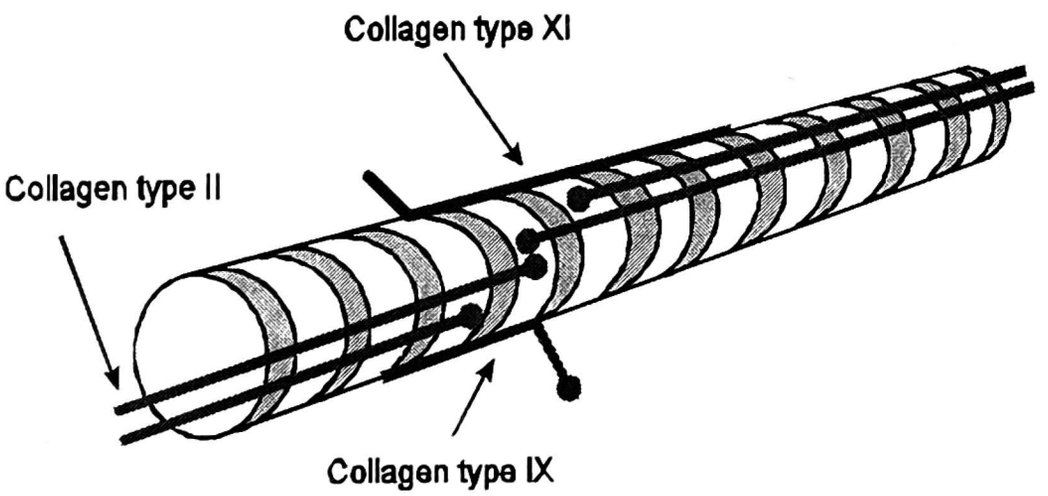

Figure 1. Collagenous components of the cartilage. Collagens provide the structural framework for hyaline cartilage. Collagen type II forms fibrils that contain small amounts of collagens IX and XI

Collagen type XVI and type XIX share some similarities in structure. Collagen type XIX is expressed in brain, eye and testis (MAYNE, BREWTON 1993).

Collagen type VI is unique because this protein forms a structure of beaded filaments, which were found in most connective tissues (KIELTY et al. 1993).

The anchoring fibrils that link basement membranes to the underlying extracellular matrix contain **collagen type VII.** Collagen type VII is expressed in numerous external tissues such as skin, oral mucosa and cervix. This large collagen is encoded by 118 exons (CHRISTIANO et al. 1994).

Collagens type XIII and XVII are classified as transmembrane collagens (MACITs) because they have the transmembrane domain, suggesting that these proteins are not secreted into the extracellular matrix. Collagen type XIII is expressed in a wide variety of tissues. Collagen type XVII is expressed in hemidesmosomes of the skin (PIHLAJANIEMI, REHN 1995).

The MULTIPLEXINs are called **collagens XV and XVIII.** Both collagens consist of the multiple triple-helical domains and the non-collagenous interruptions. They are distributed in a wide variety of tissues. Collagen type XV is expressed predominantly in the adrenal gland, kidney and pancreas and collagen XVIII in the liver, kidney and placenta (PROCKOP, KIVIRIKKO 1995).

Clinical consequences of mutations in the collagen genes

Diseases caused by mutations in some genes encoding the fibrillar collagens

Disorders caused by defects of collagens type I and II belong to the osteochondrodysplasias group. The osteodysplasias are characterized by bone abnormalities resulting from defects in collagen type I. The chondrodysplasias feature abnormal skeletal formation and linear growth. Abnormalities of collagen type II are mostly found in this category (HORTON, HECHT 1993).

The mutations detected in the genes encoding collagens type I and type II present a wide spectrum: single base-pair substitution in the codons for obligatory glycine, point mutations in C-terminal domain that interfere with molecular assembly, deletions and insertions, null allele mutations and mutations in splicing consensus sequences.

Osteogenesis imperfecta (OI) is a generalized disorder of connective tissue characterized by an increased fragility of bones. OI is also manifested in other tissues containing collagen type I, by blue sclera, hearing loss, dentinogenesis imperfecta (DI), hyperextensible joints, hernias and easy bruising. The degree of bone fragility and the presence of the other symptoms depend on OI subtype presented by the patient. The most severe forms of OI reveal bones and other tissues so fragile that death occurs in the uterus or shortly after birth (PROCKOP, KIVIRIKKO 1995).

OI results from the mutations in one of the two genes (*COL1A1* and *COL1A2*) for the type I procollagen. Most frequent are single base substitutions that convert a codon for an obligatory glycine, in the region of the -Gly-X-Y- repeats of the central triple-helical domain, to a codon for an amino acid with a bulkier side chain (KUIVANIEMI et al. 1991).

In 1991, SPOTILA et al. described the heterozygous mutation in the *COL1A2* gene that converted the Gly^{619} to Ser in a 52-year-old woman with severe postmenopausal **osteoporosis**. The patient also presented other features, such as slightly blue sclerae and mild hearing loss. Other mutations in the *COL1A1* and

COL1A2 genes were detected in patients with premenopausal osteoporosis and premature osteoporosis (SHAPIRO et al. 1992). The linkage analysis of many families with autosomal dominant osteopenia excluded involvement of the type I collagen gene and the vitamin D receptor gene in the pathomechanism of this disorder.

Chondrodysplasias form a heterogenous group of inherited disorders affecting the growing cartilage. The majority of chondrodysplasias are caused by mutations in various types of collagen genes. The classification of chondrodysplasias is based on physical appearance, age of onset, mode of inheritance and radiographic findings. Chondrodysplasias are rather rare, and their nomenclature is quite confusing, since more than 150 different disorders have been defined. Collagenopathies type II comprise some chondrodysplasias, consisting of hereditary disorders caused by defects in collagen type II. The following disorders are classified as collagenopathies type II: achondrogenesis type II, hypochondrogenesis, spondyloepiphyseal dysplasia congenita, spondyloepimetaphyseal dysplasia congenita, Stickler syndrome, Marshall syndrome and osteoarthritis (SPRANGER 1994, VIKKULA et al.1994, HORTON et al. 1996).

Achondrogenesis type II is a perinatally lethal skeletal dysplasia characterized by extremely short limbs, large head, short trunk, barrel-shaped chest, micrognathia (one or both jaws are unusually small), cleft palate and low nasal bridge. Radiographs show ossification deficiencies in tubular bones, the spine and the pelvis (EYRE et al. 1986, HORTON, HECHT 1993). The manifestation of **hypochondrogenesis** is similar to achondrogenesis type II, but the symptoms are less severe. Affected individuals may survive for several months, but usually they die after birth. Achondrogenesis and hypochondrogenesis are caused by substitutions that convert glycine in the central triple-helical domain.

Spondyloepiphyseal dysplasia (SEDC) is a heterogeneous group of disorders affecting the spine and epiphyses. If the methaphyses are also affected, the term **spondyloepimetaphyseal dysplasia (SEMDC)** is used. SEDC is manifested by a short trunk, a barrel-shaped chest, short limbs and a short neck. Radiological examination of the spine shows ossification deficiency, platyspondyly (flattening of the vertebral bodies) and hypoplasia of the odontoid process. A delayed ossification of pelvic bones and hip joints is observed. The phenotype of SEDC and SEMDC varies from a severely to a slightly shortened stature with an early-onset degenerative osteoarthritis. The mutations detected in the patients with SEDC and SEMDC are substitutions that convert glycine to a bulkier amino acid in the central, triple-helical domain of α1(II) chain, a substitution that converts arginin to cystein, RNA splicing mutations, in-frame deletions or in-frame insertions (KUIVANIEMI et al. 1991, VIKKULA et al. 1994). In patients from the Polish population, we found a novel glycine to arginine substitution in the triple-helical domain of α1(II) chain. The mutation was detected in a patient with spondyloepimetaphyseal dysplasia congenita (data not published). Till now, only

a few mutations have been detected in patients with SEMDC, probably because of the low incidence of this disease.

Stickler syndrome, also known as hereditary progressive arthro-ophthalmopathy, is characterized by high myopia (short-sightedness), vitreoretinal degeneration and frequently retinal detachments and cataracts. Other features are midfacial hypoplasia, micrognathia, cleft palate and sensorineural hearing defect. The skeletal abnormalities are manifested by an irregularity of the vertebral bodies, mild epiphyseal dysplasia, overtubulation of long bones, juvenile progressive arthropathy and laxity of joints. So far, nine mutations have been identified in the *COL2A1* gene in the patients with Stickler syndrome and all the mutations led to a premature termination of translation (AHMAD et al. 1991). The linkage analysis excluded the *COL2A1* gene in several families with Stickler syndrome. Therefore, the original Stickler syndrome is now classified as Stickler syndrome type I with severe ocular symptoms and linkage to *COL2A1* locus, and as Stickler syndrome type II with lack of the ocular symptoms and linkage to loci other than *COL2A1*. In 1996, RICHARDS et al. demonstrated the linkage of Stickler syndrome to the *COL11A1* gene encoding the α1(XI) chain of collagen XI. In 1998, SIRKO-OSADSA et al. detected mutations in the *COL11A2* gene in patients with Stickler syndrome type II.

Marshall syndrome shares many similarities with Stickler syndrome. The ocular, facial and auditory features are similar in both syndromes, but short stature, intracranial ossification, abnormal frontal sinuses and thickened calvaria are unique to Marshall syndrome. In 1997, the linkage of Marshall syndrome to the *COL2A1* and *COL11A1* genes was hypothesised. This hypothesis was confirmed in 1998 by GRIFFITH et al., by the identification of a splicing mutation in the *COL11A1* gene, that led to a deletion of 18 amino acids in the region of the triple-helical domain of the α1 (XI) chain. The mutation co-segregated with Marshall syndrome.

The latest studies of the patients with Marshall syndrome and Stickler syndrome led to the identification of fifteen novel mutations in the *COL11A1* gene. Ten of the novel mutations were detected in patients with Marshall syndrome, five in patients with overlapping Marshall and Stickler phenotypes (ANNUNEN et al. 1999).

The mild form of collagenopathy type II is **osteoarthritis (OA)**. Osteoarthritis is manifested by joint pain and tenderness, limited range of motion, stiffness and articular crepitus. Radiological examination revealed narrowing of the joint space, formation of subchondral cysts, subchondral bone sclerosis and osteophytes. Osteoarthritis is classified as primary when the predisposing factors are unknown, while the secondary form of OA is caused usually by infections, trauma, obesity, metabolic disease or joint disorders. Primary osteoarthritis is caused by mutations in the *COL2A1* gene (ALA-KOKKO et al. 1990, BLEASEL et al. 1998). The mutations convert arginine to cysteine, the amino acid that is not present in the central triple-helical domains of collagens.

Mutations in the *COL3A1* gene cause **Ehlers-Danlos syndrome** type IV (EDS IV). The patients with EDS IV can die from a rupture of large arteries and other hollow organs. Ehlers-Danlos syndrome type IV is also manifested by skin and joint abnormalities, for example, cigarette paper-like scarring of the skin, large ecchymoses over bony protuberances and thin skin. This severe form of EDS is caused by mutations that convert glycine to bulkier amino acids, RNA splicing mutations and large deletions (KUIVANIEMI et al. 1991).

The subset of patients with **arterial aneurysms** and **intracranial aneurysms** have mutations in the *COL3A1* gene (KONTUSAARII et al. 1990, KUIVANIEMI et al. 1991).

Diseases caused by mutations in some genes encoding the non-fibrillar collagens

Mutations in the genes *COL4A3*, *COL4A4*, *COL4A5* and *COL4A6* encoding collagen type IV cause **Alport syndrome**, a hereditary glomerular nephritis, characterized by progressive loss of kidney function and loss of hearing. The mutations include substitutions, large deletions, inversions, insertions, and duplications (LEMMINK et al. 1997).

Dystrophic form of epidermolysis bullosa is caused by mutations in the *COL7A1* gene encoding collagen type VII. The manifestations of this disorder include severe blistering and scarring of the skin from a minor trauma. Histological analysis of the skin in patients with epidermolysis bullosa shows that the anchoring fibril linking the basement membrane to the anchoring plaques in the skin are reduced or completely absent (CHRISTIANO et al. 1994).

About 15 mutations have been found in the *COL10A1* gene, encoding collagen type X, in patients with **Schmidt metaphyseal chondrodysplasia**. The typical features of this disease are shortness of stature, shortening of tubular bones and tibial bowing (varus deformity). The mutations were identified in the region encoding the C-terminal noncollagenous domain of the protein (DHARMAVARAM et al.1994).

Conclusions

The classification of collagens in relation to their encoding genes requires further detailed studies at the molecular level. Current results indicate that particular diseases, caused by defects of collagen structure, are genetically heterogenous. Molecular studies of collagen genes revealed that mutations in the same gene can be manifested by a various phenotypes. By contrast, we have observed that one phenotype can be caused by mutations in various collagen genes.

The molecular analysis of this important group of proteins has significant practical aspects. Since collagenopathies are usually diseases with a severe course,

predominantly affecting bone and cartilage, genetic counselling is of great importance. Recognizing the molecular background of collagenopathies can help in a proper diagnosis and, as a result, in an improvement of genetic counselling for the families at risk.

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