Review article

Genetic disorders of collagen

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SUMMARY   Osteogenesis imperfecta, Ehlers-Danlos syndrome, and Marfan syndrome form a group of genetic disorders of connective tissue. These disorders exhibit remarkable clinical heterogeneity which reflects their underlying biochemical and molecular differences. Defects in collagen types I and III have been found in all three syndromes.

The term 'collagen disease' should be confined to a small group of disorders caused by structural or metabolic defects of collagen. Our understanding of the pathobiology of the genetic collagen disorders has been slow in its evolution, particularly when compared to other genetic disorders like the haemoglobinopathies. This is partly due to the biological complexity of the connective tissues and partly to our limited knowledge of the various structural, functional, and genetic features of these proteins. To a certain extent knowledge of the latter has been increased by several fundamental findings, such as the discovery of the tissue heterogeneity of collagens, the elucidation of the numerous biosynthetic steps leading to collagen fibrillogenesis, and last, but not least important, by the isolation and characterisation of several human collagen genes. In this review we will cover basic aspects of the biochemistry and molecular biology of the human collagen system and discuss findings from some well documented variants of heritable disorders of collagen. We will also attempt to relate this basic information to the practice of clinical genetics.

Collagen: the biochemistry

Tissue specific expression of different collagen types is partly responsible for the unique physical properties of the various connective tissues. To date, at least 10 different collagen types, whose subunits are

encoded by approximately 20 distinct genes, have been identified.[1 2] While very little is known about the contribution of most of these proteins to the structural integrity of the extracellular matrix, this role is quite obvious for the so-called fibrillar collagens, types I, II, and III. The designation fibrillar is derived from the type of supramolecular aggregates (fibrils) they form in several connective tissues, such as tendon, ligaments, skin, cartilage, and bone.[3] Four distinct genes encode the subunits of the three fibrillar collagens (table). Typically, a fibrillar collagen is synthesised as a precursor molecule (preprocollagen) which comprises a short signal peptide, two terminal (carboxy and amino) propeptides, and a central α chain segment (fig 1). The structure of the central segment, also known as the triple helical domain, is characterised by the presence of a glycine residue in every third position.

TABLE 1   Chromosomal assignment of human collagen genes.[4]

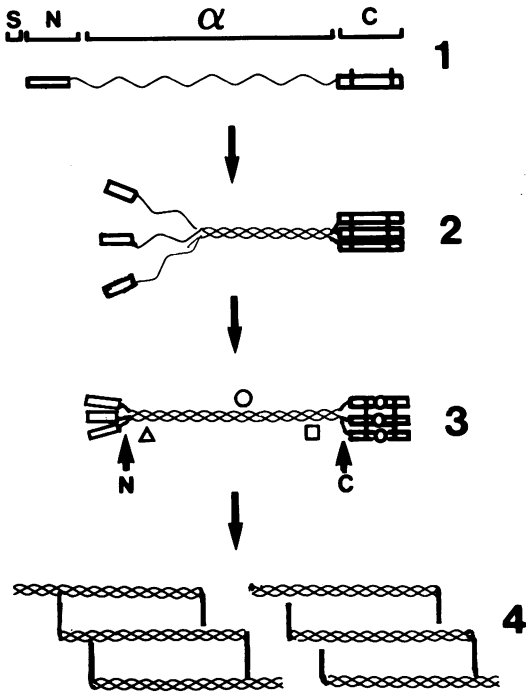| Gene	Region	RFLPs and allele (+) frequency
1 COL1A1 [proα1(I)]	17q2·1→17q2·2	MspI, 0·77 RsaI, 0·86
2 COL1A2 [proα2(I)]	7q2·1→7q2·2	EcoRI, 0·34 RsaI, 0·68 MspI, 0·86 StuI, 0·92
3 COL2A1 [proα1(II)]	12q1·31→12q1·32	HindIII, 0·34 PvuII, 0·55 3'f△ Variable in different ethnic groups
4 COL3A1 [proα1(III)]	2q3·1→2q3·23	EcoRI, 0·61 AvaII, 0·26
5 COL4A1 [proα1(IV)]	13q3·4	—
6 COL5A2 [proα2(V)]	2q2·4→2q3·1	—

FIG 1 *Schematic representation of fibrillogenesis. (1) Preprocollagen molecule with indication of the various protein domains: S=signal peptide, N=N-propeptide, α=α chain, C=C-propeptide. (2) Triple helix formation. (3) Post-translational modifications (circles, squares, and triangle) and removal of terminal propeptides (N and C). (4) Self-assembly into fibrils.*

The amino acid sequence of the α chains, which are 1000 amino acids long, is therefore commonly represented as $(Gly-X-Y)_{333}$.[3] Many of the X positions in this sequence are occupied by the rigid ring amino acid proline. Many of the Y positions are occupied by the analogous ring amino acid 4-hydroxyproline. The remaining X and Y positions are largely occupied by charged and hydrophobic amino acids.[5] Eventually, identical or similar chains assemble together to form a homo- or hetero-trimer, respectively. The C-terminal propeptide plays a fundamental role in this initial biosynthetic step by registering the three α chains through the formation of interchain disulphide bonds between the cysteynyl residues. The triple helical domain of the α chain, on the other hand, is necessary for the formation of the rope-like triple helix and the subsequent extracellular self-assembly of the molecules into fibrils (fig 1).[5] The formation of stable triple helix requires: (1) the presence of the glycine residues in the restricted space where the three α

chains come together, (2) the presence of interactive hydrophobic and polar charged amino acid residues in the X and Y positions, which provide critical electrostatic and hydrophobic interactions and direct the self-assembly of collagen molecules into a quarter staggered array, and (3) the presence of proline and hydroxyproline residues which confer stability to the triple helical structure.[5] Unlike the other two domains, the N-peptides do not interact with each other, thus exhibiting a high degree of diversity of structure and length. Besides containing the signal peptide, common to most secretory proteins, the N-propeptide, in conjunction with the C-terminal counterpart, prevents intracellular fibrillogenesis and possibly regulates at various levels the process of fibril formation. As the nascent polypeptides appear in the lumen of the rough endoplasmic reticulum, the post-translational modifications of the molecules begin to take place: hydroxylation of the proline and lysine residues, glycosylation of the hydroxylysine residues, and glycosylation of the C-propeptides. These intracellular modifications occur while the pro α chains assemble through the establishment of disulphide bonds in the C-propeptides. Three important post-translational modifications seem to occur extracellularly: removal of the N- and C-propeptides mediated by specific endopeptidases and the oxidation of lysine residues by the enzyme lysyl oxidase.[5] The fibrillogenesis appears to occur spontaneously.

Collagen: the genes

The fibrillar collagens are encoded by single copy genes whose coding sequences are fragmented into 52 units (exons),[6] resulting in genes which span between 18 and 40 kilobases (kb) of chromosomal DNA (fig 2).[7-10] Such an extremely complex structure has raised several important questions about the evolution of the collagens. It is now clear that the introns of the fibrillar collagen genes, regardless of their size, are located in identical positions. This implies that the four genes originated from a primordial multi-exon structure indentical to that observed at present. All the fibrillar collagen genes are single copy in the human genome. The numerous exons of the triple helical domain are all related to a basic 54 base pair unit that codes for 18 amino acid residues or six in-phase triplets of Gly-X-Y repeats. This observation led to the hypothesis that this segment of the collagen genes evolved by tandem duplication of an ancestral 54 base pair unit. Since the same gene structure is not seen in the non-fibrillar collagens, the functional significance of this 18 amino acid coding unit
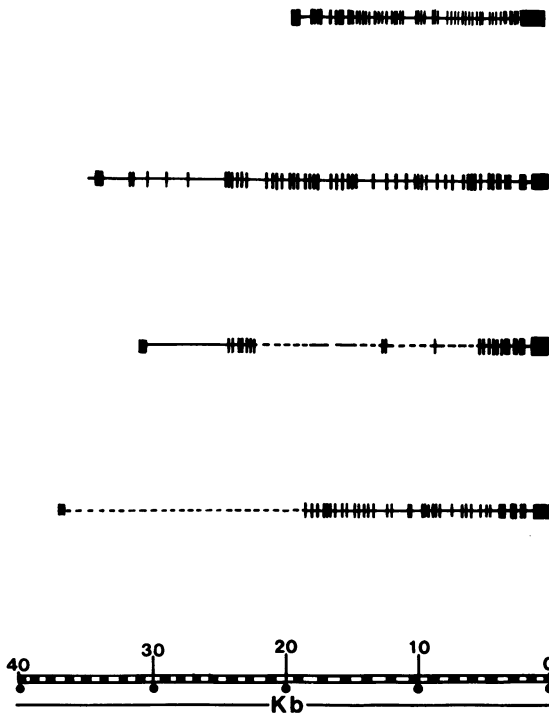
FIG 2 *Exon-intron arrangement of the fibrillar collagen genes. From top to bottom: COL1A1, COL1A2, COL2A1, and COL3A1.*

remains to be elucidated, as does the evolutionary reason that determined the final number of duplications. To some extent the study of collagen mutations resulting in pathological phenotypes has shed new light on these and other structural as well as biosynthetic features of the fibrillar collagens.

Connective tissue disorders include a large variety of syndromes with heterogeneous and sometimes overlapping modes of expression. Abnormalities in the structure and in the biosynthesis of the fibrillar collagens have been associated with osteogenesis imperfecta (OI), Marfan syndrome, Ehlers-Danlos syndromes (EDS), and possibly the chondrodystrophies. In the following sections we will attempt to discuss some mutations of collagen types I and III in relation to specific clinical phenotypes and show how genetic linkage can be used in the study of these disorders.

Osteogenesis imperfecta

The term osteogenesis imperfecta describes a group of disorders which are highly heterogeneous in their phenotypic manifestations.[11] Bone fragility is the cardinal clinical manifestation of the OI syndromes.

In addition, short stature, joint laxity, easy bruising, blue sclerae, presenile hearing loss, and dentinogenesis imperfecta may also be present. This phenotypic diversity has prompted numerous attempts at classification, the most recent and widely accepted one having been proposed by Sillence *et al*.[11] Accordingly, OI is divided into four phenotypic groups, although many affected subjects do not fit such rigid categorisation.

OI type I is inherited as an autosomal dominant trait and is characterised by postnatal onset of fractures, blue sclerae, joint laxity, and, in some cases, by hearing loss and dentinogenesis imperfecta. Barsh *et al*[12] have reported decreased synthesis of proα1(I) chains intracellularly, which results in decreased synthesis of mature triple helical type I collagen molecules. The synthesised proα1(I) chains are approximately 50% of the normal amount and this observation correlates with the finding that the proα1(I) to proα2(I) mRNA ratio in the three cell lines studied is 1:1, rather than the expected 2:1.[13] An example of an amino acid substitution in an X or possibly Y position of the proα1(I) chain has been provided by Nicholls *et al*.[14] Cultured fibroblasts from a patient with OI type I synthesised in addition to the normal α1(I) chain, an extra disulphide linked dimer, which could be reduced to monomeric form by mercaptoethanol. The authors concluded that this mutation resulted in the presence of cysteine, an amino acid not normally found in the triple helical domain of either the proα1(I) or the proα2(I) chains.

OI type II is the lethal perinatal type, which is characterised by extreme bone fragility resulting in death in utero or shortly after birth. It has been suggested that this type of OI is inherited as an autosomal recessive trait but recent clinical,[15][16] genetic,[15][17] biochemical,[18–22] and molecular studies[23][24] have shown that this particular OI phenotype has both recessive and dominant forms, the latter representing new mutations. Since extensive studies have been performed in cultured fibroblasts from a number of subjects with OI type II we will refer to some of them in detail. Defects in both the proα1(I) and proα2(I) chains have been shown in variants with OI type II. Furthermore, both structural rearrangements (insertions and deletions) and amino acid substitutions have been described.[18][19] The most extensively studied variant with OI type II (CRL-1262) has been found to be heterozygous for an internal deletion of approximately 0·5 kb in the proα1(I) procollagen gene.[23] This observation is consistent with previous findings which indicated that the proband's cultured fibroblasts synthesised two different species of proα1(I) chains, a normal and a shortened one.[20][21] These

investigations also showed that the amount of type I procollagen secreted was substantially reduced. Using S1 nuclease mapping experiments, Chu *et al*[23] located this proα1(I) deletion between amino acid residues 325 and 410. More recently the same authors, by cloning and sequencing the mutant proα1(I) allele, showed that the deletion (643 bp long) is contained between two introns of the triple helical domain and that it eliminated exons 27, 28, and 29 (aa 411–328). Interestingly, the breakpoints of the rearrangement were found to be located in two almost perfect inverted repeats (AGAGCCACA– TGTGGCCACT). This observation strongly suggests that the self-complementary nature of the quasipalindromic sequence may have favoured the formation of a secondary structure in turn used as substrate for the deletion.[25]

Another OI type II variant has been reported recently where disulphide bonded α1(I) dimers have been found in the pepsin extracted dermis collagen. A Gly→Cys substitution in position 988[22][24] in the cyanogen bromide peptide α1(I)-CB6 (aa 816–1014) resulted in an interruption of the (Gly-X-Y) triplet periodicity and subsequent impairment of triple helix formation. Biochemical studies on the parents' cultured cell fibroblasts did not reveal any abnormalities, although it should be noted that one parent has Marfan syndrome.[22] It can, therefore, be assumed that the OI phenotype is due to a spontaneous mutation in the COL1A1 gene. The two OI variants discussed share a common pathogenetic mechanism whereby 75% of the triple helical molecules are virtually non-functional since they cannot form a stable triple helix and subsequently cannot be secreted.

OI type III is also heterogeneous and is clinically characterised by significant bone fragility with fractures frequently occurring in utero, short stature, and usually significant skeletal deformity, dentinogenesis imperfecta, hearing loss, and blue sclerae. In the majority of families studied, the proband is the only affected subject. Autosomal recessive inheritance has also been suggested[11] and documented.[26] Biochemical studies in fibroblasts derived from a patient with OI type III showed secretion of type I procollagen molecules consisting of proα2(I) chains intracellularly.[27] Northern blot hybridisation showed that the patient's fibroblasts contained normal levels of proα2(I) mRNA, efficiently translatable in a heterologous cell free system.[28] A combination of nuclease S1 mapping and the cloning and sequencing of the mutant allele showed a four base pair frame shift deletion resulting in a complete alteration of the codon phase for the last 34 amino acids of the C-propeptide of the proα2(I) chain.[26][29] In addition, the deletion elimin-

ated the last cysteine in the C-propeptide. This observation dramatically demonstrates the importance of the interchain disulphide bonds in the formation of the triple helix. The mutation was inherited as an autosomal recessive trait from the parents, who were third cousins and phenotypically normal.

OI type IV is inherited as an autosomal dominant trait and is characterised by mild to moderate bone fragility, usually postnatal onset of fractures, short stature, white sclerae, hearing loss, and dentinogenesis imperfecta.[11][30] Tsipouras *et al*,[31] using a restriction fragment length polymorphism (RFLP) associated with the COL1A2 gene, showed cosegregation of a RFLP allele with the OI phenotype. This suggested that the abnormal phenotype was due to a mutation in the COL1A2 gene. Further studies from cultured skin fibroblasts from two affected subjects of that family showed a small (approximately 10 amino acid) deletion in the amino terminal end of the α2(I)-CB3,5 peptide between amino acid residues 402 and 550.[32–34]

Marfan syndrome

Marfan syndrome is now recognised as a group of clinical phenotypes with overlapping manifestations characterised by arachnodactyly, dolichostenomelia, pectus deformities, scoliosis, aortic root dilatation, mitral valve prolapse, myopia, and lens dislocation or subluxation. Congenital contractural arachnodactyly and the Marfanoid hypermobility syndrome have been suggested as allelic forms of Marfan syndrome.[35] In one case, the phenotype has been linked to a structural defect of type I collagen, namely a 20 amino acid in frame insertion of 50% of the proα2(I) chains, located within the α2(I)-CB4 peptide. This defect seems to interfere with collagen crosslinking by shifting the normal registering of the procollagen chains.[36] A decrease in non-reducible cross links and increased extractability of skin collagen from subjects with Marfan syndrome has been reported by Boucek *et al*.[37] This may be secondary to a defect(s) in the primary structure of either of the proα2(I) collagen chains. Elastin has also been implicated in the aetiology of Marfan syndrome. Increased urinary excretion of desmosine, an amino acid cross link unique to elastin, and decrease of desmo- and iso-desmosine in elastin extracted from aortic tissue have been shown by Abraham *et al*.[38] Elastin and collagen are closely related in the aorta and it is possible that a mutation which alters the structure of either of the two macromolecules could change the morphology or function of one or the other.

Tsipouras *et al*[39] used RFLPs associated with the

COL1A2 gene to study a large, three generation family with Marfan syndrome. Genetic linkage to the COL1A2 gene was excluded, providing further evidence about the genetic heterogeneity of this condition.

Ehlers-Danlos syndrome

Ehlers-Danlos syndrome appears to be an even more heterogeneous group of disorders than OI. The clinical manifestations of these disorders include laxity of joints, soft and extensible skin which may also be fragile, and excessive bruising. The syndrome has been classified into at least 11 clinical types.[40] The most severe form of EDS is clearly type IV. It is transmitted as an autosomal dominant or autosomal recessive trait.[40] Patients with this disease have minimal joint laxity and very thin transparent skin. The most dramatic manifestation is the spontaneous rupture of large vessels and hollow organs such as the intestines. Biochemical studies in a number of variants show decreased type III procollagen secreted into the medium.[41] This may be due to a structural alteration in the proα1(III) chains interfering in the triple helix formation. Evidence for a structural alteration in the proα1(III) chain was recently obtained from one variant of EDS type IV, in which the rate of secretion of type III procollagen into the cultured fibroblasts was about normal.[42] However, the proα1(III) chains were found to consist of both the normal species and also of another species which migrated more slowly on polyacrylamide gel. The peptide pattern obtained after digestion of the proα1(III) chains with CNBr is suggestive of a small peptide insertion. Studies using COL3A1 associated RFLPs in two families with an autosomal dominant form of Ehlers-Danlos type IV syndrome showed linkage of the phenotype to a RFLP allele. Biochemical studies in cultured fibroblasts from affected subjects from the two families showed defects in the synthesis and secretion of type III collagen.[43]

Ehlers-Danlos syndrome type VII is characterised by short stature, extensible skin, generalised joint hypermobility, and multiple joint dislocations. Since in most families affected subjects represent sporadic cases, both autosomal dominant and autosomal recessive modes of inheritance are possible. Unprocessed type I N-procollagen accumulates extracellularly either because of a deficiency of the N-proteinase activity (EDS VIIA)[44] or because structural defects of type I procollagen impair the cleavage of the N-peptide by an otherwise normal N-proteinase (EDS VIIB).[45 46] In two EDS VIIB patients it was determined that the defects were due to a small deletion around the N-proteinase cleavage site of

either the proα1(I) or the proα2(I) chains.[40 41] In a third EDS VIIB patient, a structural alteration in the proα2(I) N-proteinase cleavage site was originally reported,[47] a finding not substantiated by our molecular analysis of this variant (F Ramirez, unpublished data). This apparent discrepancy could either be explained by an error in the original biochemical studies or by the possibility that procollagen processing depends on one additional criterion; that is, proper folding of the heterotrimer. In other words, it could be hypothesised that some mutations distantly located from the N-proteinase cleavage site may influence the processing of procollagen by changing the three dimensional structure of the substrate.

Discussion

This review provides details of the aetiology of some genetic disorders of connective tissue and emphasises the genetic heterogeneity observed in this group. Most of the information has been derived from analysis of osteogenesis imperfecta variants and therefore relates to the effect of different mutations on the biosynthesis of type I procollagen. However, given the structural homologies of the various fibrillar collagens and the genes encoding for them, some of the following conclusions could be assumed to apply in all fibrillar collagens.

(1) The biological consequences of defects in the C-peptide and the triple helical domain of the proα1(I) chain are significant. Defects in these two regions have been found in variants with lethal perinatal osteogenesis imperfecta (OI type II), severe osteogenesis imperfecta (OI type III), and one variant of Marfan syndrome. Two explanations can be offered. (a) The two regions play a key role in the formation of triple helix, the foremost requirement for normal fibrillogenesis. (b) Type I collagen is a heterotrimer containing two proα1(I) and one proα2(I) chains. Thus, a mutation in one proα1(I) allele would result in 75% of all type I collagen molecules being abnormal by virtue of carrying at least one altered proα1(I) chain. Defects affecting the N-peptide, on the other hand, result in a distinct pathological phenotype, Ehlers-Danlos syndrome type VII, regardless of the nature of the chain involved.

(2) The relative pools of normal and abnormal collagen molecules available for fibrillogenesis may be a determining factor for the clinical severity of a disorder. Thus, a 50% reduction in total normal type I collagen results in mild dominant osteogenesis imperfecta (OI type I), while defects in the proα2(I) chain with the subsequent extracellular presence of

both normal and abnormal molecules have been described in moderate to severe osteogenesis imperfecta (OI type IV).

Applications in the practice of clinical genetics

Our knowledge of the molecular and biochemical heterogeneity of the genetic disorders of connective tissue is having an impact on our understanding of their nature and our ability to provide more accurate genetic counselling and genotype diagnosis for persons at risk. Linkage studies using RFLPs associated with the various fibrillar collagen genes (table) can be used for the delineation of the genetic heterogeneity of these disorders, as in dominant osteogenesis imperfecta,[48] and also for the genotypic diagnosis of persons at risk in informative families. In a family with OI type IV linked to a COL1A2 allele, genotype analysis was performed in chorionic villus tissue from a fetus at risk for this condition (P Tsipouras, unpublished data). In two families with Ehlers-Danlos syndrome type IV genetic linkage was established to a COL3A1 allele facilitating the genotypic diagnosis in two infants at risk of having inherited the mutation resulting in EDS IV.[41] This condition will not manifest clinically until late childhood or early adolescence and this approach offers genotype diagnosis at the incipient stage of the disorder. Since biochemical diagnosis from cultured skin fibroblasts is not always feasible, linkage studies offer an alternative in certain families.

OI type II has long been held to be transmitted as an autosomal recessive trait with 25% recurrence risk. The study of a number of mutants from subjects affected with this condition has shown that the great majority result from heterozygous mutations in either the COL1A1 or the COL1A2 genes.[18] [19] It also appears that these heterozygous mutations occur de novo since biochemical studies in parental fibroblasts have failed to disclose any abnormality (P H Byers and P Tsipouras, unpublished data). Empirical recurrence risks derived from two studies ranged from 3 to 7%.[15] [17] It should be emphasised that an autosomal recessive variety of lethal perinatal OI may exist, but it presents with a distinct clinical and radiological phenotype.[15] [16] In order to facilitate genetic counselling of parents who have had a child with OI type II, an attempt should be made to obtain a skin biopsy from the proband for biochemical studies. If this is not possible, the parents should be told about the low recurrence risk of de novo mutations. If biochemical studies have been performed on the proband, an amniocentesis is warranted in addition to ultrasonographic monitoring. Amniocytes in culture synthesise type I procol-

lagen and defects in the proα1(I) or the proα2(I) chains could therefore be detected.[49] Otherwise, ultrasonographic monitoring should be offered in all subsequent pregnancies followed by fetal radiographs in case of positive findings. OI type II can be accurately diagnosed during the second trimester of pregnancy by ultrasound.[49]

In this review we have discussed only those disorders clearly linked to structural mutations of collagen genes. However, defects in various post-translational steps have been shown in other connective tissue disorders.[50] It is also possible to predict that even in disorders strictly considered to be caused by collagen abnormalities, future studies may determine the possible involvement of other gene products, thus greatly enhancing the already established heterogeneity of these syndromes.

Note added in proof

Sykes *et al* (*Lancet* 1986;i:69–72) used RFLPs associated with the COL1A1 and COL1A2 genes to demonstrate the genetic heterogeneity of the autosomal dominant osteogenesis imperfecta.

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