Collagen IV related disease and therapies

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List of Abbreviations

AS: Alport Syndrome

ADAS: Autosomal dominant Alport syndrome

ARAS : Autosomal recessive Alport syndrome

Arg: Arginine

Asp: Aspartic acid

BM: basement membrane

bp : base pairs

CSVD: cerebral small vessel disease

EC: endothelial cell

ECM: extracellular matrix

ER: endoplasmic reticulum

FDA: Food and Drug Administration

GBM: glomerular basement membrane

GP: Goodpasture syndrome

HANAC: hereditary angiopathy, nephropathy, aneurysm and cramps

IAC: integrin adhesion complexes

ICH: intracerebral haemorrhage

kB: kilo base pairs

kDa: kilodalton

miR: miRNA

nm: nanometer

P3H: prolyl3-hydroxylase

P4H : prolyl4-hydroxylase

PADMAL: Pontine autosomal dominant microangiopathy and leukoencephalopathy

PDI : protein disulfide isomerase

TBMN: Thin basement membrane nephropathy

TGFβ1: transforming growth factor beta 1

VR3: variable region 3

XLAS: X-linked Alport syndrome

4PBA: sodium 4-phenylbutyrate

Abstract

While traditionally the function of the extracellular matrix and the basement membrane was considered to be providing structural support, it is now clear that this only covers one aspect of its multiple functions. This is also illustrated by our growing knowledge of the role of collagen IV, a major component of basement membranes, in development, health and disease. With the extracellular matrix and collagen IV increasingly being recognised as key players in a growing number of diseases from stroke and vascular defects to kidney disease, deafness and eye abnormalities, it is paramount that we increase our fundamental understanding of these complex molecules ranging from their biosynthesis to their role in human disease. Recently, exciting progress has been made in delineating the mechanisms by which mutations in collagen IV cause disease, and these are being exploited to develop mechanism-based treatments. Yet many important questions remain that need addressing to develop treatments for diseases associated which collagen IV.

1. Introduction

The vertebrate genome encodes 28 types of collagen, which is the most abundant protein of the human body, of which type IV collagen is evolutionarily the most ancient [1-3]. Collagen IV is a major protein component found almost exclusively in the basement membrane (BM), constituting up to 50% of its protein mass, although recent evidence from Drosophila has proposed a non-BM mediated role in adipocyte adhesion [4].

The BM is a specialized sheet like structure in the extracellular matrix (ECM), present in almost all tissues. BMs underlie epithelial and endothelial cell layers, surround adipocytes [5, 6], neurons [7], skeletal muscle fibres [8], cardiomyocytes [9] and smooth muscle cells [10], and are present in the synaptic cleft of neuromuscular junctions [11, 12]. BMs have several important functions, including compartmentalizing tissues, providing structural support and modulating cell behaviour, signalling and tissue repair [13-15]. For a more in depth review on BM function, we direct the reader to [16, 17]. Major BM components are laminin, nidogen, the heparan sulfate proteoglycan perlecan, and collagen of which collagen IV is the major type [18]. In vertebrates laminin is required for initial BM assembly and collagen IV recruitment [19, 20] although recent elegant data from C. elegans showed this may not apply to all BMs [21]. Collagen IV is necessary for BM maintenance and nidogens act as a bridge between laminin and collagen IV [18, 20, 22]. Perlecan is the predominant proteoglycan and its deposition is dependent on collagen IV in at least some BMs [23].

Each BM has its own composition and combined with the interactions of these BM components with cells and other secreted proteins (e.g, growth factors), allows BMs to carry out their different functions. Thus, each BM is uniquely tailored. The importance of BM and collagen IV for biology and physiology is illustrated by the diseases associated with alterations in the BM and collagen IV. In general, these are rare severe multisystemic disorders for which treatments are urgently needed. However, it is also becoming clear that the BM and collagen IV play an important role in common forms of disease in the general population [24-31].

Here we will provide an overview of collagen IV biology and focus primarily on Mendelian disorders due to collagen IV mutations. Collagen IV also has been analysed in a wide spectrum of pathologies including fibrosis, diabetes, cardiomyopathy to name but a few. While some of these will be mentioned, these are not the focus of this review.

2. Collagen IV

In 1966 Dr. Nicholas Kefalides described collagen IV in vertebrates while studying extracted glomerular basement membranes from dogs as a glycoprotein with abnormal levels of hydroxyproline and hydroxylysine [32]. He and others subsequently identified the end of collagen IV protomers are not cleaved in contrast to fibrillary collagens [33, 34]. Since then collagen IV has remained the topic of intense research ranging from its fundamental biochemistry, gene and protein structure function, as well as its expanding role in pathophysiology and disease.

The transition from single cell organisms to multicellular tissues necessitated a cellular microenvironment [2, 35] which was provided by the ECM. The spongin variant of collagen IV was a primordial component [36] and enabled ECM assembly and genesis of multicellular tissues [1-3]. Ctenophora represent one of the earliest branching extant animal phyla in which a BM and collagen IV have been identified, and their features are comparable between non-bilaterian and bilateria animal phyla [2, 37-39]. In contrast, related unicellular groups do not encode collagen IV [2]. Remarkably, ctenophores express up to twenty collagen IV genes [2], compared to six in vertebrates, illustrating conservation and adaptation dating back >0.5 billion years [40, 41].

3. Gene organisation and regulation

In vertebrates three pairs of paralogous genes (*COL4A1, COL4A2, COL4A3, COL4A4, COL4A5* and *COL4A6*), encode the collagen IV alpha chains α1(IV)-α6(IV) (Figure 1).

The genes are arranged in a head-to-head configuration separated by a communal promoter and transcribed in opposite directions [42-47]. *COL4A1* is paired with *COL4A2*, *COL4A3* with

COL4A4, and *COL4A5* with *COL4A6* (Figure 1). The alpha chains interact to generate three collagen IV protomers, α1α1α2(IV), α3α4α5(IV) and α5α5α6(IV), that form the different collagen IV networks in the BM.

This genomic organisation is the result of three independent duplication events in evolution following the duplication and inversion of the ancestral *COL4A1* gene to give rise to *COL4A2* [46]. The next duplication resulted in the *COL4A3/COL4A4* pair with the final duplication generating the more closely related *COL4A5/COL4A6* [46]. Based on intron-exon structure and protein sequences the α-chains can be divided into the α1(IV) group containing α1(IV), α3(IV), and α5(IV), and the α2(IV) group with α2(IV), α4(IV) α6(IV) [48].

3.1 *COL4A1* and *COL4A2*

The *COL4A1* and *COL4A2* genes consist of 52 and 48 exons respectively, and are located in a ~370 kB locus on human chromosomal region 13q34 and mouse chromosome 8. Throughout the 1980s and 1990s experiments using plasmid expression systems provided the first insights into the regulation of *COL4A1* and *COL4A2* transcription. *COL4A1* and *COL4A2* are separated by a mere 127 nucleotides, in which there is a bi-directional promoter (Figure 1) [42, 49]. The promoter has a palindromic sequence with an A/T intense region 30 base pairs (bp) upstream from the transcription start site and accordingly lacks the usual TATA box [42, 49, 50]. The promoter contains three key elements, found also in other BM proteins: a CT box, a CCAAT box and GC box, that bind the transcription factors CTCBF, CCAAT binding protein and Sp1 respectively [42, 49, 51, 52] (Figure 1). The bi-directional promoter can be considered as a small region of two overlapping gene specific promoters [52] that are not transcriptionally active [42]. Transcription depends on gene specific enhancers in introns 1 of *COL4A1* and *COL4A2* that promote binding of CTCBF, CCAAT binding protein and Sp1 [42, 51, 52] in the short promoter [52]. These cis-acting enhancers act cooperatively and competitively [53], perhaps in part, by causing steric hindrance, enabling gene specific transcription. There is also a silencer in intron 3 of *COL4A2* that affects transcription of both

genes [53, 54] (Figure 1). These cis- and trans-acting elements enable a tightly controlled regulation of transcription with different levels of transcriptional activity of *COL4A1 versus COL4A2*. This correlates with a ~2:1 ratio of *COL4A1 versus COL4A2* mRNA levels reflecting α1α1α2(IV) composition [53, 55]. Collagen IV is also transcriptionally regulated by members of the Smad family (including Smad1 and Smad2) and DDR1 [56-58], but their binding sites remain poorly defined.

COL4A1/*COL4A2* expression is also regulated post-transcriptionally including by binding of micro-RNAs (miR) to the 3'UTR of *COL4A1* including miR-29 [59, 60]. The miR-29 family consists of miR-29a, b, and c. *In vitro COL4A1* mRNA levels were regulated by miR-29c [61], which is also active in endothelial cells [62]. However, our knowledge regarding the post-transcriptional regulation of collagen IV expression by non-coding RNAs remains poor.

3.2 *COL4A3* and *COL4A4*

The *COL4A3* and *COL4A4* genes are located on human chromosomal region 2q36, and in mice on chromosome 1 [63, 64]. They are located in a ~314 kB locus, are transcribed in opposite direction and separated by a 164 bp bi-directional promoter (Figure 1). Similar to *COL4A1/COL4A2,* the promoter contains a CpG island, GC boxes, CTC boxes and a CCAAT box, and no TATA box (Figure 1). *COL4A3* and *COL4A4* compromise of 52 and 48 exons respectively but two alternative first exons have been described for *COL4A4*, which can give rise to two *COL4A4* isoforms [47].

3.3 *COL4A5* and *COL4A6*

The *COL4A5* and *COL4A6* genes consist of 51 and 46 exons respectively and are found on the X chromosome within a single ~510 kB locus in humans (Xq22) and mice [46, 65, 66]. Their promoter is more complex and larger (~1400 bp) than for *COL4A1/COL4A2* and *COL4A3/COL4A4*, as *COL4A6* has two promoters (Figure 1). This enables a tissue specific *COL4A6* expression including divergent expression from *COL4A5* [67, 68]. The two promoters lead to usage of two transcription start sites generating two alternative transcripts with different

first *COL4A6* exons, and two α6(IV) isoforms with different signal peptides [45, 68, 69]. As for the other gene pairs, the promoter has characteristics of those for housekeeping genes including the absence of the TATA box and presence of CCAAT, CTC and GC boxes (Figure 1).

It should however be noted, that in part due to the complex organisation of the different loci and the large size of the introns, our knowledge of the regulation of gene expression at the transcriptional level remains incomplete.

4. Collagen IV protein domain structure and biosynthesis

Seminal rotary shadowing electron microscopy experiments revealed that collagen IV proteins have a rod-like structure with a length of ~400 nm in which a central triple helical collagen domain of around ~330 nm is book-ended by two globular domains that, in contrast to fibrillar collagens, remain attached in the mature protein [70] (Figure 2). Collagen IV α-chains have similar domain structures and 50–70% homology at the amino acid level. Each α-chain, a rope like structure, consists of a short N-terminal ~120 amino acid 7S domain, a central collagenous triple helical domain (~1400 residues), and a C-terminal globular non-collagenous (NC) 1 domain (~230 residues) (Figure 2) [70-75]. The collagen domain, the defining feature of all collagens, is made up of a 3 amino acid repeat (Gly-Xaa-Yaa) in which every third residue is a glycine, and Xaa and Yaa can be any residue. Each chain also has functional subdomains and potential binding sites for interacting proteins [75]. The NC1 domains contain a cryptic peptide that can be released upon proteolytic cleavage. Following the initial identification of the anti-angiogenic fragment tumstatin from α3(IV) [76], it became apparent that similar anti-angiogenic fragments (24-28 kDa) can be released from the other α-chains [77]: arresten from α1(IV), canstatin from α2(IV), tetrastatin from α4(IV), pentastatin from α5(IV), and hexastatin from α6(IV) [77-83].

In contrast to archetypical fibrillar collagens, the collagen domain contains 21–26 interruptions of the Gly-Xaa-Yaa triplet repeat [44, 46 Parkin, 2011 #202, 84-88]. It has been proposed that

all collagens have evolved from a single 54 bp ancestral exon and that alternative splicing leading to incorporation of intronic sequences has generated these interruptions to the Gly-Xaa-Yaa repeat [89, 90]. The interruptions vary in length from 1 to 24 nucleotides and while their sequence is relatively poorly conserved between α-chains, their positions are highly conserved [91]. These interruptions provide flexibility to collagen IV to form a network, while also enabling proteolytic cleavage of the triple helix or serve as cell binding sites [73]. The interruptions have also been proposed to strengthen lateral association between chains by enabling intra-chain crosslinking [92-94].

As with all secreted proteins, during translation the nascent polypeptide is translocated into the endoplasmic reticulum (ER) *via* transient signal peptides. In the ER, three collagen IV α-chains interact to form a triple helical molecule called a protomer. Six different chains can theoretically form 56 different combinations of triple helices, however only three heterotrimers exist: α1α1α2(IV), α3α4α5(IV) and α5α5α6(IV). The formation of protomers begins at the C-terminal NC1 domain and in a zip like fashion the α-chains wrap around each other to form a triple helix through to the N-terminal 7S domain. Following protomer folding and secretion in ECM, protomers self-assemble into a three-dimensional network (Figure 2).

4.1 NC1 domains and initiation of protomer folding

The collagen IV NC1 domain is a globular ~13 nm domain [70], ~229 amino acid residues for α1(IV) and α2(IV), that is highly conserved in evolution (~95% identity) and between α-chains [44, 95-97]. The first stage of protomer formation is the interaction between NC1 domains of three α-chains [98]. The 12 cysteine residues within the NC1 domains from intra- and inter-chain disulphide bonds made by protein disulfide isomerase (PDI). The intrachain bonds form a knot to give a compact NC1 domain structure similar to a 4 leaf clover [99]. The interchain bisulfide bonds are important for alignment of the three α-chains before triple helix formation. Recognition motifs within the NC1 domain of an α-chain establishes protomer composition by selecting which α-chain it will bind to. This includes domain swapping interactions whereby each domain extends a β-hairpin motif into a corresponding docking site located in the variable

region 3 (VR3) of the NC1 domain of the adjacent α-chain [100]. Non-covalent interactions occur between the VR3 and the β-hairpin that determine chain specificity. For example, the α2(IV) chain β-hairpin loop binds to the α1(IV) VR3 to initiate α1α1α2(IV) formation (Sundaramoorty et al 2002; Than et al 2002) whereby the reduced affinity of α2(IV) for α2(IV), as compared to α1(IV), drives the 2:1 α-chain composition [100]. For α3α4α5(IV) and α5α5α6(IV) this is driven by α4(IV) and α6(IV) respectively [100, 101].

The NC1 domain also prevents inappropriate intracellular cross linking/network formation and initiates extracellular network assembly. This is related to the difference in chloride (Cl^-) concentration between inside the cell (~12 mM) and the ECM (~100 mM) [102]. The low intracellular Cl^- concentration leads to the formation of an intrachain salt bridge between residue Arg76 and Asp78 (and to a lesser extent Glu40) that blocks protomer oligomerisation [103]. Once exposed to the higher extracellular Cl^- concentration the salt bridge destabilises to enable initiation of network formation in the ECM [103].

4.2 Post-translational modification of collagen IV α-chains

The accurate intracellular assembly and folding of collagens requires a large set of proteins and much of what we know has been obtained through analysis of fibrillar collagens (see also Chapter 2 in this book by Sergey Leikin and co-authors). Over the years, excellent reviews [104-106] have covered this topic in depth and we will provide a brief collagen IV orientated overview. It should be noted that much of the folding and secretion of collagen IV remains unclear, and this represents a knowledge gap that needs to be addressed to increase our fundamental understanding of biology and diseases in which collagen IV is implicated.

Multiple enzymes, co-factors and chaperones are implicated in the successful assembly and secretion of collagen IV. Following the interaction between NC1 domains, triple helix formation occurs which is dependent on the Gly-Xaa-Yaa repeat. This protein folding requires post-translational modification of the single unfolded α-chains (Figure 3). Within the Gly-Xaa-Yaa repeat, Xaa and Yaa can be any residue but most often are proline (~28%) and hydroxy-

proline (38%) respectively [107]. Proline can be hydroxylated at the fourth or third carbon of the proline ring by the enzymes prolyl4-hydroxylase (P4H) and prolyl3-hydroxylase (P3H) respectively (Figure 3) [107-110]. This hydroxylation provides thermal stability to the triple helix by promoting electrostatic interactions [107].

Vertebrates have three isoforms of P4H and P3H (P3H1-P3H3). P4H is composed of two sub-units (α and β) whereby PDI makes up the β subunit [109] (Figure 3). The importance of proline hydroxylation has become clear through phenotypes of model organisms deficient for the hydroxylases *C. elegans* deficient for *P4H* are embryonic lethal with defects similar to worms with mutations in *Emb-9* (*COL4A1* ortholog) [111], while in mice heterozygous *P4ha1* deficiency caused embryonic lethality and defective collagen IV assembly [112]. Interestingly, mutations in the catalytic domain of P4H1 cause a connective tissue/myopathy disorder affecting tendon, bone, muscle and eye with reduced collagen IV staining in muscle BMs [113].

P3H acts on proline in the Xaa position of the collagen repeat (Gly-Xaa-HyP) after prolyl-4-hydroxylation has occurred. Isoform 1 of P3H (P3H1) together with cyclophilin B and cartilage-associate protein (CRTAP) forms a protein complex with chaperone activity [114], and mutations in these proteins cause recessive osteogenesis imperfecta [106]. Prolyl-3-hydroxylation causes a slight decrease in thermal stability [114] and is involved in mediating interactions with other ECM components including nidogen in the BM [115-117]. The expression pattern of P3H2 overlaps to a large extent with that of collagen IV [110] and P3H2 deficient mice develop a myopia-like phenotype with altered collagen IV hydroxylation in the lens capsule [118]. The absence of overt phenotypes can be explained by a functional redundancy [117], possibly with P3H3, which has a similar expression pattern [119]. For a detailed review on proline hydroxylases we refer the reader to [120].

Lysine residues that are at the Yaa position of the collagen triplet can also undergo hydroxylation by lysyl hydroxylases to provide binding sites for intermolecular collagen crosslinks, influencing the biomechanical properties of the ECM, and subsequent carbohydrate attachment (Figure 3) [104]. Mammals have three isoforms of lysyl

hydroxylases, LH1-LH3, which are encoded by the genes *PLOD1-3* (procollogen-lysine 1, 2-oxoglutarate 5-dioxygenase). The importance of lysine hydroxylation is underscored by the embryonic lethality around 9.5 day postcoitum, and aberrant BM and collagen IV secretion in mice deficient for Lh3 [121]. Defects in collagen processing and secretion also occur in lysyl hydroxylase deficient worms and cause contraction-induced muscle defects similar to that observed with collagen IV mutations [122]. In contrast to LH1 and LH2, LH3 is a multi-functional enzyme that also possesses galactosyl- (GT) and glycosyltransferase (GGT) activities responsible for modification of hydroxylysine to galactosylhydroxylysyl or glucosylgalactosylhydroxylysyl, which is fundamental for collagen IV secretion and BM structure. Very elegant mouse studies determined that the lethality of Lh3 deficient embryos was due to lack of GT and GGT activities, and not LH activity, highlighting the critical importance of the glycosyltransferase activities [123].

The clinical importance of galactosylation of hydroxylysine has also become apparent by the identification of mutations in *COLGALT1*, which encodes collagen β (1-O) galactosyltransferase 1, in patients with cerebral small vessel disease (Miyatake et al 2018). Intriguingly, the clinical characteristics correspond with *COL4A1* syndrome and functional analysis in cell lines supported that the mutations affect α1(IV) processing [124]. While this requires validation in vascular cell types and *in vivo*, and we need to establish if the disease is due to processing defects of collagen IV alone (as compared to collagen IV and other collagens), it suggests mutations in collagen modifying enzymes may contribute to *COL4A1* syndrome.

4.3 Collagen triple helix folding

Collagen triple helix formation commences following binding between NC1 domains. The α-chains wrap around each other in C to N-terminal direction to generate the central triple helical collagen domain whereby the glycine residue is located in the centre of the helix. Areas that

have formed a triple helix will cease to be subject to post-translational hydroxylation and glycosylation.

For triple helix formation to occur all proline residues require to be in trans conformation but proline occurs in nascent collagen polypeptide in a cis or trans conformation. Peptidylprolyl isomerases (PPIases) mediate this conformational change and mutations in the PPIases Cyclophilin B, FK506 binding protein, and parvulins [125] cause the classical collagen disorder osteogenesis imperfecta [106], illustrating the importance of PPI for collagen folding.

Several chaperones are also involved in collagen folding. This includes PDI, which has chaperone activity in addition to its other roles, and Heat Shock Protein 47 (HSP47). HSP47 is a collagen specific chaperone that binds and stabilises collagen IV protomers, amongst other collagens, in the ER and during their transit to the cis-Golgi [126](see also Chapter 2 in this book by Sergey Leikin and co-authors). The decrease in pH in the cis-Golgi causes HSP47 to dissociate from the collagen and for it to be recycled back to the ER. Mutations in the gene encoding HSP47, *SERPINH1*, cause osteogenesis imperfecta [106] while in mice and cells deficiency of HSP47 caused accumulation of collagen IV in the ER due to a lower secretion rate and absence in the BM [127, 128]. The quality of the secreted collagen was also affected as it has reduced thermal stability and increased sensitivity to protease digestion, supporting a critical stabilising role for HSP47 [129]. The mouse phenotype (including lethality at 11.5 day postcoitum) was very similar to mice deficient for α1α1α2(IV) [20]. These data elegantly underscore the importance of the HSP47 for collagen IV processing and secretion, and the "quality" of secreted collagen (Figure 3).

4.4 Transport from the intracellular to the extracellular space

The regulation of the secretion of large cargos such as collagens has received increased attention over the past decade with very elegant and paradigm shifting papers [130-133]. This is a very active and fast moving area of collagen and cell biology research. Much of this research has centred on collagen I and collagen VII although effects on collagen IV secretion

have been documented [134]. For a more detailed overview we refer the reader to Chapter 2 in this book by Sergey Leikin and co-authors in this book and [135].

Collagen IV translocates from the rough ER *via* the ERGIC (ER Golgi Intermediate Compartment) to the Golgi for secretion to the ECM. Smaller proteins are secreted via coat protein complex II (COPII) secretory vesicles that are generated at ER exit sites (ERES) and have a diameter of 60–90 nm [135]. However, collagens are extraordinarily large semi-rigid proteins (e.g. collagen IV ~ 400nm) too big for normal COPII vesicles. Thus, their transport necessitates specialized transport vesicles to accommodate these large cargos. An important role for Transport and Golgi organization 1 protein (TANGO1, also called MIA3) has been identified [130], as its deletion in mice leads to intracellular accumulation of collagen VII and other collagens including type IV. TANGO1 can recruit collagens via its luminal SH3 domain, and in parallel its cytoplasmic domain recruits other proteins to form extended COPII vesicles [130, 135, 136]. Recent evidence revealed a role for HSP47 in this process by acting as an anchor molecule between the SH3 domain of TANGO1 and collagens, allowing packaging of collagen molecules in the growing carrier (Figure 3). The cytoplasmic domains of TANGO1 interact with Sec23A/Sec24C, the outer molecules of COPII vesicles. This brings collagen into contact with the COPII vesicle to allow them to grow sufficiently to accommodate the cargo and subsequently transport the cargo from the ERES to the ERGIC vesicles [135]. Recent data however identified an alternative but not mutually exclusive mechanism that does not involve large carriers, but whereby collagen accumulates at the ERES in a TANGO1-COPII dependent manner and then flows more directly between ER and GOLGI [135]. This is made possible through proposed fusion of membranes of the ERES and ERGIC adopting a tunnel like structure [132, 135, 137].

4.5 Extracellular network formation

Following secretion the NC1 domains of two protomers associate end-to-end forming an NC1 hexamer, and the 7S domains of four protomers form a 7S tetramer, giving rise to a lattice style collagen IV network (Figure 2).

The increased extracellular chloride concentration in the ECM “breaks” the Arg76-Asp78 intra-chain salt bridge in the NC1 domains, and Arg76 is re-orientated towards an opposing NC1 trimer. Six salt bridges are formed across two NC1 trimers between Arg76 residue, and Glu175 and Asn187 from the other protomer [138, 139]. Additionally each bound chloride ion directly engages six electrostatic interactions per hexamer [103]. Combined, this allows for two protomers to bind, and the structural integrity of the NC1 hexamers is further strengthened by a sulfilimine covalent bond between methionine 93 and hydroxylysine 211 of opposing NC1 trimers. This sulfilimine bond is catalysed by peroxidasin [140] and is fundamental to the structural integrity and tensile force of the collagen IV scaffold as illustrated by distorted BM, tissue morphology and lethality in drosophila due to loss sulfilimine bonds [139, 141].

Following collagen IV dimer formation, four protomers bind through their N-terminal 7S domains at an angle to generate the lattice configuration of the network (Figure 2). The 7S domains, which consists of a lysine and cysteine rich region and a triple helical region, interact in a parallel and anti-parallel fashion [99]. Disulfide bonds between the 5 cysteine residues as well as lysine–hydroxylysine crosslinking ensures resistance against proteolytic cleavage by enzymes such as collagenase, and provides mechanical stability. The lysine–hydroxylysine crosslinking is performed by lysyl oxidase-like-2 (LOXL2) [139, 142], which has also been implicated in angiogenesis [143].

The site of collagen IV production can be distant from the site of incorporation into the BM and/or cells that do not reside on the BM also contribute to the BM by secretion of its components [144]. Consequently a mechanism that can delay extracellular assembly and maintain collagen IV folding might be employed. Secreted Protein Acidic and Rich in Cysteine (SPARC) is a matricellular glycoprotein that binds collagens [145, 146] and can function as an extracellular chaperone to ensure proper spatial folding of collagen IV before incorporation into the BM [23, 147, 148]. This is supported by data from *Drosophila* and *C. elegans* whereby *SPARC* knockdown and deficiency, respectively, led to local inappropriate accumulation of collagen IV rather than diffusion to distal sites [23, 149, 150]. These studies suggest SPARC

is important for allowing collagen IV to diffuse from sites of production and assemble on the surfaces of distal tissue. Recent studies have however suggested that this mechanism may only apply to maintenance of existing BM rather than for *de novo* BM assembly [144].

5. Collagen IV receptors

Besides providing structural support, collagen IV also plays a role in cell biological processes including cell differentiation, survival, and migration, mediated by the interactions of collagen IV with cells. Collagen IV binds multiple cells types such as endothelial cells, hepatocytes, platelets, keratinocytes, pancreatic cells, mesangial, as well as tumour cells [151-157]. The collagen receptors can be divided into integrin and non-integrin receptors.

5.1 Integrin receptors

Integrin receptors provide critical mechanosensing functions and convert the spatiotemporal information from the ECM into cellular signalling through the formation of integrin adhesion complexes (IAC) [158]. These transmembrane glycoproteins exist as noncovalent heterodimers made up of α and β subunits [159]. There are 18 α and 8 β subunits that combine to form 24 receptors with different binding and expression patterns. The integrin protein family can be divided into RGD-binding integrins and collagen-binding integrins [160]. The β1 subgroup of integrins are the main collagen receptors, particularly α1β1 and α2β1, and although these can both bind collagen IV, α1β1 does so with a greater affinity [161]. Decreased expression of either of these receptors led to a decrease in adhesion and migration (α1β1), and adhesion and morphogenesis (α2β1) [162, 163]. β1 integrin has also been implicated in recruiting collagen IV to some BMs in *C. elegans* [21]. Other collagen IV binding integrins include α3β1, α10β1, and α11β1, but their functional significance and role remains unclear [164-166]. Altered integrin signalling has been put forward as a potential mechanism in Alport syndrome [167], and for some mutations in HANAC syndrome that are located close to CB3 domain of α1(IV), a major integrin binding site [73, 168-171].

5.2 Non-integrin receptors

The discoidin domain receptors (DDR1 and DDR2) are type 1 transmembrane tyrosine kinase proteins that are activated by the triple helix found in some collagens [172]. DDR1 can be activated by most collagens, such as collagen I-IV and VIII, whilst DDR2 by collagen I, II, III and V but not IV [173]. Several lines of evidence have highlighted a role for DDR1 in collagen IV biology. DDR1 knock-out mice develop renal defects including thickening of the glomerular BM (GBM) and proteinuria, that also occur in Alport syndrome due to collagen IV mutations [174]. Interestingly, recent evidence indicates a novel unexpected role for DDR1 in mediating collagen IV transcription. In fibrosis, following collagen binding, DDR1 is translocated to the nucleus, where it binds chromatin and drives collagen IV expression [58]. This not only reveals a novel role for DDR1 but also indicates an additional layer of regulation and complexity relevant to collagen disease that requires further investigation.

Besides integrins and DDRs, collagen IV can also bind and activate a G-protein coupled receptor, GPR126, which is essential for Schwann cell myelination, ear canal formation, and heart development [175]. Finally, collagen IV can bind via its 3-hydroxylated proline residue to glycoprotein VI [115, 117], an important platelet collagen receptor, and absence of this binding bas been associated with increased platelet aggregation in mice [115].

6. Collagen IV expression patterns

During vertebrate development α1α1α2(IV) is ubiquitously expressed. In mice absence of α1α1α2(IV) due to deletion of exons 1 of *Col4a1* and *Col4a2*, cause embryonic lethality around 10.5-11.5 days postcoitum due to placental defects, and embryos displayed growth retardation and haemorrhaging [20]. The critical role of α1α1α2(IV) is also nicely illustrated by *Col4a2* mutant embryos homozygous for a deletion in exon 18. Embryos survived organogenesis but died by 14.5 days postcoitum and defects occurred in almost every organ [176]. This included cardiovascular, cardiac, skeletal, lung and endocrine organ defects. The later embryonic lethality of *Col4a1/Col4a2* knock-out embryos can be explained by residual *Col4a2* expression [20, 176], presumably through alternative splicing.

During embryonic development, in some BMs α1α1α2(IV) is replaced by α3α4α5(IV) or α5α5α6(IV). For example, replacement by α3α4α5(IV) occurs in the GBM [177]. This developmental switch can also give rise to a mixed network such as in Bowman's capsule of the kidney and the lens capsule [178]. Consequently, in adults α1α1α2(IV) is present in BMs of all tissues, whereas α3α4α5(IV) occurs in the glomerular and tubular BM of the kidney, the alveolar BM of the lung, as well as BM in testis and inner ear cochlea [44]. The α5α5α6(IV) protomer is expressed in the BM of the bronchial epithelium, the oesophagus, smooth muscle cells, skin, and Bowman's capsule of the kidney and the synovia [67, 179]. The tissues that are affected in patients and mice with mutations in collagen IV reflect the expression patterns of the different networks. This means that invariably mutations cause complex multi-systemic syndromes.

7. Collagen IV pathologies

Mutations affecting every α-chain have been described and the first mutation affecting collagen IV was described in patients with Alport Syndrome (AS). Tables 1 and 2 provide an overview of the some of the animal models for collagen IV diseases and some of the clinical features of the Mendelian diseases, respectively.

7.1 COL4A1 syndrome and mutations in *COL4A1 and COL4A2*

The identification of the first mutations in *COL4A1/COL4A2* in patients was based on analysis of *Col4a1/Col4a2* mutant mouse models that were generated in large ENU mutagenesis screens [180-182] (Table 1). This represents a case whereby the mouse models led to the description of the human disease, which is underscoring the power of these models to determine disease mechanisms.

COL4A1/COL4A2 mutations cause COL4A1 syndrome, a rare dominant multi-systemic disorder that cause a wide range of abnormalities affecting the brain, eye, kidney, vasculature

and muscle (Table 2). However, COL4A1 syndrome remains poorly characterised clinically. The vast majority of mutations are missense mutations affecting the glycine residues, although missense mutations have been described in each of the protein domains and affecting the glycine, Xaa and Yaa residues of the triplet [183]. From mice studies, a general genotype-phenotype correlation has been established whereby mutations affecting the Xaa or Yaa residues cause milder disease, and that more C-terminal glycine mutations are associated with more severe intracerebral haemorrhaging [181, 184]. From patients and mice it has become apparent that *COL4A2* mutations tend to cause a milder disease [182, 185, 186]. This can be explained by α1α1α2(IV) composition whereby a *COL4A2* mutation can affect 50% of the promoter compared to 75% with a *COL4A1* mutation. Non-sense mutations have also been described [187, 188] as well as mutations in the 3'UTR [60] and copy number mutations [189, 190]. It has also become apparent that the *de novo* mutation rate in *COL4A1/COL4A2* is high (~40%) [183] which can give rise to apparent sporadic cases. Intriguingly very recently the first case has been reported of recessive form of COL4A1 disease [191].

We will provide a brief overview of some of the major clinical features of COL4A1 syndrome (Table 2) but for a more in depth overview of the clinical features, we refer the reader to [183, 192].

7.1.1 Cerebrovascular and cerebral defects in COL4A1 syndrome

Cerebrovascular disease is the predominant feature of COL4A1 syndrome (OMIM #614519,#175780, #614483) but patients can display a large variability in clinical presentation and severity due to the large amount of variable expressivity and penetrance of the disease (Table 2) [183, 185]. The different clinical characteristics of the same mutation can occur within and between families [183, 185, 186].

A key feature of COL4A1 syndrome is recurrent intracerebral haemorrhage (ICH) but the brain and cerebrovascular features also cover porencephaly (development of cyst or cavities due to prenatal or neonatal ICH), white matter defects, and cortical defects (Table 2) [183, 193-195].

The cortical defects are associated with signs of white matter vascular insult [192], supporting the vascular involvement in disease pathogenesis. Neuronal migration defects also occur in *Col4a1* mutant mice [196] and cause lissencephaly [197], which can occur in COL4A1 syndrome [183, 192]. The consequences of these defects are varied and severe with (infantile) hemiparesis, seizures, developmental delay, mental retardation, hydrocephalus, cerebral palsy, and epilepsy [192, 198].

Many of the cerebral clinical features of COL4A1 syndrome are considered to be due to the cerebral small vessel disease (CSVD) caused by these mutations [183]. CSVD is a disorder of the small penetrating capillaries and arterioles and in the general population accounts for ~30% of stroke and is the leading cause of vascular cognitive decline (vascular dementia) [199, 200].

7.1.2 PADMAL and altered collagen IV levels.

While the majority (~60%) of mutations are missense mutations affecting the collagen triplet [201], nonsense mutations including splice site mutations and premature termination codon mutations that lead to reduced collagen IV levels, have also been identified in COL4A1 syndrome associated with haemorrhaging [187, 188]. In contrast, mutations affecting the miR-29 binding site of the 3′ UTR of *COL4A1* that increase *COL4A1* mRNA levels cause PADMAL (Pontine autosomal dominant microangiopathy and leukoencephalopathy, also known as multi-infarct dementia Swedish type; OMIM #618564). PADMAL is characterized by recurrent ischemic strokes causing lacunar infarcts, leukoencephalopathy, progressive dementia, motor impairment and spinal cord defects [60, 202, 203]. The different clinical features including ischemic stroke indicates PADMAL can be considered a clinical sub-entity within COL4A1 syndrome. Intriguingly, duplication and triplication of a genomic region harbouring *COL4A1* and *COL4A2* have been observed in patients with CSVD including lacunar infarcts [189, 204]. These cerebrovascular defects occur due to alteration in α1α1α2(IV) composition and level, with reduced and increased levels being pathogenic.

7.1.3 Ocular disease

Besides the vascular BMs, the eye contains BMs in the anterior and posterior structures, and the lens (Table 2). In mice and patients, mutations in *COL4A1/COL4A2* affect anterior and/or posterior ocular structures, and can lead to anterior segment dysgenesis (ASD) and microphthalmia (OMIM #180000, #175780) [181, 205-209] (Table 1 & 2). The anterior defects include congenital cataracts, iridocorneal adhesions, ciliary body defects and buphthalmos [181, 205-207, 209] some of which affecting the ocular drainage structures, causing impaired vision and glaucoma [210].

In addition to these anterior defects, arterial retinal tortuosity occurs in mouse models and patients whereby the arteries become tortuous and prone to bleeding [169, 181, 206, 207, 211], potentially causing retinal detachment and (temporary) vision loss. The retinal vascular defects reflect aspects of the cerebrovascular disease and have been proposed as a potential biomarker for cerebrovascular disease severity [184, 212]. However not all patients display ocular defects and there is a large variability in the clinical presentation [183, 213]. This variability reflects the importance of genetic background and modifiers to the ocular disease, which has been illustrated by *Col4a1* mutant mice on different genetic backgrounds [206, 214].

7.1.4 Neuromuscular disease

Up to one third of COL4A1 syndrome patients present with muscle pathology and myopathy [186] which also occurs in mice models as demonstrated by centralised nuclei, reduced grip strength and elevated serum creatine kinase levels [169, 171, 196, 215] (Table 2). The muscle phenotype can be part of congenital muscular dystrophy [196]; a spectrum of childhood diseases encompassing muscular, ocular and cerebral malformations whereby patients develop muscle weakness, hypotonia and in some cases severe myopathy which can be fatal. *COL4A1* mutations have been detected in dominant forms of muscle-eye-brain disease (MEB; OMIM #175780) and Walker Warburg Syndrome (WWS). In contrast to the more common recessive diseases forms, α1α1α2(IV) associated WWS/MEB is independent of glycosylation

of dystroglycan [196]. In mice the myopathy was associated with myelination and conductivity defects in the peripheral nerves [215], but vascular defects were also associated with the myopathy [171]. In addition, the neuromuscular junction plays a key role in muscle contraction, and α1α1α2(IV) is present in the synaptic cleft, and plays a role in clustering of synaptic vesicles and nerve terminal maturation [11]. Finally, in *C. elegans* mutations in *Col4a1* and *Col4a2* orthologs (*emb-9* and *let-2* respectively) result in muscle fibre rupture [216], while in Drosophila, mutations in *Col4a1* or *Col4a2* orthologs (*Cg25c* and *Viking*, respectively) causes myopathy [217] with defective muscle attachment due to altered collagen IV-integrin signalling [218]. Combined, these data indicate an intricate and complex mechanisms of the myopathy with vascular, muscular, and neuronal contributions.

7.1.5 HANAC and kidney defects

In the kidney α1α1α2(IV) is expressed in all BMs during development but is replaced by α3α4α5(IV) in the GBM, and forms a mixed network with α5α5α6(IV) in Bowman's capsule [74]. In patients much of the renal component of COL4A1 syndrome, has been characterised in HANAC syndrome (hereditary angiopathy, nephropathy, aneurysm and cramps; OMIM #611773) which, similar to PADMAL, has been proposed as a clinical sub-entity of COL4A1 syndrome [169, 170]. HANAC syndrome patients can develop cerebrovascular, eye, kidney and muscular defects (Table 2), but compared to non-HANAC COL4A1 syndrome patients, the brain is relatively mildly affected in HANAC syndrome [169, 170, 219]. HANAC is due to mutations in exons 24 and 25 of *COL4A1* in or near the CB3 region of α1(IV), an important domain for integrin signalling [169, 170], supporting that location of mutations in the α-chain affects clinical outcome. This is also supported by the mouse model of HANAC syndrome, harbouring the G498V mutation, which is the only *Col4a1* mutant mouse model that is viable when homozygous mutant [171, 212, 220].

In mice, *Col4a1* mutations causes a progressive kidney pathology with early onset defects to Bowman's capsule that are associated with MMP activation and activation of the parietal

epithelial cells and tubular dysfunction [181, 220-222] that could reflect hydronephrosis. The glomeruli are prone to cyst formation, while subtle defects in the GBM have also been observed [221, 222]. As these defects occur in multiple mouse models, this indicates they are not specific to HANAC. *COL4A1* mutant mice also develop apparent dysmorphia of the papilla / atrophy of the medulla [220, 221] and the recent identification of *COL4A1* mutations in patients with vesicoureteral reflux (VUR), in which urine flows retrograde, from the bladder into the ureters/kidneys [223], provides a potential mechanism to this apparent hydronephrosis. These patients were diagnosed with CAKUT (Congenital anomalies of the kidney and urinary tract) [223], extending the spectrum of features of COL4A1 syndrome and highlighting the importance of α1α1α2(IV) for kidney morphogenesis as well as a developmental origin to some of these defects.

7.1.6 Additional clinical features in COL4A1 syndrome

In contrast to the vascular, eye, kidney and muscle defects, only few reports have highlighted a cardiac component to COL4A1 syndrome. Arrhythmia has been noted in HANAC syndrome and patients that harbour a deletion of a locus containing *COL4A1* and *COL4A2* develop congenital cardiac defects [169, 224, 225]. Support for severely reduced α1α1α2(IV) levels causing cardiac defects is also supported by mouse studies [176].

The broad expression pattern of α1α1α2(IV) coupled with defects in almost every tissue in mice deficiency for α2(IV) [176], and the variable expressivity and penetrance of COL4A1 syndrome [183], indicates that mutations may lead to defects in tissues that have so far remained poorly or uncharacterised. For example BM defects have been observed in the skin of a patient with a *COL4A2* mutation and in oesophagus of *Col4a1* mutant mice, but no overt disease was described in this particular family and mouse respectively [181, 185]. It is therefore likely that additional clinical features of COL4A1 syndrome will be uncovered, which will be important for molecular diagnosis and management of patients.

7.1.7 The role of α1α1α2(IV) in common disease

The identification of *de novo* mutations in apparent sporadic cases [183, 226] suggested that variants in α1α1α2(IV) could occur and contribute to common forms of disease. Analysis of ~100 patients with sporadic ICH identified rare pathogenic mutations in *COL4A1* and *COL4A2* [227, 228], indicating very rare missense mutations in α1α1α2(IV) contribute to sporadic forms of adult ICH and haemorrhagic stroke. In addition large scale genetic analysis uncovered that common variants in *COL4A1-COL4A2* are risk factors for ICH [28, 31, 229] and white matter hyperintensities [230] in the general population. The proportion of ICH patients in which collagen IV plays a role as well as the identity and mechanisms of the variants remains unknown and requires investigation of larger cohorts. First inroads into the frequency of pathogenic variants in *COL4A1* in the general population have been made by Paré and colleagues who determined that within ~100,000 samples from the Genome Aggregation Database, the prevalence varies across different ethnicities and was highest in African/African-American at 0.3% [231].

In addition to stroke, *COL4A1* and *COL4A2* have also been associated with vascular calcification, vascular stiffness, coronary artery disease and myocardial infarct in the general population [24, 26, 27, 232]. In the case of myocardial infarct and coronary artery disease, the intronic variant in *COL4A2* reduced expression. The lower C*OL4A2* levels were associated with smooth muscle cell apoptosis and the presence of more unstable atherosclerotic plaques, which are known to increase the risk of myocardial infarct [233]. These data clearly demonstrate that these genes and the basement membrane are important determinant of vascular health and physiology.

7.2 Mutations and diseases of α3α4α5(IV) and α5α5α6(IV)

7.2.1 Alport syndrome

Mutations in *COL4A3, COL4A4 and COL4A5* affect the α3α4α5(IV) network and cause Alport syndrome (AS) (OMIM # 301050, # 203780, # 104200) (Table 2). This multi-systemic disease leads to progressive glomerulonephritis accompanied by sensorineural hearing loss and

ocular pathology [74, 174]. AS patients display altered thickness of the GBM leading to progressive splitting, and patients develop haematuria, proteinuria and eventually end-stage kidney disease. Ocular pathology in AS patients includes a thin fragile lens capsule termed anterior lenticonus as well as temporal retinal thinning and dot and fleck retinopathy [234]. The ocular phenotypes can be used for disease diagnosis as their presence correlates with renal failure before the age of 30 [234].

AS can occur as X-linked Alport syndrome (XLAS) caused by *COL4A5* mutations which affect both α3α4α5(IV) and α5α5α6(IV) networks. Mutations in *COL4A3* or *COL4A4* cause autosomal recessive AS (ARAS) and autosomal dominant AS (ADAS) and affect only the α3α4α5(IV) network [74]. The distribution of these cases is as follows: 80% XLAS, 15% ARAS, and 5% ADAS, with a combined prevalence of AS of 1–9/100,000 [74]. The inheritance patterns affect disease severity. XLAS is more severe than autosomal AS, reflecting the effects on two collagen IV networks. For the autosomal forms, ARAS is more severe than ADAS whereby early onset end stage renal disease occurs in ARAS, and ADAS patients may not progress to end stage renal disease [74]. As expected for X-linked disorders, XLAS causes a more severe disease in males than in females, who can be considered heterozygous carriers. In females the X chromosome inactivation pattern, which causes a characteristic mosaic expression pattern of α3α4α6(IV) [235], influences disease severity and results in a wide range of clinical features.

AS can be caused by deletion, insertion, splice site and missense mutations, and the type of mutation impacts disease severity and age of onset [236]. Nonsense mutations are associated with a more severe disease and earlier age of onset, juvenile in the case of *COL4A5* mutations, compared with missense mutations. This establishes that absence or reduced levels of α3α4α5(IV) are a driving force of disease [74]. The majority of missense mutations affect the glycine residue in the Gly-Xaa-Yaa repeat [237]. The position of the glycine mutation in the collagen domain impacts on disease severity with C-terminal mutations being associated with a more severe disease [236]. ARAS patients can harbour compound heterozygous or

homozygous *COL4A3* or *COL4A4* mutations [74, 236]. More recently it has become clear that AS can also be due to digenic inheritance whereby the two *COL4A3*-*COL4A5* mutations are located on different or same chromosomes [238-240]. Furthermore, molecular analysis of ADAS families also provided intriguing data that digenic inheritance may involve non-collagen IV genes. In this case a *COL4A4* mutation, which was insufficient to cause disease by itself in some pedigree members, was co-inherited with a hypomorphic mutation in *LAMA5* (laminin alpha 5 chain) [241], which plays a key role in maintaining the glomerular filtration barrier. The second mutation may also not be limited to other BM components as a hypomorphic mutation in *NPHS2* (encoding the podocin protein in which mutations cause congenital nephrotic syndrome) was also found to be co-inherited with a heterozygous *COL4A4* mutation [242]. This led the authors to propose a model whereby digenic or even oligogenic inheritance with other BM components and filtration barrier components should be considered for cases whereby the pathogenic effects of the collagen IV mutation is milder [241].

Until recently autosomal dominant thin basement membrane nephropathy (TBMN, also known as familial benign haematuria; OMIM # 141200) was considered a separate entity. However 40% of TBMN patients carry heterozygous *COL4A3* or *COL4A4* mutations and the clinical features of haematuria and BM thinning also occur in carriers in families with AS [243]. Furthermore, a similar fraction of ADAS (24%) and TBMN patients (20%) develop end stage renal disease [244, 245], and heterozygous *Col4a3*$^{+/-}$ mice, which exhibit TBMN, develop chronic renal failure and have a reduced life expectancy [246]. In light of these findings the criteria for AS were reconsidered and TBMN is now considered part of AS [247].

During development the GBM consists of α1α1α2(IV) and this is gradually replaced by α3α4α5(IV). The GBM of AS patients contains no α3α4α5(IV) but the embryonic α1α1α2(IV) chain persists, which is less cross-linked and more susceptible to proteolytic degradation and damage due to increased urine flow, leading to GBM damage [177]. This disease mechanism is supported by the structural defects in mature GBM of X-linked Alport's syndrome patients that contains α1α1α2(IV), and filtration defects in *Col4a3*$^{-/-}$ mice in areas of the GBM that are

structurally normal [248]. These differences in network characteristics are also illustrated by the lack of improved outcome in ARAS patients that have mosaic α5α5α6(IV) expression [249]. However there do appear to be species differences as in the *Col4a3*$^{-/-}$ AS mouse model induced α5α5α6(IV) expression reduced disease severity [250]. These species differences will complicate translation of findings between mice and patients, which will need to be considered for disease mechanism-based treatment strategies.

7.2.2. X-linked Alport syndrome with diffuse leiomyomatosis

X-linked Alport syndrome may occur combined with diffuse leiomyomatosis (OMIM #308940), which is characterized by benign smooth muscle tumours leading to oesophageal dysfunction and genital leiomyomas [74]. The mutations are deletions that affect *COL4A5* and *COL4A6* leading to the original hypothesis that absence of α3α4α5(IV) and α5α5α6(IV) causes the disease [251]. However, this was challenged by deletions extending beyond exon 3 of *COL4A6* that do not cause diffuse leiomyomatosis [252], and by the identification of a deletion in *COL4A5* that caused AS- diffuse leiomyomatosis [253]. However, in the latter study *COL4A6* expression was not assessed. Moreover recent analysis of deletion breakpoints within a substantial cohort of AS- diffuse leiomyomatosis patients does support that inactivation of *COL4A5* and *COL4A6* is causative [254] and disease could be due to effects on regulatory elements [254].

7.2.3 Goodpasture Syndrome

Goodpasture syndrome (GP, OMIM # 233450) is an autoimmune disorder in which autoantibodies attack the GBM leading to glomerulonephritis and pulmonary haemorrhage. GP is a rare disorder (1-2 cases per million per year) [255, 256], however it can be fatal without medical intervention, whereby patients quickly progress to end stage renal failure. A combination treatment of plasma exchange and immunosuppression improves renal outcome and survival time [257]. In GP, autoantibodies target epitopes within the NC1 domain of α3(IV) and α5(IV) in α3α4α5(IV) in the GBM [243]. These epitopes are normally hidden by the NC1

hexamer structure, and thus require to be unmasked. Several mechanisms have been put forward including reactive oxygen species, hexamer structure and stability [258, 259]. While the exact aetiology remains to be determined, studies suggest a conformational change in α3α4α5(IV) results from perturbation of sulfilimine crosslinking of NC1 through the enzyme peroxidasin, or excess phosphorylation of the NC1 domain by increased expression of goodpasture binding protein [140, 256, 260]. This would expose the cryptic epitopes only accessible to the autoantibodies.

Auto-immunity can also be a major complication for AS patients with nonsense mutations that have undergone a renal transplant. The presence of “foreign” collagen IV chains post-transplantation can cause anti-GBM nephritis. For ARAS the epitopes are also in the NC1 domain of α3(IV) and α4(IV) [261] whilst for X-linked AS the epitope is in α5(IV) [262, 263]. However unlike in GP, these epitopes are accessible in the NC1 hexamer [263].

Recent genetic data has significantly increased our understanding of the role of α3α4α5(IV) and α5α5α6(IV) in biology and disease. For α3α4α5(IV), mutations in *COL4A3 and COL4A4* have been associated with kidney disorders including diabetic kidney disease [264, 265], focal segmental sclerosis (Voskarides et al 2007) and steroid-resistant nephrotic syndrome [266]. This has been complemented in the identification of altered axonogenesis in zebrafish due to *COL4A5/COL4A6* mutations [267] (Table 1). Excitingly missense mutations in *COL4A6* that affect a glycine residue within the collagen domain have been recently identified as a cause for non-syndromic hearing loss [268].

8 Disease mechanisms

8.1 Developmental origin to adult disease

A feature of both AS and COL4A1 syndrome are the early onset of the disease and evidence has been gathered pointing to a significant role for developmental defects or origin for these diseases.

For AS the maintenance of the embryonic α1α1α2(IV) network in the GBM renders the GBM susceptible to biomechanical strain [177]. In the case of COL4A1 syndrome, mice and patients develop ICH at birth or during embryonic development, and congenital or prenatal porencephaly [180, 182, 204, 226, 269-272]. The cortical malformation (e.g. lissencephaly) [192] is also associated with developmental neuronal migration defects [196, 197]. Furthermore induced expression of mutant *Col4a1* in a conditional mouse model revealed that expression of the mutant allele pre-weaning was necessary to induce adult ICH [184] and for the anterior segment dysgenesis in the eye, around or before 10.5-12.5 days postcoitum [214].

Angiogenesis is the biological process of forming new blood vessels from pre-existing capillary networks. During sprouting angiogenesis, endothelial cells (EC) degrade the vascular BM, invade into the surrounding tissue and proliferate in response to angiogenic factors to form tubes [273, 274]. This process of EC activation, proliferation, and survival is dependent on the ECM [77]. Altered angiogenesis is known to lead to defects in vascular patterning [274], which can cause arterial tortuosity syndrome [275], and VEGF (vascular endothelial growth factor) signalling, a key player in angiogenesis is implicated in retinal vascular tortuosity [276], a feature of COL4A1 syndrome [181, 206, 207, 211]. *Col4a1* mutant mice exhibit increased levels of pro-angiogenic growth factors including VEGF, and increased angiogenesis in the brain during embryonic development [184, 211]. This strongly argues for a role of altered embryonic –post-natal angiogenesis in COL4A1 syndrome.

The mechanisms by which *Col4a1* mutations affect angiogenesis remain unclear, but collagen IV is implicated in the regulation of angiogenesis. For example, endothelial cell migration and proliferation is modulated by collagen IV [277] with collagen IV levels correlating with the amount of tube formation, e.g the addition of exogenous collagen IV promoted tube formation [278]. Collagen IV can also exert anti-angiogenic effects through release of cryptic peptide [77, 279]. This includes the anti-angiogenic peptide arresten and canstatin, generated by proteolytic cleavage of NC1 domains of α1(IV) and α2(IV), respectively [77]. These molecules can bind integrin $\alpha_1\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_5$, and transmembrane integrin like receptor CMG2

(capillary morphogenesis gene 2) and may alter angiogenesis by affecting MAPK signalling [77, 280]. Therefore, the reduced levels of these peptides due to lower levels of collagen IV in the ECM of *Col4a1* mutant mice [180, 184, 206, 221, 270], represents one possible mechanism by which these mutations affect angiogenesis. In addition collagen IV binds BMP (bone morphogenic protein) [14, 281], which is a member of the TGFβ family of proteins, known to affect angiogenesis [282]. Thus, effects of the mutation on growth factor signalling mediated by collagen IV also represent an attractive hyp othesis.

8.2 Cellular origins of collagen IV diseases

Both COL4A1 and Alport syndromes are multi-systemic disorders and the collagen IV proteins are expressed by multiple different cell types. A key aspect in terms of elucidating disease mechanism is therefore determining the cellular origin of the disease. This is important as it will directly establish which cell types will need to be targeted by any treatments.

Using an elegant conditional *Col4a1* mutant mouse model, Gould and colleagues identified that expression of mutant *Col4a1* in EC or smooth muscle alone is sufficient to cause ICH, with a more minor effect from expression in astrocytes [184]. Interestingly, altered vascular function is implicated in CSVD [283] and mutant *Col4a1* expression affects endothelial and vascular function with altered vasodilation of the peripheral vasculature [270]. The smooth muscle cell defects in *Col4a1* mutant mice include hypermuscularisation of the transitional segment of the vasculature (a segment between arterioles and capillaries), predicted to increase vascular resistance. Combined with smooth muscle cell apoptosis upstream of this segment, which weakens the vascular wall, this then, could lead to haemorrhage at this weakened site [284]. Importantly the same mechanism was observed in sporadic patients with intracerebral haemorrhage [284]. Combined, these data highlight that both structural and functional vascular defects may contribute to the vascular disease.

The vascular defects also contribute to the myopathy [171] and retinal defects in the eye, while the lens defects play a key role in the development of the ASD defect in the eye including increased intra-ocular pressure [211, 214]. This confirms that COL4A1 syndrome is more than a vascular disorder and thus that the mechanisms of disease will need to be established for the different cell types and tissues.

For AS recent genetic evidence indicated that induced expression of *Col4a3* in EC of *Col4a3*$^{-/-}$ mice, did not ameliorate disease [285]. This not only indicates that EC do not contribute to the deposition of the α3α4α5(IV) in the GBM but also that treatments will need to be aimed at the podocyte.

8.3 Molecular disease mechanisms

Collagen IV is folded in the ER and secreted into the ECM. It is therefore logical to assume that ECM defects are a key feature of collagen pathologies, either due to reduced levels of collagen or secretion of mutant protein. It has been shows that delay in protein folding due to collagen missense mutations can lead to excessive post-translational modification of the protomer and disease [286, 287] (Figure 3). However missense mutations in secreted protein can lead to ER retention and ER stress, which can become pathogenic and has been implicated in several ECM disorders [288-290] (Figure 4).

Thus, conceptually collagen IV mutations could act via ER stress and/or ECM defects. In the following section we will describe in more detail the different molecular disease mechanisms of the collagen IV pathologies.

8.3.1 ECM defects and quantity and quality of the collagen network

<u>COL4A1 syndrome</u>

A central feature of mouse models and patients with *COL4A1/COL4A2* mutations are BM structural defects that affect every tissue analysed to date including eye, kidney, muscle and vasculature [171, 180, 181, 206, 212, 220, 221, 270, 291, 292]. These defects are associated

with and can be due to severely reduced collagen IV levels in the BM [20, 176, 180, 184, 185, 187, 196, 270]. The reduced level of collagen IV is a clear mechanism for the non-sense mutations that have been identified in patients [183, 188] (Figure 4). However it also applies to missense mutations. Analysis of mice and patients cells with *Col4a1/COL4A1* or *Col4a2*/*COL4A2* missense mutations respectively revealed that incorporation of a single mutant α-chain in α1α1α2(IV) is sufficient for the mutant protein to be retained in the ER [171, 180, 185, 206, 220] (Figure 4). The severity of this retention is influenced by the position of the mutation, whereby in general more C-terminal mutations cause a higher degree of intracellular retention, and more severe ICH [184, 209]. This indicates that at least in some genetic backgrounds reduced levels of collagen IV in the BM can cause cerebrovascular disease. The quantitative difference are not limited to reduced levels however as increased collagen IV levels can also be pathogenic, for example in PADMAL [60].

Besides these quantitative differences, missense mutations will also affect the quality of the protomer, providing an additional disease mechanism through possible secretion of mutant α1α1α2(IV) (Figure 4). For example, the network can be less stable as mutant α1α1α2(IV) displays reduced thermal stability reflected by the temperature sensitive nature of some phenotypes in *C. elegans*, Drosophila and cell culture models [209, 216, 218, 293]. The mutation could also affect a functional domain within the protomer that impacts on a specific function such as ECM-cell signalling. This could explain how some missense mutations may cause a severe phenotype despite being located in the N-terminal end of the protein, and provide a mechanism for some of the clinical variability with COL4A1 syndrome. For example a N-terminal *Col4a1* glycine mutation in mice caused a mild cerebrovascular phenotype but a severe myopathy, which was associated with altered GPCR signalling leading to the myelination defects and neuromuscular component of COL4A1 syndrome [209, 215]. Similarly altered integrin signalling occurs in Drosophila [218] and mice [220], and has been postulated to play a key role in HANAC [170]. This indicates that aberrant signalling is likely a key feature of COL4A1 syndrome.

ECM composition and turnover are key features for the BM to carry out its functional and biomechanical roles. Proteases such as MMPs and their inhibitors including TIMPs influence ECM composition and turnover [294]. In kidneys of the HANAC mouse model, the ECM and Bowman's capsule defects are associated with increased MMP2/9 activity, which are major collagen IV degrading MMPs, and activation of the epithelial cells, illustrating effects on altered cell state [220].

These data illustrate the large variety in qualitative and quantitative effects by which *COL4A1/COL4A2* mutations cause compositional and structural matrix defects in COL4A1 syndrome.

Alport Syndrome

ECM defects are also a constant feature of AS and the increased severity of non-sense mutations provides compelling evidence for reduced levels or absence of α3α4α5(IV) and associated structural GBM defects being a major causative mechanism (Figure 4). The persistence of α1α1α2(IV), which is less resistant to proteolytic cleavage [177], also highlights the altered and reduced biomechanical stability, leading to BM structural defects as the GBM is unable to deal with the biomechanical strain. In addition to structural defects, the ECM composition is also altered with aberrant laminin α2 expression [295].

These structural and compositional defects affect cell phenotype within the affected kidney. They cause endothelin A receptor expression in glomerular cells and an altered more invasive behaviour of mesangial cells [296]. Podocytes also adopt a more invasive phenotype with loss of foot processes [297], associated with focal adhesion kinase activation, cytokine (e.g. TGFβ) production indicating an inflammatory state and MMP activation [298], highlighting ECM remodelling and onset of fibrosis. The observed fibrosis and MMP activation are influenced by LOXL2 (lysyl oxidase like 2) collagen cross-linking activity [299]. The altered cell phenotypes and their responses may also be determined in part by altered ECM-cell signalling, for example *via* integrins α1β1 and α2β1, as well as DDR receptors [237, 300].

8.3.2. ER stress as disease mechanism for collagen IV disorders

Missense mutations in secreted protein can affect protein folding such as triple helix formation in the case of collagen. This can elicit a stress response called ER stress which leads to activation of a signalling response called the unfolded protein response (UPR). Briefly, the UPR is a conserved homeostatic response that aims to restore proteostasis, ER homeostasis and cell function by pausing general protein synthesis, degrading misfolded proteins *via* a process called ERAD, and increasing the production of molecular chaperones involved in protein folding [301]. However, when cells and the UPR fails to reinstate ER homeostasis and prolonged UPR activation occurs, the UPR triggers apoptosis and can become pathogenic. For excellent reviews on the UPR and its role in disease we refer the reader to [302, 303].

In terms of COL4A1 syndrome, analysis by ourselves and others identified that in mice with *Col4a1* mutations, reduced secretion and collagen retention were associated with ER stress and UPR activation in the vasculature [184, 270] (Figure 4). Importantly, an allelic series of *Col4a1* mouse models revealed that ICH severity correlated with levels of ER retention, which broadly corresponded to the position of the mutation in the collagen domain, rather than extracellular collagen IV levels [184, 209]. We described that in a family with a *COL4A2* mutation, ER stress and not ECM defects was associated with ICH [185]. In this case ECM defects were detected in an unaffected carrier of the disease [185], providing evidence that ECM defects *per se* may not always be sufficient. It should however be noted that not every glycine mutation induces ER retention and stress, in particular those that are more located towards the N-terminal end of the collagen domain [184, 209], and that this genotype-phenotype relation did not apply to the myopathy [209]. There is less information for non-glycine mutations although a lysine mutation affecting the Yaa position of the collagen triplet can also induce ER stress in the vasculature [270].

Overall these data highlight a trend with more severe protein folding defects associated with mutation located towards the C-terminal end of the collagen domain, and indicate that ER retention and stress can have a strong modifier effect for ICH.

Comparative analysis between ECM defects and ER stress revealed tissue specific mechanisms occur as in mice the myopathy and Bowman's Capsule defects were associated with ECM defects, and ICH with ER stress [184, 215, 221]. Analysis of the kidney defects also provided evidence for cell specific mechanism within the same tissue whereby the tubular disease was associated with ER stress and the defects in Bowman's capsule with ECM defects [221]. This may reflect the sensitivity of some cell types or tissues to ER retention and/or BM defects, but the nature and position of the mutation is also likely to affect this (see also ECM defects 8.3.1). Mutations affecting the Yaa residue of the collagen repeat and a mutation in the NC1 domain caused a milder eye disease and ICH, respectively, than glycine mutations [181, 209].

Overall these data highlight a complexity whereby the nature of the mutation and cell specific effects combine to cause disease. However, it remains unclear what the relative contribution of ER stress (and/or ECM) defects to the different COL4A1 disease mechanisms is. Is it pathogenic or an epiphenomenon? This represents an important knowledge gap to guide treatment development.

For AS, similar questions are being raised given the presence of missense mutations in X-linked and autosomal dominant forms of AS. A key advance was made by transfection of a *COL4A3* cDNA containing a glycine mutation in cultured podocyte cell line which revealed ER stress induction [304]. Importantly, this was confirmed in a novel mouse model harbouring the same *Col4a3* mutation as well as patient podocytes. Intriguingly, the UPR was also activated when *Col4a3* was either knocked-down or over-expressed, suggesting imbalance between the different collagen IV alpha chains may be sufficient in some cases to induce ER stress [304]. Investigation of *COL4A5* glycine mutations in primary patient dermal fibroblasts confirmed induction of ER stress and autophagy [305]. However how these intracellular effects contribute to AS remains unclear.

8.4 Environmental and genetic modifiers

Investigating genetic and environmental modifiers of disease provides important insight into disease mechanism and potential therapeutic targets and approaches, but can also inform on clinical management of patients to reduce risk of disease progression. Genetic analysis of families and mouse models with *COL4A1/COL4A2* mutations support that eye, muscular and cerebrovascular diseases are dependent on genetic background. In mice the CAST genetic background was able to effectively suppress the severe defects caused by *Col4a1* mutations [184, 196, 206], while our analysis in patients revealed that a *COL4A2* glycine mutation that causes ICH and porencephaly is also subject to genetic modifiers [185]. The identity of these modifiers in vertebrates remains unclear although for the ocular disease a locus was identified on mouse chromosome 1 [206] while it can also involve ER retention and ER stress in patients [185]. In *C. elegans* other BM components modify *Col4a1/emb9* phenotypes [306].

Data from animal studies also uncovered environmental modifiers on cerebrovascular disease. This includes vaginal birth as a risk factor with Caesarian delivery reducing neonatal ICH [291], while exercise and use of blood thinners increased disease severity [184]. In addition, age can also be considered as a modifier given the age dependent nature of the kidney disease, vascular dysfunction and cerebrovascular disease [184, 212, 221, 270].
These modifiers underlie the reduced penetrance and clinical variability of COL4A1 syndrome. This implies that absence of disease in one individual with a particular *COL4A1* or *COL4A2* variant does not exclude this variant from being pathogenic *per se*. Consequently, the contribution of rare *COL4A1/COL4A2* variants to disease in the general population may be higher than anticipated and at least some variants of unknown significance may be pathogenic.

AS development is also influenced by modifier effects of other BM components. This was nicely illustrated by inducing α5α5α6(IV) expression in the GBM of *Col4a3*$^{-/-}$ mice to reduce phenotype severity with a delayed age of onset of renal failure [250]. In contrast increased levels of laminin α5 and α1 exacerbated disease [248], while a laminin □2 missense variant,

which was not pathogenic itself, increased progression to kidney failure in *Col4a3*$^{-/-}$ mice and proteinuria in female *Col4a5*$^{+/-}$ mice [307].

In conclusion, the data of AS and COL4A1 syndrome provide compelling evidence that BM, and potentially other ECM components as well as intracellular pathways can act as genetic modifiers of collagen IV disorders. This also argues for a more complete sequencing analysis of BM/ECM components in patients to provide more accurate molecular diagnosis.

9. Therapeutic strategies

Treatment for hereditary disorders can be divided into approaches aimed at managing disease progression and providing symptom relief, strategies to modulate disease mechanisms and gene therapy approaches [290] (Table 3). Gene therapy approaches are appealing conceptually as they are independent of the disease mechanism and provide an actual cure. However the multi-systemic nature of collagen IV pathologies and the fact that different cell types underlie the different clinical features, e.g. EC for ICH and lens capsule for ASD defects in COL4A1 syndrome, complicates the use of these approaches as multiple cell types require to be targeted.

9.1 Gene therapy and therapies targeting downstream mechanisms

To date the multi-systemic nature of COL4A1 syndrome combined with a very young age of onset complicates disease management which focuses on symptom relief including a shunt to drain excess fluid for hydrocephalus, anti-convulsing medicine for seizures etc. Given that hypertension is the major risk factor for haemorrhage [308], blood pressure lowering drugs can reduce the high risk of stroke. Finally, physical or speech impediments are managed by physiotherapy and speech therapy respectively.

For AS patients, there has been more success in managing disease progression and treatment [309, 310]. Following initial small clinical trials targeting the renin angiotensin system to reduce blood pressure in AS [311-313], a recent large multi-centre phase 3 clinical trial using ramipril with a follow-up period of 6 years indicated reduced progression of proteinuria,

lower decline in filtration rate, and slower progression to renal failure in AS children [314]. This provides compelling data as to the clinical benefit of angiotensin-converting enzyme (ACE) inhibitors for AS.

Fibrosis is a feature of AS, and many other ECM disorders and several approaches have been adopted to target fibrosis in AS mouse models. This includes cerivastatin- a HMG-CoA reductase inhibitor that targets the TGFβ1 pathway, and vasopeptidase inhibitor AVE7688, which had anti-fibrotic and anti-inflammatory effects [315, 316], while targeting STAT3 signalling also attenuates disease progression in mice [317]. Finally, phase 3 clinical trials are progressing with bardoxolone (BARD) [318], which has anti-fibrotic and anti-inflammatory effects. BARD does increase glomerular filtration rates in AS patients, which has raised debate as to whether this represents a potential risk [319].

Finally, gene therapy based approaches are also being explored for AS (Table 3). This has included reducing fibrosis by targeting miR-21, which promotes fibrosis [320], as its levels are increased in kidneys of AS patients and correlate with disease severity [321]. Initial support came from administration of an anti-miR21 in *Col4a3*$^{-/-}$ mice that attenuates kidney disease progression [321, 322]. This has formed the basis for the currently ongoing clinical trial using RG-012, also known as lademirsen, designed to block the activity of miR-21 (clinicaltrials.gov Identifier NCT03373786).

In mice proof-of-concept gene therapy approaches targeting the actual GBM included a transgene system to induce α3α4α5(IV) expression by podocytes and promote its incorporation into a defective GBM. This not only restored the missing α3α4α5(IV) network but also slowed kidney disease progression and extended life span [323]. Induction of α3α4α5(IV) expression has also been explored through transplanting amniotic fluid stem cells into *Col4a5*$^{-/-}$ mice which delayed interstitial fibrosis, kidney decline and prolonged animal survival [324]. Transfer of bone marrow derived stem cells post-irradiation also appeared to repair BM defects in *Col4a3*$^{-/-}$ mice [325], but this has been debated as irradiation itself also prolonged

life span [326, 327]. Further research will be required to confirm the potential of stem cell transplantation and reconcile these differences.

9.2 Targeting ER stress

Dominant mutations in AS and COL4A1 syndromes can cause intracellular retention with ER stress and UPR activation [206, 209, 221, 270, 290, 304, 305]. This suggests that enabling protein folding could alleviate intracellular pathology and be beneficial to the ECM by promoting collagen IV secretion. This could be achieved through chemical chaperones, which enhance protein folding or stability by mimicking the molecular chaperones in the ER [328] or via promoting misfolded protein degradation, as has been applied for *Col10a1* via carbamazepine in mouse models of metaphyseal chrondrodysplasia type Schmid [289]. As several chemical chaperones and carbamazepine are FDA-approved, these would represent attractive therapeutic approach for transition into clinical trials.

For collagen IV disease, the emphasis has been on the compound sodium 4-phenylbutyrate (4PBA), which is FDA approved for treatment of urea cycle disorders, but also possesses chemical chaperone activity [328]. 4PBA increases ER folding capacity, stabilizing folded protein and increasing ER-associated degradation (ERAD) [328]. *In vitro* treatment of primary patient cells harbouring a *COL4A2* mutation established that 4PBA reduced ER retention and ER stress [185], which was confirmed for an allelic series of mutations in mouse embryonic fibroblasts [209]. Importantly in mice chronic preventative 4PBA treatment reduced adult and paediatric ICH for some missense mutations [184, 222, 329]. 4PBA was also effective in reducing ICH severity once the disease was established [222], suggesting 4PBA could be beneficial for patients post-diagnosis with COL4A1 syndrome. Furthermore a short term transient treatment also reduced severity with lasting effect, but it should be noted that increased efficacy was obtained when treatment commences early in life [329]. However, efficacy of PBA was dose-dependent [329] and did not prevent or treat the ASD and Bowman's capsule defects [222]. This suggests that for patients with multi-systemic features a combinatorial treatment targeting both ECM defects and ER stress will be required [222].

4PBA not only reduces ER stress but also promotes secretion of collagen IV [209, 215, 222]. Worryingly, 4PBA treatment of mice with a glycine mutation reduced the ability of the dermal BM to withstand mechanical stress [222]. Moreover, 4PBA treatment increased severity of myopathy in a mouse model with a more N-terminal glycine mutation that did not cause ER stress [215]. One explanation may be that PBA caused secretion of mutant protein. PBA treatment may thus be contra-indicative for clinical features of COL4A1 syndrome due to ECM defects and missense mutations that do not induce ER stress.

This is also a consideration for AS due to missense mutations where treatment of patient cells with 4PBA was associated with reduced ER stress [305]. Given the mechanical stress to which the GBM is exposed, it is now key to determine if PBA has efficacy *in vivo* or whether it also negatively affects the GBM due to increased deposition of mutant protein.

In conclusion these intervention studies highlighted that a detailed understanding of the disease mechanisms will be required. A one size fits all therapeutic approach will be unlikely and patient stratification based on the molecular mechanisms of the mutations will be necessary.

10 Concluding remarks

It has been 30 years since the identification of the first collagen IV mutation in disease. Since then it has become apparent that these “simple” Mendelian diseases are complex multi-systemic disorders characterised by tissue and cell-specific disease mechanisms as well as mutation specific quantitative and qualitative effects on the collagen network. While it may seem that we have come a long way in increasing our understanding of the mechanisms of collagen IV biosynthesis and collagen IV disease, the hard truth is that major gaps in our knowledge remain. These range from gaps in our knowledge relating to fundamental aspects of collagen secretion and its regulation, (e.g. is collagen IV biosynthesis, like collagen I [133], under the control of circadian rhythm? What is the half-life of collagen IV in the ECM and how is BM degradation/turnover regulated?) to the disease mechanisms (e.g. what is the relative contribution of ER stress and ECM defects? What are the genetic modifiers?).

We are still in desperate need for treatment for collagen IV disorders and the role of collagen IV in human physiology and pathophysiology remains incomplete. In particular, besides the Mendelian disorders many questions remain about the role of these molecules in common forms of disease. Large population cohorts will undoubtedly be a powerful resource in addressing these. But even when it comes to Alport and COL4A1 syndromes, increased in-depth molecular mechanistic insight will be needed to develop the effective treatments that patients deserve and need. Key questions remain regarding the role of ER stress and ECM defects in disease and the mechanisms by which these defects exert their effects. Comparison across different diseases will be powerful and may allow us to transition from treatments based on clinical feature to phenotype-agnostic therapeutics that target key mechanisms. In many ways we have only just begun to lay the foundations for an exciting time in ECM research.

This chapter was written during the lockdown in the UK caused by the COVID19 pandemic and we hope that you are all safe and healthy.

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Table 1: Frequently used animal models of collagen-related genetic diseases. Due to space limitation, only the original reference describing the model has been provided.

BM component	Affected gene	Animal model	Disease Phenotype (or human equivalent)	References
Collagen IV	*Col4a1*	Mouse missense mutations	COL4A1 syndrome HANAC syndrome	[180-182, 220]
	Col4a1/ Col4a2 double null	Mouse	Embryonically lethal, growth retardation vascular defects	[20]
	Col4a1 (Cg25c) and *Col4a2 (Vkg)*	Drosophila missense and loss of function mutation	Intestinal defects, myopathy	[217]
	emb-9; let-2 (Cola4a1, Col4a2)	*C. elegans* missense mutations	Embryonic lethal	[216]
	Col4a2	Mouse missense mutations	COL4A1 syndrome	[182]
		Exon 18 deletion	Embryonic lethality Multiple organ defect	Reissig 2019
	Col4a3	Mouse knock-out and missense mutation	ARAS and ADAS	[304, 330, 331]
	Col4a3 & *Col4a4* double null	Mouse	juvenile form of AS	[332]
	Col4a4	Mouse missense mutation	ARAS	[333]
	Col4a5	Mouse knock-out and nonsense mutation	X-linked Alport Syndrome	[334, 335]
	Col4a5	Zebrafish in frame deletion	Defective retinal axon guiding	[336]
	Col4a6	Zebrafish in frame deletion	Defective axon guiding, cerebellar granule cells defects	[267]

Table 2 Overview of some of the clinical features of the Mendelian diseases due to mutations in collagen IV.

Disease	Tissue	feature	Reference
COL4A1 syndrome	Cerebrovascular	Intracerebral haemorrhage Cerebral small vessel disease Porencephaly	[183, 192]
	Brain white matter defect	White matter hyperintensities leukoencephalopathy	[183, 192]
	Brain cortical defects	Lissencephaly, hydranencephaly Schizencephaly Polymicrogyria focal cortical dysplasia nodular heterotopia	[183, 192]
	Eye	Arterial Tortuosity Anterior segment dysgenesis Retinal haemorrhage glaucoma High myopia Micro-opthalmia	[183 , 207]
	Muscle	Increased CK Muscle cramps Myopathy Walker Warburg Syndrome Muscle Eye Brain disease	[183, 193 , 196]
	Heart	Supraventricular arrhythmia Congenital heart defect, common arterial trunk	[169]. [183, 224]
	Kidney	Congenital anomalies of the kidney and urinary tract Urinary retention Glomerulopathy Cystic kidney disease Haematuria Tubular defect	[183, 223]) [337]
	PADMAL	Lacunar infarct Ischemic stroke Leukoencephalopathy	[60, 202]

	HANAC	CSVD, intracranial aneurysms, retinal tortuosity, ASD, muscle cramps, Raynaud phenomenon renal cysts, hematuria	[169, 183 , 219]
Alport syndrome			
	Kidney	Proteinuria, hematuria, end stage kidney disease	[74, 338]
	Eye	Posterior cataract, lenticonus Dot fleck retinopathy	[74, 338]
	Ear	Sensorineural deafness	[74, 338]
Alport syndrome - diffuse leiomyomatosis		Alport syndrome Smooth muscle cell tumour	[74]

Table 3: Mechanism-based therapeutic strategies for collagen-related diseases. Clinical trials are indicated in bold.

Disease	Mechanism-target	Treatment	References
COL4A1 Syndrome	ER retained protein, ER stress pathway	Chemical chaperones eg. 4-phenylbutyrate (4PBA)	[184, 185, 222] Labelle-Dumais 2019
Alport Syndrome	ER retained protein, ER stress	4PBA	Wang, 2017 #58
	Blood pressure	Angiotensin-converting enzyme inhibitors e.g. **Ramipril** Angiotensin II type 1 receptor blockers e.g **Losartan**	[309, 312-314]
	Fibrosis	HMG-CoA-reductase inhibitor (cerivastatin) Vasopeptidase inhibitor AVE7688 anti–miR-21 oligonucleotides STAT3 inhibitor eg. stattic	[315] [316] [322] [317]
	Oxidative stress, inflammation and fibrosis:	Nrf2 activator eg. bardoxolone methyl (BARD)	[318]
	Functional replacement	Gene therapy:	[323]
		Stem cell Bone marrow-derived stem cells Amniotic fluid stem cells	[325] [324]

References

1. Aouacheria A, Geourjon C, Aghajari N, Navratil V, Deleage G, Lethias C, et al. Insights into early extracellular matrix evolution: spongin short chain collagen-related proteins are homologous to basement membrane type IV collagens and form a novel family widely distributed in invertebrates. Mol Biol Evol. 2006;23(12):2288-302. doi: 10.1093/molbev/msl100.
2. Fidler AL, Darris CE, Chetyrkin SV, Pedchenko VK, Boudko SP, Brown KL, et al. Collagen IV and basement membrane at the evolutionary dawn of metazoan tissues. Elife. 2017;6. doi: 10.7554/eLife.24176.
3. Fidler AL, Boudko SP, Rokas A, Hudson BG. The triple helix of collagens - an ancient protein structure that enabled animal multicellularity and tissue evolution. J Cell Sci. 2018;131(7). doi: 10.1242/jcs.203950.
4. Dai J, Ma M, Feng Z, Pastor-Pareja JC. Inter-adipocyte Adhesion and Signaling by Collagen IV Intercellular Concentrations in Drosophila. Curr Biol. 2017;27(18):2729-40 e4. doi: 10.1016/j.cub.2017.08.002.
5. Haraida S, Nerlich AG, Wiest I, Schleicher E, Lohrs U. Distribution of basement membrane components in normal adipose tissue and in benign and malignant tumors of lipomatous origin. Mod Pathol. 1996;9(2):137-44.
6. Noro A, Sillat T, Virtanen I, Ingerpuu S, Back N, Konttinen YT, et al. Laminin production and basement membrane deposition by mesenchymal stem cells upon adipogenic differentiation. J Histochem Cytochem. 2013;61(10):719-30. doi: 10.1369/0022155413502055.
7. Nguyen H, Ostendorf AP, Satz JS, Westra S, Ross-Barta SE, Campbell KP, et al. Glial scaffold required for cerebellar granule cell migration is dependent on dystroglycan function as a receptor for basement membrane proteins. Acta Neuropathol Commun. 2013;1:58. doi: 10.1186/2051-5960-1-58.
8. Sanes JR. The basement membrane/basal lamina of skeletal muscle. J Biol Chem. 2003;278(15):12601-4. doi: 10.1074/jbc.R200027200.
9. Walker CA, Spinale FG. The structure and function of the cardiac myocyte: a review of fundamental concepts. J Thorac Cardiovasc Surg. 1999;118(2):375-82. doi: 10.1016/S0022-5223(99)70233-3.
10. Hedin U, Roy J, Tran PK, Lundmark K, Rahman A. Control of smooth muscle cell proliferation--the role of the basement membrane. Thromb Haemost. 1999;82 Suppl 1:23-6.
11. Fox MA, Sanes JR, Borza DB, Eswarakumar VP, Fassler R, Hudson BG, et al. Distinct target-derived signals organize formation, maturation, and maintenance of motor nerve terminals. Cell. 2007;129(1):179-93. doi: 10.1016/j.cell.2007.02.035.
12. Ricard-Blum S. The collagen family. Cold Spring Harb Perspect Biol. 2011;3(1):a004978. doi: 10.1101/cshperspect.a004978.
13. Vracko R. Basal lamina scaffold-anatomy and significance for maintenance of orderly tissue structure. Am J Pathol. 1974;77(2):314-46.
14. Wang X, Harris RE, Bayston LJ, Ashe HL. Type IV collagens regulate BMP signalling in Drosophila. Nature. 2008;455(7209):72-7. doi: 10.1038/nature07214.
15. Yurchenco PD. Basement membranes: cell scaffoldings and signaling platforms. Cold Spring Harb Perspect Biol. 2011;3(2). doi: 10.1101/cshperspect.a004911.
16. Jayadev R, Sherwood DR. Basement membranes. Curr Biol. 2017;27(6):R207-R11. doi: 10.1016/j.cub.2017.02.006.
17. Pozzi A, Yurchenco PD, Iozzo RV. The nature and biology of basement membranes. Matrix Biol. 2017;57-58:1-11. doi: 10.1016/j.matbio.2016.12.009.
18. Van Agtmael T, Bruckner-Tuderman L. Basement membranes and human disease. Cell Tissue Res. 2010;339(1):167-88. doi: 10.1007/s00441-009-0866-y.

19. Smyth N, Vatansever HS, Murray P, Meyer M, Frie C, Paulsson M, et al. Absence of basement membranes after targeting the LAMC1 gene results in embryonic lethality due to failure of endoderm differentiation. J Cell Biol. 1999;144(1):151-60. doi: 10.1083/jcb.144.1.151.
20. Poschl E, Schlotzer-Schrehardt U, Brachvogel B, Saito K, Ninomiya Y, Mayer U. Collagen IV is essential for basement membrane stability but dispensable for initiation of its assembly during early development. Development. 2004;131(7):1619-28. doi: 10.1242/dev.01037.
21. Jayadev R, Chi Q, Keeley DP, Hastie EL, Kelley LC, Sherwood DR. alpha-Integrins dictate distinct modes of type IV collagen recruitment to basement membranes. J Cell Biol. 2019;218(9):3098-116. doi: 10.1083/jcb.201903124.
22. Dai J, Estrada B, Jacobs S, Sánchez-Sánchez BJ, Tang J, Ma M, et al. Dissection of Nidogen function in Drosophila reveals tissue-specific mechanisms of basement membrane assembly. PLoS Genet. 2018;14(9):e1007483. doi: 10.1371/journal.pgen.1007483.
23. Pastor-Pareja JC, Xu T. Shaping cells and organs in Drosophila by opposing roles of fat body-secreted Collagen IV and perlecan. Dev Cell. 2011;21(2):245-56. doi: 10.1016/j.devcel.2011.06.026.
24. Yamada Y, Kato K, Oguri M, Fujimaki T, Yokoi K, Matsuo H, et al. Genetic risk for myocardial infarction determined by polymorphisms of candidate genes in a Japanese population. J Med Genet. 2008;45(4):216-21. doi: 10.1136/jmg.2007.054387.
25. Tarasov KV, Sanna S, Scuteri A, Strait JB, Orrù M, Parsa A, et al. COL4A1 Is Associated With Arterial Stiffness by Genome-Wide Association Scan. Circ Cardiovasc Genet. 2009;2(2):151-8. doi: doi:10.1161/CIRCGENETICS.108.823245.
26. O'Donnell CJ, Kavousi M, Smith AV, Kardia SL, Feitosa MF, Hwang SJ, et al. Genome-wide association study for coronary artery calcification with follow-up in myocardial infarction. Circulation. 2011;124(25):2855-64. doi: 10.1161/CIRCULATIONAHA.110.974899.
27. Schunkert H, Konig IR, Kathiresan S, Reilly MP, Assimes TL, Holm H, et al. Large-scale association analysis identifies 13 new susceptibility loci for coronary artery disease. Nat Genet. 2011;43(4):333-8. doi: 10.1038/ng.784.
28. Rannikmae K, Davies G, Thomson PA, Bevan S, Devan WJ, Falcone GJ, et al. Common variation in COL4A1/COL4A2 is associated with sporadic cerebral small vessel disease. Neurology. 2015;84(9):918-26. doi: 10.1212/WNL.0000000000001309.
29. Traylor M, Zhang CR, Adib-Samii P, Devan WJ, Parsons OE, Lanfranconi S, et al. Genome-wide meta-analysis of cerebral white matter hyperintensities in patients with stroke. Neurology. 2016;86(2):146-53. doi: 10.1212/wnl.0000000000002263.
30. Rannikmae K, Sivakumaran V, Millar H, Malik R, Anderson CD, Chong M, et al. COL4A2 is associated with lacunar ischemic stroke and deep ICH: Meta-analyses among 21,500 cases and 40,600 controls. Neurology. 2017;89(17):1829-39. doi: 10.1212/WNL.0000000000004560.
31. Chung J, Marini S, Pera J, Norrving B, Jimenez-Conde J, Roquer J, et al. Genome-wide association study of cerebral small vessel disease reveals established and novel loci. Brain. 2019;142(10):3176-89. doi: 10.1093/brain/awz233.
32. Kefalides NA. A collagen of unusual composition and a glycoprotein isolated from canine glomerular basement membrane. Biochem Biophys Res Commun. 1966;22(1):26-32. doi: 10.1016/0006-291x(66)90597-3.
33. Kefalides NA. Structure and biosynthesis of basement membranes. Int Rev Connect Tissue Res. 1973;6:63-104. doi: 10.1016/b978-0-12-363706-2.50008-8.
34. Minor RR, Clark CC, Strause EL, Koszalka TR, Brent RL, Kefalides NA. Basement membrane procollagen is not converted to collagen in organ cultures of parietal yolk sac endoderm. J Biol Chem. 1976;251(6):1789-94.
35. Hynes RO. The evolution of metazoan extracellular matrix. J Cell Biol. 2012;196(6):671-9. doi: 10.1083/jcb.201109041.
36. Exposito JY, Le Guellec D, Lu Q, Garrone R. Short chain collagens in sponges are encoded by a family of closely related genes. J Biol Chem. 1991;266(32):21923-8.

37. Ryan JF, Pang K, Schnitzler CE, Nguyen AD, Moreland RT, Simmons DK, et al. The genome of the ctenophore Mnemiopsis leidyi and its implications for cell type evolution. Science. 2013;342(6164):1242592. doi: 10.1126/science.1242592.
38. Moroz LL, Kocot KM, Citarella MR, Dosung S, Norekian TP, Povolotskaya IS, et al. The ctenophore genome and the evolutionary origins of neural systems. Nature. 2014;510(7503):109-14. doi: 10.1038/nature13400.
39. Draper GW, Shoemark DK, Adams JC. Modelling the early evolution of extracellular matrix from modern Ctenophores and Sponges. Essays Biochem. 2019;63(3):389-405. doi: 10.1042/EBC20180048.
40. Boute N, Exposito JY, Boury-Esnault N, Vacelet J, Noro N, Miyazaki K, et al. Type IV collagen in sponges, the missing link in basement membrane ubiquity. Biol Cell. 1996;88(1-2):37-44. doi: 10.1016/s0248-4900(97)86829-3.
41. Nauroy P, Hughes S, Naba A, Ruggiero F. The in-silico zebrafish matrisome: A new tool to study extracellular matrix gene and protein functions. Matrix Biol. 2018;65:5-13. doi: https://doi.org/10.1016/j.matbio.2017.07.001.
42. Poschl E, Pollner R, Kuhn K. The genes for the alpha 1(IV) and alpha 2(IV) chains of human basement membrane collagen type IV are arranged head-to-head and separated by a bidirectional promoter of unique structure. EMBO J. 1988;7(9):2687-95.
43. Hostikka SL, Eddy RL, Byers MG, Hoyhtya M, Shows TB, Tryggvason K. Identification of a distinct type IV collagen alpha chain with restricted kidney distribution and assignment of its gene to the locus of X chromosome-linked Alport syndrome. Proc Natl Acad Sci U S A. 1990;87(4):1606-10. doi: 10.1073/pnas.87.4.1606.
44. Mariyama M, Leinonen A, Mochizuki T, Tryggvason K, Reeders ST. Complete primary structure of the human alpha 3(IV) collagen chain. Coexpression of the alpha 3(IV) and alpha 4(IV) collagen chains in human tissues. J Biol Chem. 1994;269(37):23013-7.
45. Sugimoto M, Oohashi T, Ninomiya Y. The genes COL4A5 and COL4A6, coding for basement membrane collagen chains alpha 5(IV) and alpha 6(IV), are located head-to-head in close proximity on human chromosome Xq22 and COL4A6 is transcribed from two alternative promoters. Proc Natl Acad Sci U S A. 1994;91(24):11679-83. doi: 10.1073/pnas.91.24.11679.
46. Zhou J, Leinonen A, Tryggvason K. Structure of the human type IV collagen COL4A5 gene. J Biol Chem. 1994;269(9):6608-14.
47. Momota R, Sugimoto M, Oohashi T, Kigasawa K, Yoshioka H, Ninomiya Y. Two genes, COL4A3 and COL4A4 coding for the human alpha3(IV) and alpha4(IV) collagen chains are arranged head-to-head on chromosome 2q36. FEBS Lett. 1998;424(1-2):11-6. doi: 10.1016/s0014-5793(98)00128-8.
48. Netzer KO, Suzuki K, Itoh Y, Hudson BG, Khalifah RG. Comparative analysis of the noncollagenous NC1 domain of type IV collagen: identification of structural features important for assembly, function, and pathogenesis. Protein Sci. 1998;7(6):1340-51. doi: 10.1002/pro.5560070610.
49. Soininen R, Huotari M, Hostikka SL, Prockop DJ, Tryggvason K. The structural genes for alpha 1 and alpha 2 chains of human type IV collagen are divergently encoded on opposite DNA strands and have an overlapping promoter region. J Biol Chem. 1988;263(33):17217-20.
50. Heikkila P, Soininen R, Tryggvason K. Directional regulatory activity of cis-acting elements in the bidirectional alpha 1(IV) and alpha 2(IV) collagen gene promoter. J Biol Chem. 1993;268(33):24677-82.
51. Fischer G, Schmidt C, Opitz J, Cully Z, Kuhn K, Poschl E. Identification of a novel sequence element in the common promoter region of human collagen type IV genes, involved in the regulation of divergent transcription. Biochem J. 1993;292 (Pt 3):687-95. doi: 10.1042/bj2920687.
52. Schmidt C, Fischer G, Kadner H, Genersch E, Kuhn K, Poschl E. Differential effects of DNA-binding proteins on bidirectional transcription from the common promoter region of human collagen type IV genes COL4A1 and COL4A2. Biochim Biophys Acta. 1993;1174(1):1-10. doi: 10.1016/0167-4781(93)90085-r.

53. Pollner R, Schmidt C, Fischer G, Kuhn K, Poschl E. Cooperative and competitive interactions of regulatory elements are involved in the control of divergent transcription of human Col4A1 and Col4A2 genes. FEBS Lett. 1997;405(1):31-6. doi: 10.1016/s0014-5793(97)00152-x.
54. Haniel A, Welge-Lussen U, Kuhn K, Poschl E. Identification and characterization of a novel transcriptional silencer in the human collagen type IV gene COL4A2. J Biol Chem. 1995;270(19):11209-15. doi: 10.1074/jbc.270.19.11209.
55. Schmidt C, Pollner R, Poschl E, Kuhn K. Expression of human collagen type IV genes is regulated by transcriptional and post-transcriptional mechanisms. FEBS Lett. 1992;312(2-3):174-8. doi: 10.1016/0014-5793(92)80929-b.
56. Abe H, Matsubara T, Iehara N, Nagai K, Takahashi T, Arai H, et al. Type IV collagen is transcriptionally regulated by Smad1 under advanced glycation end product (AGE) stimulation. J Biol Chem. 2004;279(14):14201-6. doi: 10.1074/jbc.M310427200.
57. Jiang W, Zhang Y, Wu H, Zhang X, Gan H, Sun J, et al. Role of cross-talk between the Smad2 and MAPK pathways in TGF-beta1-induced collagen IV expression in mesangial cells. Int J Mol Med. 2010;26(4):571-6. doi: 10.3892/ijmm_00000501.
58. Chiusa M, Hu W, Liao HJ, Su Y, Borza CM, de Caestecker MP, et al. The Extracellular Matrix Receptor Discoidin Domain Receptor 1 Regulates Collagen Transcription by Translocating to the Nucleus. J Am Soc Nephrol. 2019;30(9):1605-24. doi: 10.1681/ASN.2018111160.
59. Takahashi M, Eda A, Fukushima T, Hohjoh H. Reduction of type IV collagen by upregulated miR-29 in normal elderly mouse and klotho-deficient, senescence-model mouse. PLoS One. 2012;7(11):e48974. doi: 10.1371/journal.pone.0048974.
60. Verdura E, Herve D, Bergametti F, Jacquet C, Morvan T, Prieto-Morin C, et al. Disruption of a miR-29 binding site leading to COL4A1 upregulation causes pontine autosomal dominant microangiopathy with leukoencephalopathy. Ann Neurol. 2016;80(5):741-53. doi: 10.1002/ana.24782.
61. Griffiths M, Van Sinderen M, Rainczuk K, Dimitriadis E. miR-29c overexpression and COL4A1 downregulation in infertile human endometrium reduces endometrial epithelial cell adhesive capacity in vitro implying roles in receptivity. Sci Rep. 2019;9(1):8644. doi: 10.1038/s41598-019-45155-6.
62. Licholai S, Szczeklik W, Sanak M. miR-29c-3p is an Effective Biomarker of Abdominal Aortic Aneurysm in Patients Undergoing Elective Surgery. Microrna. 2016;5(2):124-31. doi: 10.2174/2211536605666160901103754.
63. Turner N, Mason PJ, Brown R, Fox M, Povey S, Rees A, et al. Molecular cloning of the human Goodpasture antigen demonstrates it to be the alpha 3 chain of type IV collagen. J Clin Invest. 1992;89(2):592-601. doi: 10.1172/JCI115625.
64. Kamagata Y, Mattei MG, Ninomiya Y. Isolation and sequencing of cDNAs and genomic DNAs encoding the alpha 4 chain of basement membrane collagen type IV and assignment of the gene to the distal long arm of human chromosome 2. J Biol Chem. 1992;267(33):23753-8.
65. Oohashi T, Sugimoto M, Mattei MG, Ninomiya Y. Identification of a new collagen IV chain, alpha 6(IV), by cDNA isolation and assignment of the gene to chromosome Xq22, which is the same locus for COL4A5. J Biol Chem. 1994;269(10):7520-6.
66. Zhang X, Zhou J, Reeders ST, Tryggvason K. Structure of the human type IV collagen COL4A6 gene, which is mutated in Alport syndrome-associated leiomyomatosis. Genomics. 1996;33(3):473-9. doi: 10.1006/geno.1996.0222.
67. Ninomiya Y, Kagawa M, Iyama K, Naito I, Kishiro Y, Seyer JM, et al. Differential expression of two basement membrane collagen genes, COL4A6 and COL4A5, demonstrated by immunofluorescence staining using peptide-specific monoclonal antibodies. J Cell Biol. 1995;130(5):1219-29. doi: 10.1083/jcb.130.5.1219.
68. Sund M, Maeshima Y, Kalluri R. Bifunctional promoter of type IV collagen COL4A5 and COL4A6 genes regulates the expression of alpha5 and alpha6 chains in a distinct cell-specific fashion. Biochem J. 2005;387(Pt 3):755-61. doi: 10.1042/BJ20041870.

69. Segal Y, Zhuang L, Rondeau E, Sraer JD, Zhou J. Regulation of the paired type IV collagen genes COL4A5 and COL4A6. Role of the proximal promoter region. J Biol Chem. 2001;276(15):11791-7. doi: 10.1074/jbc.M007477200.
70. Timpl R, Wiedemann H, van Delden V, Furthmayr H, Kuhn K. A network model for the organization of type IV collagen molecules in basement membranes. Eur J Biochem. 1981;120(2):203-11. doi: 10.1111/j.1432-1033.1981.tb05690.x.
71. Risteli J, Bachinger HP, Engel J, Furthmayr H, Timpl R. 7-S collagen: characterization of an unusual basement membrane structure. Eur J Biochem. 1980;108(1):239-50. doi: 10.1111/j.1432-1033.1980.tb04717.x.
72. Raija S, Haka-Risku T, Prockop DJ, Tryggvason K. Complete primary structure of the α1-chain of human basement membrane (type IV) collagen. FEBS Lett. 1987;225(1-2):188-94. doi: 10.1016/0014-5793(87)81155-9.
73. Vandenberg P, Kern A, Ries A, Luckenbill-Edds L, Mann K, Kuhn K. Characterization of a type IV collagen major cell binding site with affinity to the alpha 1 beta 1 and the alpha 2 beta 1 integrins. J Cell Biol. 1991;113(6):1475-83. doi: 10.1083/jcb.113.6.1475.
74. Hudson BG, Tryggvason K, Sundaramoorthy M, Neilson EG. Alport's syndrome, Goodpasture's syndrome, and type IV collagen. N Engl J Med. 2003;348(25):2543-56. doi: 10.1056/NEJMra022296.
75. Parkin JD, San Antonio JD, Pedchenko V, Hudson B, Jensen ST, Savige J. Mapping structural landmarks, ligand binding sites, and missense mutations to the collagen IV heterotrimers predicts major functional domains, novel interactions, and variation in phenotypes in inherited diseases affecting basement membranes. Hum Mutat. 2011;32(2):127-43. doi: 10.1002/humu.21401.
76. Maeshima Y, Colorado PC, Torre A, Holthaus KA, Grunkemeyer JA, Ericksen MB, et al. Distinct antitumor properties of a type IV collagen domain derived from basement membrane. J Biol Chem. 2000;275(28):21340-8. doi: 10.1074/jbc.M001956200.
77. Mundel TM, Kalluri R. Type IV collagen-derived angiogenesis inhibitors. Microvasc Res. 2007;74(2-3):85-9. doi: 10.1016/j.mvr.2007.05.005.
78. Petitclerc E, Boutaud A, Prestayko A, Xu J, Sado Y, Ninomiya Y, et al. New Functions for Non-collagenous Domains of Human Collagen Type IV: NOVEL INTEGRIN LIGANDS INHIBITING ANGIOGENESIS AND TUMOR GROWTHIN VIVO. J Biol Chem. 2000;275(11):8051-61. doi: 10.1074/jbc.275.11.8051.
79. Colorado PC, Torre A, Kamphaus G, Maeshima Y, Hopfer H, Takahashi K, et al. Anti-angiogenic cues from vascular basement membrane collagen. Cancer Res. 2000;60(9):2520-6.
80. He G-A, Luo J-X, Zhang T-Y, Wang F-Y, Li R-F. Canstatin-N fragment inhibits in vitro endothelial cell proliferation and suppresses in vivo tumor growth. Biochem Biophys Res Commun. 2003;312(3):801-5. doi: https://doi.org/10.1016/j.bbrc.2003.11.003.
81. Boosani CS, Sudhakar A. Cloning, purification, and characterization of a non-collagenous anti-angiogenic protein domain from human alpha1 type IV collagen expressed in Sf9 cells. Protein Expr Purif. 2006;49(2):211-8. doi: 10.1016/j.pep.2006.03.007.
82. Koskimaki JE, Karagiannis ED, Tang BC, Hammers H, Watkins DN, Pili R, et al. Pentastatin-1, a collagen IV derived 20-mer peptide, suppresses tumor growth in a small cell lung cancer xenograft model. BMC Cancer. 2010;10(1):29. doi: 10.1186/1471-2407-10-29.
83. Brassart-Pasco S, Sénéchal K, Thevenard J, Ramont L, Devy J, Di Stefano L, et al. Tetrastatin, the NC1 Domain of the α4(IV) Collagen Chain: A Novel Potent Anti-Tumor Matrikine. PLoS One. 2012;7(4):e29587. doi: 10.1371/journal.pone.0029587.
84. Brazel D, Oberbaumer I, Dieringer H, Babel W, Glanville RW, Deutzmann R, et al. Completion of the amino acid sequence of the alpha 1 chain of human basement membrane collagen (type IV) reveals 21 non-triplet interruptions located within the collagenous domain. Eur J Biochem. 1987;168(3):529-36. doi: 10.1111/j.1432-1033.1987.tb13450.x.
85. Brazel D, Pollner R, Oberbaumer I, Kuhn K. Human basement membrane collagen (type IV). The amino acid sequence of the alpha 2(IV) chain and its comparison with the alpha 1(IV) chain reveals

deletions in the alpha 1(IV) chain. Eur J Biochem. 1988;172(1):35-42. doi: 10.1111/j.1432-1033.1988.tb13852.x.
86. Hostikka SL, Tryggvason K. The complete primary structure of the alpha 2 chain of human type IV collagen and comparison with the alpha 1(IV) chain. J Biol Chem. 1988;263(36):19488-93.
87. Zhou J, Hertz JM, Tryggvason K. Mutation in the alpha 5(IV) collagen chain in juvenile-onset Alport syndrome without hearing loss or ocular lesions: detection by denaturing gradient gel electrophoresis of a PCR product. Am J Hum Genet. 1992;50(6):1291-300.
88. Zhou J, Ding M, Zhao Z, Reeders ST. Complete primary structure of the sixth chain of human basement membrane collagen, alpha 6(IV). Isolation of the cDNAs for alpha 6(IV) and comparison with five other type IV collagen chains. J Biol Chem. 1994;269(18):13193-9.
89. Yamada Y, Avvedimento VE, Mudryj M, Ohkubo H, Vogeli G, Irani M, et al. The collagen gene: evidence for its evolutinary assembly by amplification of a DNA segment containing an exon of 54 bp. Cell. 1980;22(3):887-92. doi: 10.1016/0092-8674(80)90565-6.
90. Buttice G, Kaytes P, D'Armiento J, Vogeli G, Kurkinen M. Evolution of collagen IV genes from a 54-base pair exon: a role for introns in gene evolution. J Mol Evol. 1990;30(6):479-88. doi: 10.1007/bf02101102.
91. Leinonen A, Mariyama M, Mochizuki T, Tryggvason K, Reeders ST. Complete primary structure of the human type IV collagen alpha 4(IV) chain. Comparison with structure and expression of the other alpha (IV) chains. J Biol Chem. 1994;269(42):26172-7.
92. Yurchenco PD, Furthmayr H. Self-assembly of basement membrane collagen. Biochemistry. 1984;23(8):1839-50. doi: 10.1021/bi00303a040.
93. Yurchenco PD, Ruben GC. Basement membrane structure in situ: evidence for lateral associations in the type IV collagen network. J Cell Biol. 1987;105(6 Pt 1):2559-68. doi: 10.1083/jcb.105.6.2559.
94. Yurchenco PD, Ruben GC. Type IV collagen lateral associations in the EHS tumor matrix. Comparison with amniotic and in vitro networks. Am J Pathol. 1988;132(2):278-91.
95. Oberbaumer I, Laurent M, Schwarz U, Sakurai Y, Yamada Y, Vogeli G, et al. Amino acid sequence of the non-collagenous globular domain (NC1) of the alpha 1(IV) chain of basement membrane collagen as derived from complementary DNA. Eur J Biochem. 1985;147(2):217-24. doi: 10.1111/j.1432-1033.1985.tb08739.x.
96. Pihlajaniemi T, Tryggvason K, Myers JC, Kurkinen M, Lebo R, Cheung MC, et al. cDNA clones coding for the pro-alpha1(IV) chain of human type IV procollagen reveal an unusual homology of amino acid sequences in two halves of the carboxyl-terminal domain. J Biol Chem. 1985;260(12):7681-7.
97. Schwarz-Magdolen U, Oberbaumer I, Kuhn K. cDNA and protein sequence of the NC1 domain of the alpha 2-chain of collagen IV and its comparison with alpha 1(IV). FEBS Lett. 1986;208(2):203-7. doi: 10.1016/0014-5793(86)81018-3.
98. Soder S, Poschl E. The NC1 domain of human collagen IV is necessary to initiate triple helix formation. Biochem Biophys Res Commun. 2004;325(1):276-80. doi: 10.1016/j.bbrc.2004.10.034.
99. Siebold B, Deutzmann R, Kuhn K. The arrangement of intra- and intermolecular disulfide bonds in the carboxyterminal, non-collagenous aggregation and cross-linking domain of basement-membrane type IV collagen. Eur J Biochem. 1988;176(3):617-24. doi: 10.1111/j.1432-1033.1988.tb14321.x.
100. Khoshnoodi J, Sigmundsson K, Cartailler JP, Bondar O, Sundaramoorthy M, Hudson BG. Mechanism of chain selection in the assembly of collagen IV: a prominent role for the alpha2 chain. J Biol Chem. 2006;281(9):6058-69. doi: 10.1074/jbc.M506555200.
101. Khoshnoodi J, Cartailler JP, Alvares K, Veis A, Hudson BG. Molecular recognition in the assembly of collagens: terminal noncollagenous domains are key recognition modules in the formation of triple helical protomers. J Biol Chem. 2006;281(50):38117-21. doi: 10.1074/jbc.R600025200.
102. Ziomber A, Machnik A, Dahlmann A, Dietsch P, Beck FX, Wagner H, et al. Sodium-, potassium-, chloride-, and bicarbonate-related effects on blood pressure and electrolyte homeostasis in deoxycorticosterone acetate-treated rats. Am J Physiol Renal Physiol. 2008;295(6):F1752-63. doi: 10.1152/ajprenal.00531.2007.

103. Cummings CF, Pedchenko V, Brown KL, Colon S, Rafi M, Jones-Paris C, et al. Extracellular chloride signals collagen IV network assembly during basement membrane formation. J Cell Biol. 2016;213(4):479-94. doi: 10.1083/jcb.201510065.
104. Myllyharju J, Kivirikko KI. Collagens, modifying enzymes and their mutations in humans, flies and worms. Trends Genet. 2004;20(1):33-43. doi: 10.1016/j.tig.2003.11.004.
105. Makareeva E, Aviles NA, Leikin S. Chaperoning osteogenesis: new protein-folding disease paradigms. Trends Cell Biol. 2011;21(3):168-76. doi: 10.1016/j.tcb.2010.11.007.
106. Forlino A, Marini JC. Osteogenesis imperfecta. Lancet. 2016;387(10028):1657-71. doi: 10.1016/S0140-6736(15)00728-X.
107. Shoulders MD, Raines RT. Collagen structure and stability. Annu Rev Biochem. 2009;78:929-58. doi: 10.1146/annurev.biochem.77.032207.120833.
108. Kivirikko KI, Pihlajaniemi T. Collagen hydroxylases and the protein disulfide isomerase subunit of prolyl 4-hydroxylases. Adv Enzymol Relat Areas Mol Biol. 1998;72:325-98. doi: 10.1002/9780470123188.ch9.
109. Myllyharju J. Prolyl 4-hydroxylases, key enzymes in the synthesis of collagens and regulation of the response to hypoxia, and their roles as treatment targets. Ann Med. 2008;40(6):402-17. doi: 10.1080/07853890801986594.
110. Tiainen P, Pasanen A, Sormunen R, Myllyharju J. Characterization of recombinant human prolyl 3-hydroxylase isoenzyme 2, an enzyme modifying the basement membrane collagen IV. J Biol Chem. 2008;283(28):19432-9. doi: 10.1074/jbc.M802973200.
111. Friedman L, Higgin JJ, Moulder G, Barstead R, Raines RT, Kimble J. Prolyl 4-hydroxylase is required for viability and morphogenesis in Caenorhabditis elegans. Proc Natl Acad Sci U S A. 2000;97(9):4736-41. doi: 10.1073/pnas.97.9.4736.
112. Holster T, Pakkanen O, Soininen R, Sormunen R, Nokelainen M, Kivirikko KI, et al. Loss of assembly of the main basement membrane collagen, type IV, but not fibril-forming collagens and embryonic death in collagen prolyl 4-hydroxylase I null mice. J Biol Chem. 2007;282(4):2512-9. doi: 10.1074/jbc.M606608200.
113. Zou Y, Donkervoort S, Salo AM, Foley AR, Barnes AM, Hu Y, et al. P4HA1 mutations cause a unique congenital disorder of connective tissue involving tendon, bone, muscle and the eye. Hum Mol Genet. 2017;26(12):2207-17. doi: 10.1093/hmg/ddx110.
114. Ishikawa Y, Wirz J, Vranka JA, Nagata K, Bachinger HP. Biochemical characterization of the prolyl 3-hydroxylase 1.cartilage-associated protein.cyclophilin B complex. J Biol Chem. 2009;284(26):17641-7. doi: 10.1074/jbc.M109.007070.
115. Pokidysheva E, Boudko S, Vranka J, Zientek K, Maddox K, Moser M, et al. Biological role of prolyl 3-hydroxylation in type IV collagen. Proc Natl Acad Sci U S A. 2014;111(1):161-6. doi: 10.1073/pnas.1307597111.
116. Mizuno K, Hayashi T, Peyton DH, Bachinger HP. The peptides acetyl-(Gly-3(S)Hyp-4(R)Hyp)10-NH2 and acetyl-(Gly-Pro-3(S)Hyp)10-NH2 do not form a collagen triple helix. J Biol Chem. 2004;279(1):282-7. doi: 10.1074/jbc.M308181200.
117. Montgomery NT, Zientek KD, Pokidysheva EN, Bachinger HP. Post-translational modification of type IV collagen with 3-hydroxyproline affects its interactions with glycoprotein VI and nidogens 1 and 2. J Biol Chem. 2018;293(16):5987-99. doi: 10.1074/jbc.RA117.000406.
118. Hudson DM, Joeng KS, Werther R, Rajagopal A, Weis M, Lee BH, et al. Post-translationally abnormal collagens of prolyl 3-hydroxylase-2 null mice offer a pathobiological mechanism for the high myopia linked to human LEPREL1 mutations. J Biol Chem. 2015;290(13):8613-22. doi: 10.1074/jbc.M114.634915.
119. Vranka J, Stadler HS, auml, chinger HP. Expression of Prolyl 3-hydroxylase Genes in Embryonic and Adult Mouse Tissues. Cell Struct Funct. 2009;34(2):97-104. doi: 10.1247/csf.09002.
120. Rappu P, Salo AM, Myllyharju J, Heino J. Role of prolyl hydroxylation in the molecular interactions of collagens. Essays Biochem. 2019;63(3):325-35. doi: 10.1042/EBC20180053.

121. Rautavuoma K, Takaluoma K, Sormunen R, Myllyharju J, Kivirikko KI, Soininen R. Premature aggregation of type IV collagen and early lethality in lysyl hydroxylase 3 null mice. Proc Natl Acad Sci U S A. 2004;101(39):14120-5. doi: 10.1073/pnas.0404966101.
122. Norman KR, Moerman DG. The let-268 Locus of Caenorhabditis elegans Encodes a Procollagen Lysyl Hydroxylase That Is Essential for Type IV Collagen Secretion. Dev Biol. 2000;227(2):690-705. doi: https://doi.org/10.1006/dbio.2000.9897.
123. Ruotsalainen H, Sipila L, Vapola M, Sormunen R, Salo AM, Uitto L, et al. Glycosylation catalyzed by lysyl hydroxylase 3 is essential for basement membranes. J Cell Sci. 2006;119(Pt 4):625-35. doi: 10.1242/jcs.02780.
124. Miyatake S, Schneeberger S, Koyama N, Yokochi K, Ohmura K, Shiina M, et al. Biallelic COLGALT1 variants are associated with cerebral small vessel disease. Ann Neurol. 2018;84(6):843-53. doi: 10.1002/ana.25367.
125. Perrucci GL, Gowran A, Zanobini M, Capogrossi MC, Pompilio G, Nigro P. Peptidyl-prolyl isomerases: a full cast of critical actors in cardiovascular diseases. Cardiovasc Res. 2015;106(3):353-64. doi: 10.1093/cvr/cvv096.
126. Ito S, Nagata K. Roles of the endoplasmic reticulum–resident, collagen-specific molecular chaperone Hsp47 in vertebrate cells and human disease. J Biol Chem. 2019;294(6):2133-41. doi: 10.1074/jbc.TM118.002812.
127. Nagai N, Hosokawa M, Itohara S, Adachi E, Matsushita T, Hosokawa N, et al. Embryonic lethality of molecular chaperone hsp47 knockout mice is associated with defects in collagen biosynthesis. J Cell Biol. 2000;150(6):1499-506. doi: 10.1083/jcb.150.6.1499.
128. Matsuoka Y, Kubota H, Adachi E, Nagai N, Marutani T, Hosokawa N, et al. Insufficient folding of type IV collagen and formation of abnormal basement membrane-like structure in embryoid bodies derived from Hsp47-null embryonic stem cells. Mol Biol Cell. 2004;15(10):4467-75. doi: 10.1091/mbc.e04-01-0050.
129. Fujii KK, Taga Y, Sakai T, Ito S, Hattori S, Nagata K, et al. Lowering the culture temperature corrects collagen abnormalities caused by HSP47 gene knockout. Sci Rep. 2019;9(1):17433. doi: 10.1038/s41598-019-53962-0.
130. Saito K, Chen M, Bard F, Chen S, Zhou H, Woodley D, et al. TANGO1 facilitates cargo loading at endoplasmic reticulum exit sites. Cell. 2009;136(5):891-902. doi: 10.1016/j.cell.2008.12.025.
131. Omari S, Makareeva E, Roberts-Pilgrim A, Mirigian L, Jarnik M, Ott C, et al. Noncanonical autophagy at ER exit sites regulates procollagen turnover. Proc Natl Acad Sci U S A. 2018;115(43):E10099-E108. doi: 10.1073/pnas.1814552115.
132. McCaughey J, Stevenson NL, Cross S, Stephens DJ. ER-to-Golgi trafficking of procollagen in the absence of large carriers. J Cell Biol. 2019;218(3):929-48. doi: 10.1083/jcb.201806035.
133. Chang J, Garva R, Pickard A, Yeung CC, Mallikarjun V, Swift J, et al. Circadian control of the secretory pathway maintains collagen homeostasis. Nat Cell Biol. 2020;22(1):74-86. doi: 10.1038/s41556-019-0441-z.
134. Malhotra V, Erlmann P. The pathway of collagen secretion. Annu Rev Cell Dev Biol. 2015;31:109-24. doi: 10.1146/annurev-cellbio-100913-013002.
135. McCaughey J, Stephens DJ. ER-to-Golgi Transport: A Sizeable Problem. Trends Cell Biol. 2019;29(12):940-53. doi: 10.1016/j.tcb.2019.08.007.
136. Ishikawa Y, Ito S, Nagata K, Sakai LY, Bachinger HP. Intracellular mechanisms of molecular recognition and sorting for transport of large extracellular matrix molecules. Proc Natl Acad Sci U S A. 2016;113(41):E6036-E44. doi: 10.1073/pnas.1609571113.
137. Raote I, Malhotra V. Protein transport by vesicles and tunnels. J Cell Biol. 2019;218(3):737-9. doi: 10.1083/jcb.201811073.
138. Donald JE, Kulp DW, DeGrado WF. Salt bridges: geometrically specific, designable interactions. Proteins. 2011;79(3):898-915. doi: 10.1002/prot.22927.
139. Brown KL, Cummings CF, Vanacore RM, Hudson BG. Building collagen IV smart scaffolds on the outside of cells. Protein Sci. 2017;26(11):2151-61. doi: 10.1002/pro.3283.

140. Bhave G, Cummings CF, Vanacore RM, Kumagai-Cresse C, Ero-Tolliver IA, Rafi M, et al. Peroxidasin forms sulfilimine chemical bonds using hypohalous acids in tissue genesis. Nat Chem Biol. 2012;8(9):784-90. doi: 10.1038/nchembio.1038.
141. McCall AS, Cummings CF, Bhave G, Vanacore R, Page-McCaw A, Hudson BG. Bromine is an essential trace element for assembly of collagen IV scaffolds in tissue development and architecture. Cell. 2014;157(6):1380-92. doi: 10.1016/j.cell.2014.05.009.
142. Anazco C, Lopez-Jimenez AJ, Rafi M, Vega-Montoto L, Zhang MZ, Hudson BG, et al. Lysyl Oxidase-like-2 Cross-links Collagen IV of Glomerular Basement Membrane. J Biol Chem. 2016;291(50):25999-6012. doi: 10.1074/jbc.M116.738856.
143. Bignon M, Pichol-Thievend C, Hardouin J, Malbouyres M, Brechot N, Nasciutti L, et al. Lysyl oxidase-like protein-2 regulates sprouting angiogenesis and type IV collagen assembly in the endothelial basement membrane. Blood. 2011;118(14):3979-89. doi: 10.1182/blood-2010-10-313296.
144. Matsubayashi Y, Louani A, Dragu A, Sanchez-Sanchez BJ, Serna-Morales E, Yolland L, et al. A Moving Source of Matrix Components Is Essential for De Novo Basement Membrane Formation. Curr Biol. 2017;27(22):3526-34 e4. doi: 10.1016/j.cub.2017.10.001.
145. Hohenester E, Maurer P, Timpl R. Crystal structure of a pair of follistatin-like and EF-hand calcium-binding domains in BM-40. EMBO J. 1997;16(13):3778-86. doi: 10.1093/emboj/16.13.3778.
146. Bradshaw AD. The role of SPARC in extracellular matrix assembly. J Cell Commun Signal. 2009;3(3-4):239-46. doi: 10.1007/s12079-009-0062-6.
147. Isabella AJ, Horne-Badovinac S. Dynamic regulation of basement membrane protein levels promotes egg chamber elongation in Drosophila. Dev Biol. 2015;406(2):212-21. doi: 10.1016/j.ydbio.2015.08.018.
148. Chioran A, Duncan S, Catalano A, Brown TJ, Ringuette MJ. Collagen IV trafficking: The inside-out and beyond story. Dev Biol. 2017;431(2):124-33. doi: 10.1016/j.ydbio.2017.09.037.
149. Shahab J, Baratta C, Scuric B, Godt D, Venken KJT, Ringuette MJ. Loss of SPARC dysregulates basal lamina assembly to disrupt larval fat body homeostasis in Drosophila melanogaster. Dev Dyn. 2015;244(4):540-52. doi: 10.1002/dvdy.24243.
150. Morrissey MA, Jayadev R, Miley GR, Blebea CA, Chi Q, Ihara S, et al. SPARC Promotes Cell Invasion In Vivo by Decreasing Type IV Collagen Levels in the Basement Membrane. PLoS Genet. 2016;12(2):e1005905. doi: 10.1371/journal.pgen.1005905.
151. Murray JC, Stingl G, Kleinman HK, Martin GR, Katz SI. Epidermal cells adhere preferentially to type IV (basement membrane) collagen. J Cell Biol. 1979;80(1):197-202. doi: 10.1083/jcb.80.1.197.
152. Rubin K, Hook M, Obrink B, Timpl R. Substrate adhesion of rat hepatocytes: mechanism of attachment to collagen substrates. Cell. 1981;24(2):463-70. doi: 10.1016/0092-8674(81)90337-8.
153. Santoro SA. Identification of a 160,000 dalton platelet membrane protein that mediates the initial divalent cation-dependent adhesion of platelets to collagen. Cell. 1986;46(6):913-20. doi: 10.1016/0092-8674(86)90073-5.
154. Herbst TJ, McCarthy JB, Tsilibary EC, Furcht LT. Differential effects of laminin, intact type IV collagen, and specific domains of type IV collagen on endothelial cell adhesion and migration. J Cell Biol. 1988;106(4):1365-73. doi: 10.1083/jcb.106.4.1365.
155. Dedhar S, Saulnier R, Nagle R, Overall CM. Specific alterations in the expression of alpha 3 beta 1 and alpha 6 beta 4 integrins in highly invasive and metastatic variants of human prostate carcinoma cells selected by in vitro invasion through reconstituted basement membrane. Clin Exp Metastasis. 1993;11(5):391-400. doi: 10.1007/bf00132982.
156. Setty S, Kim Y, Fields GB, Clegg DO, Wayner EA, Tsilibary EC. Interactions of type IV collagen and its domains with human mesangial cells. J Biol Chem. 1998;273(20):12244-9. doi: DOI 10.1074/jbc.273.20.12244.
157. Kaido T, Yebra M, Cirulli V, Montgomery AM. Regulation of human beta-cell adhesion, motility, and insulin secretion by collagen IV and its receptor alpha1beta1. J Biol Chem. 2004;279(51):53762-9. doi: 10.1074/jbc.M411202200.

158. Humphries JD, Chastney MR, Askari JA, Humphries MJ. Signal transduction via integrin adhesion complexes. Curr Opin Cell Biol. 2019;56:14-21. doi: 10.1016/j.ceb.2018.08.004.
159. Campbell ID, Humphries MJ. Integrin structure, activation, and interactions. Cold Spring Harb Perspect Biol. 2011;3(3). doi: 10.1101/cshperspect.a004994.
160. Zeltz C, Gullberg D. The integrin-collagen connection - a glue for tissue repair? J Cell Sci. 2016;129(6):1284. doi: 10.1242/jcs.188672.
161. Kern A, Eble J, Golbik R, Kuhn K. Interaction of Type-Iv Collagen with the Isolated Integrin-Alpha-1-Beta-1 and Integrin-Alpha-2-Beta-1. Eur J Biochem. 1993;215(1):151-9. doi: DOI 10.1111/j.1432-1033.1993.tb18017.x.
162. Keely PJ, Fong AM, Zutter MM, Santoro SA. Alteration of Collagen-Dependent Adhesion, Motility, and Morphogenesis by the Expression of Antisense Alpha(2) Integrin Messenger-Rna in Mammary Cells. J Cell Sci. 1995;108:595-607.
163. Gardner H, Kreidberg J, Koteliansky V, Jaenisch R. Deletion of integrin alpha 1 by homologous recombination permits normal murine development but gives rise to a specific deficit in cell adhesion. Dev Biol. 1996;175(2):301-13. doi: DOI 10.1006/dbio.1996.0116.
164. Elices MJ, Urry LA, Hemler ME. Receptor functions for the integrin VLA-3: fibronectin, collagen, and laminin binding are differentially influenced by Arg-Gly-Asp peptide and by divalent cations. J Cell Biol. 1991;112(1):169-81. doi: 10.1083/jcb.112.1.169.
165. Tulla M, Pentikäinen OT, Viitasalo T, Käpylä J, Impola U, Nykvist P, et al. Selective Binding of Collagen Subtypes by Integrin α1I, α2I, and α10I Domains. J Biol Chem. 2001;276(51):48206-12. doi: 10.1074/jbc.M104058200.
166. Tiger C-F, Fougerousse F, Grundström G, Velling T, Gullberg D. α11β1 Integrin Is a Receptor for Interstitial Collagens Involved in Cell Migration and Collagen Reorganization on Mesenchymal Nonmuscle Cells. Dev Biol. 2001;237(1):116-29. doi: https://doi.org/10.1006/dbio.2001.0363.
167. Cosgrove D, Liu S. Collagen IV diseases: A focus on the glomerular basement membrane in Alport syndrome. Matrix Biol. 2017;57-58:45-54. doi: https://doi.org/10.1016/j.matbio.2016.08.005.
168. Knight CG, Morton LF, Peachey AR, Tuckwell DS, Farndale RW, Barnes MJ. The collagen-binding A-domains of integrins alpha(1)beta(1) and alpha(2)beta(1) recognize the same specific amino acid sequence, GFOGER, in native (triple-helical) collagens. J Biol Chem. 2000;275(1):35-40. doi: 10.1074/jbc.275.1.35.
169. Plaisier E, Gribouval O, Alamowitch S, Mougenot B, Prost C, Verpont MC, et al. COL4A1 mutations and hereditary angiopathy, nephropathy, aneurysms, and muscle cramps. N Engl J Med. 2007;357(26):2687-95. doi: 10.1056/NEJMoa071906.
170. Plaisier E, Chen Z, Gekeler F, Benhassine S, Dahan K, Marro B, et al. Novel COL4A1 mutations associated with HANAC syndrome: a role for the triple helical CB3[IV] domain. Am J Med Genet A. 2010;152A(10):2550-5. doi: 10.1002/ajmg.a.33659.
171. Guiraud S, Migeon T, Ferry A, Chen Z, Ouchelouche S, Verpont MC, et al. HANAC Col4a1 Mutation in Mice Leads to Skeletal Muscle Alterations due to a Primary Vascular Defect. Am J Pathol. 2017;187(3):505-16. doi: 10.1016/j.ajpath.2016.10.020.
172. Leitinger B, Hohenester E. Mammalian collagen receptors. Matrix Biol. 2007;26(3):146-55. doi: 10.1016/j.matbio.2006.10.007.
173. Itoh Y. Membrane-type matrix metalloproteinases: Their functions and regulations. Matrix Biol. 2015;44-46:207-23. doi: https://doi.org/10.1016/j.matbio.2015.03.004.
174. Funk SD, Lin M-H, Miner JH. Alport syndrome and Pierson syndrome: Diseases of the glomerular basement membrane. Matrix Biol. 2018;71-72:250-61. doi: https://doi.org/10.1016/j.matbio.2018.04.008.
175. Paavola KJ, Sidik H, Zuchero JB, Eckart M, Talbot WS. Type IV collagen is an activating ligand for the adhesion G protein-coupled receptor GPR126. Sci Signal. 2014;7(338):ra76. doi: 10.1126/scisignal.2005347.

176. Reissig LF, Herdina AN, Rose J, Maurer-Gesek B, Lane JL, Prin F, et al. The Col4a2(em1(IMPC)Wtsi) mouse line: lessons from the Deciphering the Mechanisms of Developmental Disorders program. Biol Open. 2019;8(8). doi: 10.1242/bio.042895.
177. Kalluri R, Danoff TM, Okada H, Neilson EG. Susceptibility to anti-glomerular basement membrane disease and Goodpasture syndrome is linked to MHC class II genes and the emergence of T cell-mediated immunity in mice. J Clin Invest. 1997;100(9):2263-75. doi: 10.1172/JCI119764.
178. Kelley PB, Sado Y, Duncan MK. Collagen IV in the developing lens capsule. Matrix Biol. 2002;21(5):415-23. doi: 10.1016/s0945-053x(02)00014-8.
179. Poduval P, Sillat T, Beklen A, Kouri VP, Virtanen I, Konttinen YT. Type IV collagen alpha-chain composition in synovial lining from trauma patients and patients with rheumatoid arthritis. Arthritis Rheum. 2007;56(12):3959-67. doi: 10.1002/art.23072.
180. Gould DB, Phalan FC, Breedveld GJ, van Mil SE, Smith RS, Schimenti JC, et al. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. Science. 2005;308(5725):1167-71. doi: 10.1126/science.1109418.
181. Van Agtmael T, Schlotzer-Schrehardt U, McKie L, Brownstein DG, Lee AW, Cross SH, et al. Dominant mutations of Col4a1 result in basement membrane defects which lead to anterior segment dysgenesis and glomerulopathy. Hum Mol Genet. 2005;14(21):3161-8. doi: 10.1093/hmg/ddi348.
182. Favor J, Gloeckner CJ, Janik D, Klempt M, Neuhauser-Klaus A, Pretsch W, et al. Type IV procollagen missense mutations associated with defects of the eye, vascular stability, the brain, kidney function and embryonic or postnatal viability in the mouse, Mus musculus: an extension of the Col4a1 allelic series and the identification of the first two Col4a2 mutant alleles. Genetics. 2007;175(2):725-36. doi: 10.1534/genetics.106.064733.
183. Meuwissen ME, Halley DJ, Smit LS, Lequin MH, Cobben JM, de Coo R, et al. The expanding phenotype of COL4A1 and COL4A2 mutations: clinical data on 13 newly identified families and a review of the literature. Genet Med. 2015;17(11):843-53. doi: 10.1038/gim.2014.210.
184. Jeanne M, Jorgensen J, Gould DB. Molecular and Genetic Analyses of Collagen Type IV Mutant Mouse Models of Spontaneous Intracerebral Hemorrhage Identify Mechanisms for Stroke Prevention. Circulation. 2015;131(18):1555-65. doi: 10.1161/CIRCULATIONAHA.114.013395.
185. Murray LS, Lu Y, Taggart A, Van Regemorter N, Vilain C, Abramowicz M, et al. Chemical chaperone treatment reduces intracellular accumulation of mutant collagen IV and ameliorates the cellular phenotype of a COL4A2 mutation that causes haemorrhagic stroke. Hum Mol Genet. 2014;23(2):283-92. doi: 10.1093/hmg/ddt418.
186. Jeanne M, Gould DB. Genotype-phenotype correlations in pathology caused by collagen type IV alpha 1 and 2 mutations. Matrix Biol. 2017;57-58:29-44. doi: 10.1016/j.matbio.2016.10.003.
187. Verbeek E, Meuwissen ME, Verheijen FW, Govaert PP, Licht DJ, Kuo DS, et al. COL4A2 mutation associated with familial porencephaly and small-vessel disease. Eur J Hum Genet. 2012;20(8):844-51. doi: 10.1038/ejhg.2012.20.
188. Lemmens R, Maugeri A, Niessen HW, Goris A, Tousseyn T, Demaerel P, et al. Novel COL4A1 mutations cause cerebral small vessel disease by haploinsufficiency. Hum Mol Genet. 2013;22(2):391-7. doi: 10.1093/hmg/dds436.
189. Saskin A, Sillon G, Palfreeman N, Buhas D. COL4A1/2 CNVs and cerebral small vessel disease: Narrowing in on the critical chromosomal region. Neurology. 2018;90(22):1026-8. doi: 10.1212/WNL.0000000000005601.
190. Renard D, Miné M, Pipiras E, Labauge P, Delahaye A, Benzacken B, et al. Cerebral small-vessel disease associated with COL4A1 and COL4A2 gene duplications. Neurology. 2014;83(11):1029-31. doi: 10.1212/wnl.0000000000000769.
191. Yaramis A, Lochmuller H, Topf A, Sonmezler E, Yilmaz E, Hiz S, et al. COL4A1-related autosomal recessive encephalopathy in 2 Turkish children. Neurol Genet. 2020;6(1):e392. doi: 10.1212/NXG.0000000000000392.

192. Zagaglia S, Selch C, Nisevic JR, Mei D, Michalak Z, Hernandez-Hernandez L, et al. Neurologic phenotypes associated with COL4A1/2 mutations: Expanding the spectrum of disease. Neurology. 2018;91(22):e2078-e88. doi: 10.1212/WNL.0000000000006567.
193. Tonduti D, Pichiecchio A, La Piana R, Livingston JH, Doherty DA, Majumdar A, et al. COL4A1-related disease: raised creatine kinase and cerebral calcification as useful pointers. Neuropediatrics. 2012;43(5):283-8. doi: 10.1055/s-0032-1325116.
194. Yoneda Y, Haginoya K, Kato M, Osaka H, Yokochi K, Arai H, et al. Phenotypic spectrum of COL4A1 mutations: porencephaly to schizencephaly. Ann Neurol. 2013;73(1):48-57. doi: 10.1002/ana.23736.
195. Vitale G, Pichiecchio A, Ormitti F, Tonduti D, Asaro A, Farina L, et al. Cortical malformations and COL4A1 mutation: Three new cases. Eur J Paediatr Neurol. 2019;23(3):410-7. doi: 10.1016/j.ejpn.2019.02.006.
196. Labelle-Dumais C, Dilworth DJ, Harrington EP, de Leau M, Lyons D, Kabaeva Z, et al. COL4A1 mutations cause ocular dysgenesis, neuronal localization defects, and myopathy in mice and Walker-Warburg syndrome in humans. PLoS Genet. 2011;7(5):e1002062. doi: 10.1371/journal.pgen.1002062.
197. Moon HM, Wynshaw-Boris A. Cytoskeleton in action: lissencephaly, a neuronal migration disorder. Wiley Interdiscip Rev Dev Biol. 2013;2(2):229-45. doi: 10.1002/wdev.67.
198. Shah S, Ellard S, Kneen R, Lim M, Osborne N, Rankin J, et al. Childhood presentation of COL4A1 mutations. Dev Med Child Neurol. 2012;54(6):569-74. doi: 10.1111/j.1469-8749.2011.04198.x.
199. Wardlaw JM, Smith C, Dichgans M. Mechanisms of sporadic cerebral small vessel disease: insights from neuroimaging. Lancet Neurol. 2013;12(5):483-97. doi: 10.1016/S1474-4422(13)70060-7.
200. Wardlaw JM, Smith C, Dichgans M. Small vessel disease: mechanisms and clinical implications. Lancet Neurol. 2019;18(7):684-96. doi: 10.1016/S1474-4422(19)30079-1.
201. Kinoshita K, Ishizaki Y, Yamamoto H, Sonoda M, Yonemoto K, Kira R, et al. De novo p.G696S mutation in COL4A1 causes intracranial calcification and late-onset cerebral hemorrhage: A case report and review of the literature. Eur J Med Genet. 2020;63(4):103825. doi: 10.1016/j.ejmg.2019.103825.
202. Siitonen M, Börjesson-Hanson A, Pöyhönen M, Ora A, Pasanen P, Bras J, et al. Multi-infarct dementia of Swedish type is caused by a 3'UTR mutation of COL4A1. Brain. 2017;140(5):e29-e. doi: 10.1093/brain/awx062.
203. Zhao YY, Duan RN, Ji L, Liu QJ, Yan CZ. Cervical Spinal Involvement in a Chinese Pedigree With Pontine Autosomal Dominant Microangiopathy and Leukoencephalopathy Caused by a 3' Untranslated Region Mutation of COL4A1 Gene. Stroke. 2019;50(9):2307-13. doi: 10.1161/STROKEAHA.119.024875.
204. Colin E, Sentilhes L, Sarfati A, Mine M, Guichet A, Ploton C, et al. Fetal intracerebral hemorrhage and cataract: think COL4A1. J Perinatol. 2014;34(1):75-7. doi: 10.1038/jp.2013.135.
205. Sibon I, Coupry I, Menegon P, Bouchet JP, Gorry P, Burgelin I, et al. COL4A1 mutation in Axenfeld-Rieger anomaly with leukoencephalopathy and stroke. Ann Neurol. 2007;62(2):177-84. doi: 10.1002/ana.21191.
206. Gould DB, Marchant JK, Savinova OV, Smith RS, John SW. Col4a1 mutation causes endoplasmic reticulum stress and genetically modifiable ocular dysgenesis. Hum Mol Genet. 2007;16(7):798-807. doi: 10.1093/hmg/ddm024.
207. Coupry I, Sibon I, Mortemousque B, Rouanet F, Mine M, Goizet C. Ophthalmological features associated with COL4A1 mutations. Arch Ophthalmol. 2010;128(4):483-9. doi: 10.1001/archophthalmol.2010.42.
208. Deml B, Reis LM, Maheshwari M, Griffis C, Bick D, Semina EV. Whole exome analysis identifies dominant COL4A1 mutations in patients with complex ocular phenotypes involving microphthalmia. Clin Genet. 2014;86(5):475-81. doi: 10.1111/cge.12379.
209. Kuo DS, Labelle-Dumais C, Mao M, Jeanne M, Kauffman WB, Allen J, et al. Allelic heterogeneity contributes to variability in ocular dysgenesis, myopathy and brain malformations caused by Col4a1 and Col4a2 mutations. Hum Mol Genet. 2014;23(7):1709-22. doi: 10.1093/hmg/ddt560.

210. Gould DB, John SW. Anterior segment dysgenesis and the developmental glaucomas are complex traits. Hum Mol Genet. 2002;11(10):1185-93. doi: 10.1093/hmg/11.10.1185.
211. Alavi MV, Mao M, Pawlikowski BT, Kvezereli M, Duncan JL, Libby RT, et al. Col4a1 mutations cause progressive retinal neovascular defects and retinopathy. Sci Rep. 2016;6:18602. doi: 10.1038/srep18602.
212. Ratelade J, Mezouar N, Domenga-Denier V, Rochey A, Plaisier E, Joutel A. Severity of arterial defects in the retina correlates with the burden of intracerebral haemorrhage in COL4A1-related stroke. J Pathol. 2018;244(4):408-20. doi: 10.1002/path.5023.
213. Rodahl E, Knappskog PM, Majewski J, Johansson S, Telstad W, Krakenes J, et al. Variants of anterior segment dysgenesis and cerebral involvement in a large family with a novel COL4A1 mutation. Am J Ophthalmol. 2013;155(5):946-53. doi: 10.1016/j.ajo.2012.11.028.
214. Mao M, Kiss M, Ou Y, Gould DB. Genetic dissection of anterior segment dysgenesis caused by a Col4a1 mutation in mouse. Dis Model Mech. 2017;10(4):475-85. doi: 10.1242/dmm.027888.
215. Labelle-Dumais C, Schuitema V, Hayashi G, Hoff K, Gong W, Dao DQ, et al. COL4A1 Mutations Cause Neuromuscular Disease with Tissue-Specific Mechanistic Heterogeneity. Am J Hum Genet. 2019;104(5):847-60. doi: 10.1016/j.ajhg.2019.03.007.
216. Gupta MC, Graham PL, Kramer JM. Characterization of alpha1(IV) collagen mutations in Caenorhabditis elegans and the effects of alpha1 and alpha2(IV) mutations on type IV collagen distribution. J Cell Biol. 1997;137(5):1185-96. doi: 10.1083/jcb.137.5.1185.
217. Kelemen-Valkony I, Kiss M, Csiha J, Kiss A, Bircher U, Szidonya J, et al. Drosophila basement membrane collagen col4a1 mutations cause severe myopathy. Matrix Biol. 2012;31(1):29-37. doi: 10.1016/j.matbio.2011.09.004.
218. Kiss AA, Somlyai-Popovics N, Kiss M, Boldogkoi Z, Csiszar K, Mink M. Type IV Collagen Is Essential for Proper Function of Integrin-Mediated Adhesion in Drosophila Muscle Fibers. Int J Mol Sci. 2019;20(20). doi: 10.3390/ijms20205124.
219. Alamowitch S, Plaisier E, Favrole P, Prost C, Chen Z, Van Agtmael T, et al. Cerebrovascular disease related to COL4A1 mutations in HANAC syndrome. Neurology. 2009;73(22):1873-82. doi: 10.1212/WNL.0b013e3181c3fd12.
220. Chen Z, Migeon T, Verpont MC, Zaidan M, Sado Y, Kerjaschki D, et al. HANAC Syndrome Col4a1 Mutation Causes Neonate Glomerular Hyperpermeability and Adult Glomerulocystic Kidney Disease. J Am Soc Nephrol. 2016;27(4):1042-54. doi: 10.1681/ASN.2014121217.
221. Jones FE, Bailey MA, Murray LS, Lu Y, McNeilly S, Schlotzer-Schrehardt U, et al. ER stress and basement membrane defects combine to cause glomerular and tubular renal disease resulting from Col4a1 mutations in mice. Dis Model Mech. 2016;9(2):165-76. doi: 10.1242/dmm.021741.
222. Jones FE, Murray LS, McNeilly S, Dean A, Aman A, Lu Y, et al. 4-Sodium phenyl butyric acid has both efficacy and counter-indicative effects in the treatment of Col4a1 disease. Hum Mol Genet. 2019;28(4):628-38. doi: 10.1093/hmg/ddy369.
223. Kitzler TM, Schneider R, Kohl S, Kolvenbach CM, Connaughton DM, Dai R, et al. COL4A1 mutations as a potential novel cause of autosomal dominant CAKUT in humans. Hum Genet. 2019;138(10):1105-15. doi: 10.1007/s00439-019-02042-4.
224. McMahon CJ, Breathnach C, Betts DR, Sharkey FH, Greally MT. De Novo interstitial deletion 13q33.3q34 in a male patient with double outlet right ventricle, microcephaly, dysmorphic craniofacial findings, and motor and developmental delay. Am J Med Genet A. 2015;167A(5):1134-41. doi: 10.1002/ajmg.a.36978.
225. Wang YP, Wang DJ, Niu ZB, Cui WT. Chromosome 13q deletion syndrome involving 13q31qter: A case report. Mol Med Rep. 2017;15(6):3658-64. doi: 10.3892/mmr.2017.6425.
226. Meuwissen ME, de Vries LS, Verbeek HA, Lequin MH, Govaert PP, Schot R, et al. Sporadic COL4A1 mutations with extensive prenatal porencephaly resembling hydranencephaly. Neurology. 2011;76(9):844-6. doi: 10.1212/WNL.0b013e31820e7751.

227. Jeanne M, Labelle-Dumais C, Jorgensen J, Kauffman WB, Mancini GM, Favor J, et al. COL4A2 mutations impair COL4A1 and COL4A2 secretion and cause hemorrhagic stroke. Am J Hum Genet. 2012;90(1):91-101. doi: 10.1016/j.ajhg.2011.11.022.
228. Weng YC, Sonni A, Labelle-Dumais C, de Leau M, Kauffman WB, Jeanne M, et al. COL4A1 mutations in patients with sporadic late-onset intracerebral hemorrhage. Ann Neurol. 2012;71(4):470-7. doi: 10.1002/ana.22682.
229. Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. Nat Genet. 2018;50(4):524-37. doi: 10.1038/s41588-018-0058-3.
230. Traylor M, Malik R, Nalls MA, Cotlarciuc I, Radmanesh F, Thorleifsson G, et al. Genetic variation at 16q24.2 is associated with small vessel stroke. Ann Neurol. 2017;81(3):383-94. doi: 10.1002/ana.24840.
231. Grami N, Chong M, Lali R, Mohammadi-Shemirani P, Henshall DE, Rannikmae K, et al. Global Assessment of Mendelian Stroke Genetic Prevalence in 101 635 Individuals From 7 Ethnic Groups. Stroke. 2020;51(4):1290-3. doi: 10.1161/STROKEAHA.119.028840.
232. Tarasov KV, Sanna S, Scuteri A, Strait JB, Orru M, Parsa A, et al. COL4A1 is associated with arterial stiffness by genome-wide association scan. Circ Cardiovasc Genet. 2009;2(2):151-8. doi: 10.1161/CIRCGENETICS.108.823245.
233. Yang W, Ng FL, Chan K, Pu X, Poston RN, Ren M, et al. Coronary-Heart-Disease-Associated Genetic Variant at the COL4A1/COL4A2 Locus Affects COL4A1/COL4A2 Expression, Vascular Cell Survival, Atherosclerotic Plaque Stability and Risk of Myocardial Infarction. PLoS Genet. 2016;12(7):e1006127. doi: 10.1371/journal.pgen.1006127.
234. Savige J, Sheth S, Leys A, Nicholson A, Mack HG, Colville D. Ocular features in Alport syndrome: pathogenesis and clinical significance. Clin J Am Soc Nephrol. 2015;10(4):703-9. doi: 10.2215/CJN.10581014.
235. Rheault MN. Women and Alport syndrome. Pediatr Nephrol. 2012;27(1):41-6. doi: 10.1007/s00467-011-1836-7.
236. Gubler MC. Inherited diseases of the glomerular basement membrane. Nature Clinical Practice Nephrology. 2008;4(1):24-37. doi: 10.1038/ncpneph0671.
237. Chew C, Lennon R. Basement Membrane Defects in Genetic Kidney Diseases. Front Pediatr. 2018;6:11. doi: 10.3389/fped.2018.00011.
238. Mencarelli MA, Heidet L, Storey H, van Geel M, Knebelmann B, Fallerini C, et al. Evidence of digenic inheritance in Alport syndrome. J Med Genet. 2015;52(3):163-74. doi: 10.1136/jmedgenet-2014-102822.
239. Fallerini C, Baldassarri M, Trevisson E, Morbidoni V, La Manna A, Lazzarin R, et al. Alport syndrome: impact of digenic inheritance in patients management. Clin Genet. 2017;92(1):34-44. doi: 10.1111/cge.12919.
240. Zhao X, Chen C, Wei Y, Zhao G, Liu L, Wang C, et al. Novel mutations of COL4A3, COL4A4, and COL4A5 genes in Chinese patients with Alport Syndrome using next generation sequence technique. Mol Genet Genomic Med. 2019;7(6):e653. doi: 10.1002/mgg3.653.
241. Daga S, Fallerini C, Furini S, Pecoraro C, Scolari F, Ariani F, et al. Non-collagen genes role in digenic Alport syndrome. BMC Nephrol. 2019;20(1):70. doi: 10.1186/s12882-019-1258-5.
242. Bouchireb K, Boyer O, Gribouval O, Nevo F, Huynh-Cong E, Moriniere V, et al. NPHS2 mutations in steroid-resistant nephrotic syndrome: a mutation update and the associated phenotypic spectrum. Hum Mutat. 2014;35(2):178-86. doi: 10.1002/humu.22485.
243. Heidet L, Borza DB, Jouin M, Sich M, Mattei MG, Sado Y, et al. A human-mouse chimera of the alpha3alpha4alpha5(IV) collagen protomer rescues the renal phenotype in Col4a3-/- Alport mice. Am J Pathol. 2003;163(4):1633-44. doi: 10.1016/s0002-9440(10)63520-1.
244. Voskarides K, Damianou L, Neocleous V, Zouvani I, Christodoulidou S, Hadjiconstantinou V, et al. COL4A3/COL4A4 mutations producing focal segmental glomerulosclerosis and renal failure in thin

basement membrane nephropathy. J Am Soc Nephrol. 2007;18(11):3004-16. doi: 10.1681/ASN.2007040444.
245. Marcocci E, Uliana V, Bruttini M, Artuso R, Silengo MC, Zerial M, et al. Autosomal dominant Alport syndrome: molecular analysis of the COL4A4 gene and clinical outcome. Nephrol Dial Transplant. 2009;24(5):1464-71. doi: 10.1093/ndt/gfn681.
246. Beirowski B, Weber M, Gross O. Chronic renal failure and shortened lifespan in COL4A3+/- mice: an animal model for thin basement membrane nephropathy. J Am Soc Nephrol. 2006;17(7):1986-94. doi: 10.1681/ASN.2005101044.
247. Kashtan CE, Ding J, Garosi G, Heidet L, Massella L, Nakanishi K, et al. Alport syndrome: a unified classification of genetic disorders of collagen IV alpha345: a position paper of the Alport Syndrome Classification Working Group. Kidney Int. 2018;93(5):1045-51. doi: 10.1016/j.kint.2017.12.018.
248. Abrahamson DR, Isom K, Roach E, Stroganova L, Zelenchuk A, Miner JH, et al. Laminin compensation in collagen alpha3(IV) knockout (Alport) glomeruli contributes to permeability defects. J Am Soc Nephrol. 2007;18(9):2465-72. doi: 10.1681/ASN.2007030328.
249. Murata T, Katayama K, Oohashi T, Jahnukainen T, Yonezawa T, Sado Y, et al. COL4A6 is dispensable for autosomal recessive Alport syndrome. Sci Rep. 2016;6:29450. doi: 10.1038/srep29450.
250. Kang JS, Wang XP, Miner JH, Morello R, Sado Y, Abrahamson DR, et al. Loss of alpha3/alpha4(IV) collagen from the glomerular basement membrane induces a strain-dependent isoform switch to alpha5alpha6(IV) collagen associated with longer renal survival in Col4a3-/- Alport mice. J Am Soc Nephrol. 2006;17(7):1962-9. doi: 10.1681/ASN.2006020165.
251. Zhou J, Mochizuki T, Smeets H, Antignac C, Laurila P, de Paepe A, et al. Deletion of the paired alpha 5(IV) and alpha 6(IV) collagen genes in inherited smooth muscle tumors. Science. 1993;261(5125):1167-9. doi: 10.1126/science.8356449.
252. Heidet L, Dahan K, Zhou J, Xu Z, Cochat P, Gould JD, et al. Deletions of both alpha 5(IV) and alpha 6(IV) collagen genes in Alport syndrome and in Alport syndrome associated with smooth muscle tumours. Hum Mol Genet. 1995;4(1):99-108.
253. Sá MJN, Fieremans N, de Brouwer APM, Sousa R, Costa FTe, Brito MJ, et al. Deletion of the 5'exons of COL4A6 is not needed for the development of diffuse leiomyomatosis in patients with Alport syndrome. J Med Genet. 2013;50(11):745-53. doi: 10.1136/jmedgenet-2013-101670.
254. Nozu K, Minamikawa S, Yamada S, Oka M, Yanagita M, Morisada N, et al. Characterization of contiguous gene deletions in COL4A6 and COL4A5 in Alport syndrome-diffuse leiomyomatosis. J Hum Genet. 2017;62(7):733-5. doi: 10.1038/jhg.2017.28.
255. Bolton WK. Goodpasture's syndrome. Kidney Int. 1996;50(5):1753-66. doi: 10.1038/ki.1996.495.
256. Pedchenko V, Kitching AR, Hudson BG. Goodpasture's autoimmune disease — A collagen IV disorder. Matrix Biol. 2018;71-72:240-9. doi: https://doi.org/10.1016/j.matbio.2018.05.004.
257. Henderson SR, Salama AD. Diagnostic and management challenges in Goodpasture's (anti-glomerular basement membrane) disease. Nephrol Dial Transplant. 2018;33(2):196-202. doi: 10.1093/ndt/gfx057.
258. Kalluri R, Cantley LG, Kerjaschki D, Neilson EG. Reactive oxygen species expose cryptic epitopes associated with autoimmune goodpasture syndrome. J Biol Chem. 2000;275(26):20027-32.
259. Borza DB, Bondar O, Colon S, Todd P, Sado Y, Neilson EG, et al. Goodpasture autoantibodies unmask cryptic epitopes by selectively dissociating autoantigen complexes lacking structural reinforcement: novel mechanisms for immune privilege and autoimmune pathogenesis. J Biol Chem. 2005;280(29):27147-54.
260. Raya A, Revert-Ros F, Martinez-Martinez P, Navarro S, Roselló E, Vieites B, et al. Goodpasture Antigen-binding Protein, the Kinase That Phosphorylates the Goodpasture Antigen, Is an Alternatively Spliced Variant Implicated in Autoimmune Pathogenesis. J Biol Chem. 2000;275(51):40392-9. doi: 10.1074/jbc.M002769200.
261. Wang XP, Fogo AB, Colon S, Giannico G, Abul-Ezz SR, Miner JH, et al. Distinct epitopes for anti-glomerular basement membrane alport alloantibodies and goodpasture autoantibodies within the

noncollagenous domain of alpha3(IV) collagen: a janus-faced antigen. J Am Soc Nephrol. 2005;16(12):3563-71.
262. Brainwood D, Kashtan C, Gubler MC, Turner AN. Targets of alloantibodies in Alport anti-glomerular basement membrane disease after renal transplantation. Kidney Int. 1998;53(3):762-6.
263. Kang JS, Kashtan CE, Turner AN, Heidet L, Hudson BG, Borza DB. The alloantigenic sites of alpha3alpha4alpha5(IV) collagen: pathogenic X-linked alport alloantibodies target two accessible conformational epitopes in the alpha5NC1 domain. J Biol Chem. 2007;282(14):10670-7.
264. Guan M, Ma J, Keaton JM, Dimitrov L, Mudgal P, Stromberg M, et al. Association of kidney structure-related gene variants with type 2 diabetes-attributed end-stage kidney disease in African Americans. Hum Genet. 2016;135(11):1251-62. doi: 10.1007/s00439-016-1714-2.
265. Wang Y, Zhang J, Zhao Y, Wang S, Zhang J, Han Q, et al. COL4A3 Gene Variants and Diabetic Kidney Disease in MODY. Clin J Am Soc Nephrol. 2018;13(8):1162-71. doi: 10.2215/cjn.09100817.
266. Bullich G, Trujillano D, Santin S, Ossowski S, Mendizabal S, Fraga G, et al. Targeted next-generation sequencing in steroid-resistant nephrotic syndrome: mutations in multiple glomerular genes may influence disease severity. Eur J Hum Genet. 2015;23(9):1192-9. doi: 10.1038/ejhg.2014.252.
267. Takeuchi M, Yamaguchi S, Yonemura S, Kakiguchi K, Sato Y, Higashiyama T, et al. Type IV Collagen Controls the Axogenesis of Cerebellar Granule Cells by Regulating Basement Membrane Integrity in Zebrafish. PLoS Genet. 2015;11(10):e1005587. doi: 10.1371/journal.pgen.1005587.
268. Rost S, Bach E, Neuner C, Nanda I, Dysek S, Bittner RE, et al. Novel form of X-linked nonsyndromic hearing loss with cochlear malformation caused by a mutation in the type IV collagen gene COL4A6. Eur J Hum Genet. 2014;22(2):208-15. doi: 10.1038/ejhg.2013.108.
269. Bilguvar K, DiLuna ML, Bizzarro MJ, Bayri Y, Schneider KC, Lifton RP, et al. COL4A1 mutation in preterm intraventricular hemorrhage. J Pediatr. 2009;155(5):743-5. doi: 10.1016/j.jpeds.2009.04.014.
270. Van Agtmael T, Bailey MA, Schlötzer-Schrehardt U, Craigie E, Jackson IJ, Brownstein DG, et al. Col4a1 mutation in mice causes defects in vascular function and low blood pressure associated with reduced red blood cell volume. Hum Mol Genet. 2010;19(6):1119-28. doi: 10.1093/hmg/ddp584.
271. Vermeulen RJ, Peeters-Scholte C, Van Vugt JJ, Barkhof F, Rizzu P, van der Schoor SR, et al. Fetal origin of brain damage in 2 infants with a COL4A1 mutation: fetal and neonatal MRI. Neuropediatrics. 2011;42(1):1-3. doi: 10.1055/s-0031-1275343.
272. Khalid R, Krishnan P, Andres K, Blaser S, Miller S, Moharir M, et al. COL4A1 and fetal vascular origins of schizencephaly. Neurology. 2018;90(5):232-4. doi: 10.1212/WNL.0000000000004890.
273. Senger DR, Davis GE. Angiogenesis. Cold Spring Harb Perspect Biol. 2011;3(8):a005090. doi: 10.1101/cshperspect.a005090.
274. Hogan BM, Schulte-Merker S. How to Plumb a Pisces: Understanding Vascular Development and Disease Using Zebrafish Embryos. Dev Cell. 2017;42(6):567-83. doi: 10.1016/j.devcel.2017.08.015.
275. Coucke PJ, Willaert A, Wessels MW, Callewaert B, Zoppi N, De Backer J, et al. Mutations in the facilitative glucose transporter GLUT10 alter angiogenesis and cause arterial tortuosity syndrome. Nat Genet. 2006;38(4):452-7. doi: 10.1038/ng1764.
276. Hartnett ME, Martiniuk D, Byfield G, Geisen P, Zeng G, Bautch VL. Neutralizing VEGF decreases tortuosity and alters endothelial cell division orientation in arterioles and veins in a rat model of ROP: relevance to plus disease. Invest Ophthalmol Vis Sci. 2008;49(7):3107-14. doi: 10.1167/iovs.08-1780.
277. Pedchenko V, Zent R, Hudson BG. Alpha(v)beta3 and alpha(v)beta5 integrins bind both the proximal RGD site and non-RGD motifs within noncollagenous (NC1) domain of the alpha3 chain of type IV collagen: implication for the mechanism of endothelia cell adhesion. J Biol Chem. 2004;279(4):2772-80. doi: 10.1074/jbc.M311901200.
278. Grant DS, Lelkes PI, Fukuda K, Kleinman HK. Intracellular mechanisms involved in basement membrane induced blood vessel differentiation in vitro. In Vitro Cell Dev Biol. 1991;27A(4):327-36. doi: 10.1007/bf02630910.
279. Silva RLE, Kanan Y, Mirando AC, Kim J, Shmueli RB, Lorenc VE, et al. Tyrosine kinase blocking collagen IV-derived peptide suppresses ocular neovascularization and vascular leakage. Sci Transl Med. 2017;9(373). doi: 10.1126/scitranslmed.aai8030.

280. Finnell JG, Tsang TM, Cryan L, Garrard S, Lee SL, Ackroyd PC, et al. A Canstatin-Derived Peptide Provides Insight into the Role of Capillary Morphogenesis Gene 2 in Angiogenic Regulation and Matrix Uptake. ACS Chem Biol. 2020;15(2):587-96. doi: 10.1021/acschembio.0c00064.
281. Bunt S, Hooley C, Hu N, Scahill C, Weavers H, Skaer H. Hemocyte-secreted type IV collagen enhances BMP signaling to guide renal tubule morphogenesis in Drosophila. Dev Cell. 2010;19(2):296-306. doi: 10.1016/j.devcel.2010.07.019.
282. Goumans MJ, Zwijsen A, Ten Dijke P, Bailly S. Bone Morphogenetic Proteins in Vascular Homeostasis and Disease. Cold Spring Harb Perspect Biol. 2018;10(2). doi: 10.1101/cshperspect.a031989.
283. Berry C, Sidik N, Pereira AC, Ford TJ, Touyz RM, Kaski JC, et al. Small-Vessel Disease in the Heart and Brain: Current Knowledge, Unmet Therapeutic Need, and Future Directions. J Am Heart Assoc. 2019;8(3):e011104. doi: 10.1161/JAHA.118.011104.
284. Ratelade J, Klug NR, Lombardi D, Angelim M, Dabertrand F, Domenga-Denier V, et al. Reducing Hypermuscularization of the Transitional Segment between Arterioles and Capillaries Protects Against Spontaneous Intracerebral Hemorrhage. Circulation. 2020. doi: 10.1161/CIRCULATIONAHA.119.040963.
285. Funk SD, Bayer RH, Miner JH. Endothelial cell-specific collagen type IV-alpha3 expression does not rescue Alport syndrome in Col4a3(-)(/-) mice. Am J Physiol Renal Physiol. 2019;316(5):F830-F7. doi: 10.1152/ajprenal.00556.2018.
286. Raghunath M, Bruckner P, Steinmann B. Delayed triple helix formation of mutant collagen from patients with osteogenesis imperfecta. J Mol Biol. 1994;236(3):940-9. doi: 10.1006/jmbi.1994.1199.
287. Cabral WA, Chang W, Barnes AM, Weis M, Scott MA, Leikin S, et al. Prolyl 3-hydroxylase 1 deficiency causes a recessive metabolic bone disorder resembling lethal/severe osteogenesis imperfecta. Nat Genet. 2007;39(3):359-65. doi: 10.1038/ng1968.
288. Bateman JF, Boot-Handford RP, Lamande SR. Genetic diseases of connective tissues: cellular and extracellular effects of ECM mutations. Nat Rev Genet. 2009;10(3):173-83. doi: 10.1038/nrg2520.
289. Mullan LA, Mularczyk EJ, Kung LH, Forouhan M, Wragg JM, Goodacre R, et al. Increased intracellular proteolysis reduces disease severity in an ER stress-associated dwarfism. J Clin Invest. 2017;127(10):3861-5. doi: 10.1172/JCI93094.
290. Gatseva A, Sin YY, Brezzo G, Van Agtmael T. Basement membrane collagens and disease mechanisms. Essays Biochem. 2019;63(3):297-312. doi: 10.1042/EBC20180071.
291. Gould DB, Phalan FC, van Mil SE, Sundberg JP, Vahedi K, Massin P, et al. Role of COL4A1 in small-vessel disease and hemorrhagic stroke. N Engl J Med. 2006;354(14):1489-96. doi: 10.1056/NEJMoa053727.
292. Taylor SH, Al-Youha S, Van Agtmael T, Lu Y, Wong J, McGrouther DA, et al. Tendon is covered by a basement membrane epithelium that is required for cell retention and the prevention of adhesion formation. PLoS One. 2011;6(1):e16337.
293. Kiss M, Kiss AA, Radics M, Popovics N, Hermesz E, Csiszar K, et al. Drosophila type IV collagen mutation associates with immune system activation and intestinal dysfunction. Matrix Biol. 2016;49:120-31. doi: 10.1016/j.matbio.2015.09.002.
294. Arpino V, Brock M, Gill SE. The role of TIMPs in regulation of extracellular matrix proteolysis. Matrix Biol. 2015;44-46:247-54. doi: 10.1016/j.matbio.2015.03.005.
295. Kashtan CE, Kim Y, Lees GE, Thorner PS, Virtanen I, Miner JH. Abnormal glomerular basement membrane laminins in murine, canine, and human Alport syndrome: aberrant laminin alpha2 deposition is species independent. J Am Soc Nephrol. 2001;12(2):252-60.
296. Dufek B, Meehan DT, Delimont D, Cheung L, Gratton MA, Phillips G, et al. Endothelin A receptor activation on mesangial cells initiates Alport glomerular disease. Kidney Int. 2016;90(2):300-10. doi: 10.1016/j.kint.2016.02.018.
297. Randles MJ, Collinson S, Starborg T, Mironov A, Krendel M, Konigshausen E, et al. Three-dimensional electron microscopy reveals the evolution of glomerular barrier injury. Sci Rep. 2016;6:35068. doi: 10.1038/srep35068.

298. Delimont D, Dufek BM, Meehan DT, Zallocchi M, Gratton MA, Phillips G, et al. Laminin alpha2-mediated focal adhesion kinase activation triggers Alport glomerular pathogenesis. PLoS One. 2014;9(6):e99083. doi: 10.1371/journal.pone.0099083.
299. Cosgrove D, Dufek B, Meehan DT, Delimont D, Hartnett M, Samuelson G, et al. Lysyl oxidase like-2 contributes to renal fibrosis in Col4alpha3/Alport mice. Kidney Int. 2018;94(2):303-14. doi: 10.1016/j.kint.2018.02.024.
300. Rubel D, Frese J, Martin M, Leibnitz A, Girgert R, Miosge N, et al. Collagen receptors integrin alpha2beta1 and discoidin domain receptor 1 regulate maturation of the glomerular basement membrane and loss of integrin alpha2beta1 delays kidney fibrosis in COL4A3 knockout mice. Matrix Biol. 2014;34:13-21. doi: 10.1016/j.matbio.2014.01.006.
301. Ron D, Walter P. Signal integration in the endoplasmic reticulum unfolded protein response. Nat Rev Mol Cell Biol. 2007;8(7):519-29.
302. Costa-Mattioli M, Walter P. The integrated stress response: From mechanism to disease. Science. 2020;368(6489). doi: 10.1126/science.aat5314.
303. Gonzalez-Teuber V, Albert-Gasco H, Auyeung VC, Papa FR, Mallucci GR, Hetz C. Small Molecules to Improve ER Proteostasis in Disease. Trends Pharmacol Sci. 2019;40(9):684-95. doi: 10.1016/j.tips.2019.07.003.
304. Pieri M, Stefanou C, Zaravinos A, Erguler K, Stylianou K, Lapathitis G, et al. Evidence for activation of the unfolded protein response in collagen IV nephropathies. J Am Soc Nephrol. 2014;25(2):260-75. doi: 10.1681/ASN.2012121217.
305. Wang D, Mohammad M, Wang Y, Tan R, Murray LS, Ricardo S, et al. The Chemical Chaperone, PBA, Reduces ER Stress and Autophagy and Increases Collagen IV α5 Expression in Cultured Fibroblasts From Men With X-Linked Alport Syndrome and Missense Mutations. Kidney International Reports. 2017;2(4):739-48. doi: 10.1016/j.ekir.2017.03.004.
306. Gotenstein JR, Koo CC, Ho TW, Chisholm AD. Genetic Suppression of Basement Membrane Defects in Caenorhabditis elegans by Gain of Function in Extracellular Matrix and Cell-Matrix Attachment Genes. Genetics. 2018;208(4):1499-512. doi: 10.1534/genetics.118.300731.
307. Funk SD, Bayer RH, Malone AF, McKee KK, Yurchenco PD, Miner JH. Pathogenicity of a Human Laminin beta2 Mutation Revealed in Models of Alport Syndrome. J Am Soc Nephrol. 2018;29(3):949-60. doi: 10.1681/ASN.2017090997.
308. An SJ, Kim TJ, Yoon B-W. Epidemiology, Risk Factors, and Clinical Features of Intracerebral Hemorrhage: An Update. J Stroke. 2017;19(1):3-10. doi: 10.5853/jos.2016.00864.
309. Gross O, Perin L, Deltas C. Alport syndrome from bench to bedside: the potential of current treatment beyond RAAS blockade and the horizon of future therapies. Nephrol Dial Transplant. 2014;29 Suppl 4:iv124-30. doi: 10.1093/ndt/gfu028.
310. Omachi K, Miner JH. Alport Syndrome Therapeutics: Ready for Prime-Time Players. Trends Pharmacol Sci. 2019;40(11):803-6. doi: 10.1016/j.tips.2019.07.012.
311. Proesmans W, Knockaert H, Trouet D. Enalapril in paediatric patients with Alport syndrome: 2 years' experience. Eur J Pediatr. 2000;159(6):430-3. doi: 10.1007/s004310051301.
312. Webb NJ, Lam C, Shahinfar S, Strehlau J, Wells TG, Gleim GW, et al. Efficacy and safety of losartan in children with Alport syndrome--results from a subgroup analysis of a prospective, randomized, placebo- or amlodipine-controlled trial. Nephrol Dial Transplant. 2011;26(8):2521-6. doi: 10.1093/ndt/gfq797.
313. Webb NJ, Shahinfar S, Wells TG, Massaad R, Gleim GW, McCrary Sisk C, et al. Losartan and enalapril are comparable in reducing proteinuria in children with Alport syndrome. Pediatr Nephrol. 2013;28(5):737-43. doi: 10.1007/s00467-012-2372-9.
314. Gross O, Tonshoff B, Weber LT, Pape L, Latta K, Fehrenbach H, et al. A multicenter, randomized, placebo-controlled, double-blind phase 3 trial with open-arm comparison indicates safety and efficacy of nephroprotective therapy with ramipril in children with Alport's syndrome. Kidney Int. 2020. doi: 10.1016/j.kint.2019.12.015.

315. Koepke ML, Weber M, Schulze-Lohoff E, Beirowski B, Segerer S, Gross O. Nephroprotective effect of the HMG-CoA-reductase inhibitor cerivastatin in a mouse model of progressive renal fibrosis in Alport syndrome. Nephrol Dial Transplant. 2007;22(4):1062-9. doi: 10.1093/ndt/gfl810.
316. Gross O, Koepke ML, Beirowski B, Schulze-Lohoff E, Segerer S, Weber M. Nephroprotection by antifibrotic and anti-inflammatory effects of the vasopeptidase inhibitor AVE7688. Kidney Int. 2005;68(2):456-63. doi: 10.1111/j.1523-1755.2005.00423.x.
317. Yokota T, Omachi K, Suico MA, Kamura M, Kojima H, Fukuda R, et al. STAT3 inhibition attenuates the progressive phenotypes of Alport syndrome mouse model. Nephrol Dial Transplant. 2018;33(2):214-23. doi: 10.1093/ndt/gfx246.
318. Gross O, Appel G, Block G, Chin M, Goldsberry A, Inker L, et al. A Phase 2/3 Study of the Efficacy and Safety of Bardoxolone Methyl in Patients with Alport Syndrome. Nephrology Dialysis Transplantation. 2018;33.
319. Baigent C, Lennon R. Should We Increase GFR with Bardoxolone in Alport Syndrome? J Am Soc Nephrol. 2018;29(2):357-9. doi: 10.1681/ASN.2017101062.
320. Chau BN, Xin CY, Hartner J, Ren SY, Castano AP, Linn G, et al. MicroRNA-21 Promotes Fibrosis of the Kidney by Silencing Metabolic Pathways. Sci Transl Med. 2012;4(121). doi: ARTN 121ra18

10.1126/scitranslmed.3003205.
321. Guo J, Song W, Boulanger J, Xu EY, Wang F, Zhang Y, et al. Dysregulated Expression of microRNA-21 and Disease-Related Genes in Human Patients and in a Mouse Model of Alport Syndrome. Hum Gene Ther. 2019;30(7):865-81. doi: 10.1089/hum.2018.205.
322. Gomez IG, MacKenna DA, Johnson BG, Kaimal V, Roach AM, Ren SY, et al. Anti-microRNA-21 oligonucleotides prevent Alport nephropathy progression by stimulating metabolic pathways. J Clin Invest. 2015;125(1):141-56. doi: 10.1172/Jci75852.
323. Lin X, Suh JH, Go G, Miner JH. Feasibility of repairing glomerular basement membrane defects in Alport syndrome. J Am Soc Nephrol. 2014;25(4):687-92. doi: 10.1681/ASN.2013070798.
324. Sedrakyan S, Da Sacco S, Milanesi A, Shiri L, Petrosyan A, Varimezova R, et al. Injection of Amniotic Fluid Stem Cells Delays Progression of Renal Fibrosis. J Am Soc Nephrol. 2012;23(4):661-73. doi: 10.1681/Asn.2011030243.
325. Sugimoto H, Mundel TM, Sund M, Xie L, Cosgrove D, Kalluri R. Bone-marrow-derived stem cells repair basement membrane collagen defects and reverse genetic kidney disease. Proc Natl Acad Sci U S A. 2006;103(19):7321-6. doi: 10.1073/pnas.0601436103.
326. Katayama K, Kawano M, Naito I, Ishikawa H, Sado Y, Asakawa N, et al. Irradiation prolongs survival of Alport mice. J Am Soc Nephrol. 2008;19(9):1692-700.
327. Gross O, Borza DB, Anders HJ, Licht C, Weber M, Segerer S, et al. Stem cell therapy for Alport syndrome: the hope beyond the hype. Nephrol Dial Transplant. 2009;24(3):731-4.
328. Engin F, Hotamisligil GS. Restoring endoplasmic reticulum function by chemical chaperones: an emerging therapeutic approach for metabolic diseases. Diabetes Obes Metab. 2010;12 Suppl 2:108-15.
329. Hayashi G, Labelle-Dumais C, Gould DB. Use of sodium 4-phenylbutyrate to define therapeutic parameters for reducing intracerebral hemorrhage and myopathy in Col4a1 mutant mice. Disease Models & Mechanisms. 2018;11(7):dmm034157. doi: 10.1242/dmm.034157.
330. Cosgrove D, Meehan DT, Grunkemeyer JA, Kornak JM, Sayers R, Hunter WJ, et al. Collagen COL4A3 knockout: a mouse model for autosomal Alport syndrome. Genes Dev. 1996;10(23):2981-92. doi: 10.1101/gad.10.23.2981.
331. Miner JH, Sanes JR. Molecular and functional defects in kidneys of mice lacking collagen alpha 3(IV): implications for Alport syndrome. The Journal of Cell Biology. 1996;135(5):1403-13. doi: 10.1083/jcb.135.5.1403.
332. Lu W, Phillips CL, Killen PD, Hlaing T, Harrison WR, Elder FFB, et al. Insertional Mutation of the Collagen Genes Col4a3 and Col4a4 in a Mouse Model of Alport Syndrome. Genomics. 1999;61(2):113-24. doi: https://doi.org/10.1006/geno.1999.5943.

333. Korstanje R, Caputo CR, Doty RA, Cook SA, Bronson RT, Davisson MT, et al. A mouse *Col4a4* mutation causing Alport glomerulosclerosis with abnormal collagen α3α4α5(IV) trimers. Kidney Int. 2014;85(6):1461-8. doi: 10.1038/ki.2013.493.
334. Rheault MN, Kren SM, Thielen BK, Mesa HA, Crosson JT, Thomas W, et al. Mouse Model of X-Linked Alport Syndrome. J Am Soc Nephrol. 2004;15(6):1466-74. doi: 10.1097/01.Asn.0000130562.90255.8f.
335. Hashikami K, Asahina M, Nozu K, Iijima K, Nagata M, Takeyama M. Establishment of X-linked Alport syndrome model mice with a Col4a5 R471X mutation. Biochemistry and Biophysics Reports. 2019;17:81-6. doi: https://doi.org/10.1016/j.bbrep.2018.12.003.
336. Xiao T, Baier H. Lamina-specific axonal projections in the zebrafish tectum require the type IV collagen Dragnet. Nat Neurosci. 2007;10:1529. doi: 10.1038/nn2002

https://www.nature.com/articles/nn2002#supplementary-information.
337. Rouaud T, Labauge P, Lasserve ET, Mine M, Coustans M, Deburghgraeve V, et al. Acute Urinary Retention Due to a Novel Collagen Col4a1 Mutation. Neurology. 2010;75(8):747-9. doi: DOI 10.1212/WNL.0b013e3181eee440.
338. Nozu K, Nakanishi K, Abe Y, Udagawa T, Okada S, Okamoto T, et al. A review of clinical characteristics and genetic backgrounds in Alport syndrome. Clin Exp Nephrol. 2019;23(2):158-68. doi: 10.1007/s10157-018-1629-4.

Figure 1. Genome organisation for *COL4A1/COL4A2, COL4A3/COL4A4* and *COL4A5/COL4A6*. Each pair of collagen IV genes contain a bi-directional promoter (length in nucleotides provided) and cis-acting elements. The cis-acting elements (GC box, the CAAT motif and a CTC box) bind specifically to transcription factor SP1, CAAT binding factor (CCAAT) and CTCBF. (A) *COL4A1/COL4A2* on chromosome 13. A silencer influences transcription of both *COL4A1* and *COL4A2*. (B) *COL4A3/COL4A4* on chromosome 2 contains motifs similar to *COL4A1/COL4A2* present in the bidirectional 167 nucleotide promoter. *COL4A4* has two alternative first exons. (C) *COL4A5/COL4A6* have a larger bidirectional promoter. *COL4A6* has potential alternative promoter elements depicted by the red diagonal lines and an alternative first exon.

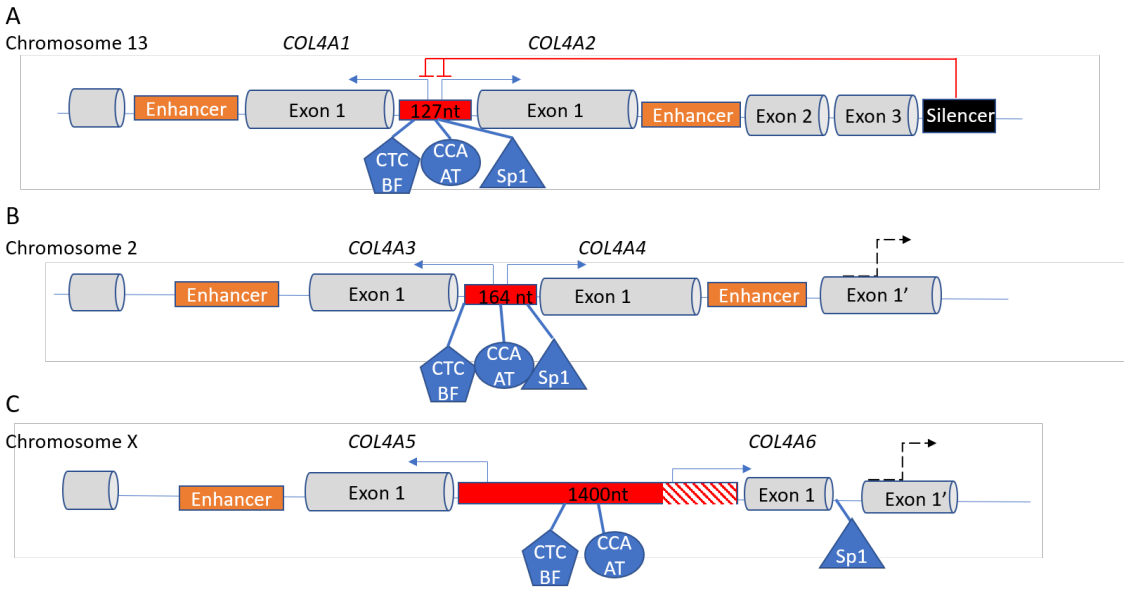

Figure 2 (A) Protein domain structure of collagen IV protomer includes a N-terminal 7S domain, a central triple helical collagen domain and a C-terminal NC1 domain. (B) Collagen network formation in the ECM includes interaction of two protomers *via* their NC1 domains to form dimers. Peroxidasin mediates the sulfilimine bond formation between the NC1 trimers. Four protomers interacts via their 7S domains to form tetramers and cross linking is mediated by LOXL2. Combined this results in a strong but flexible mesh network in the ECM (panel D).

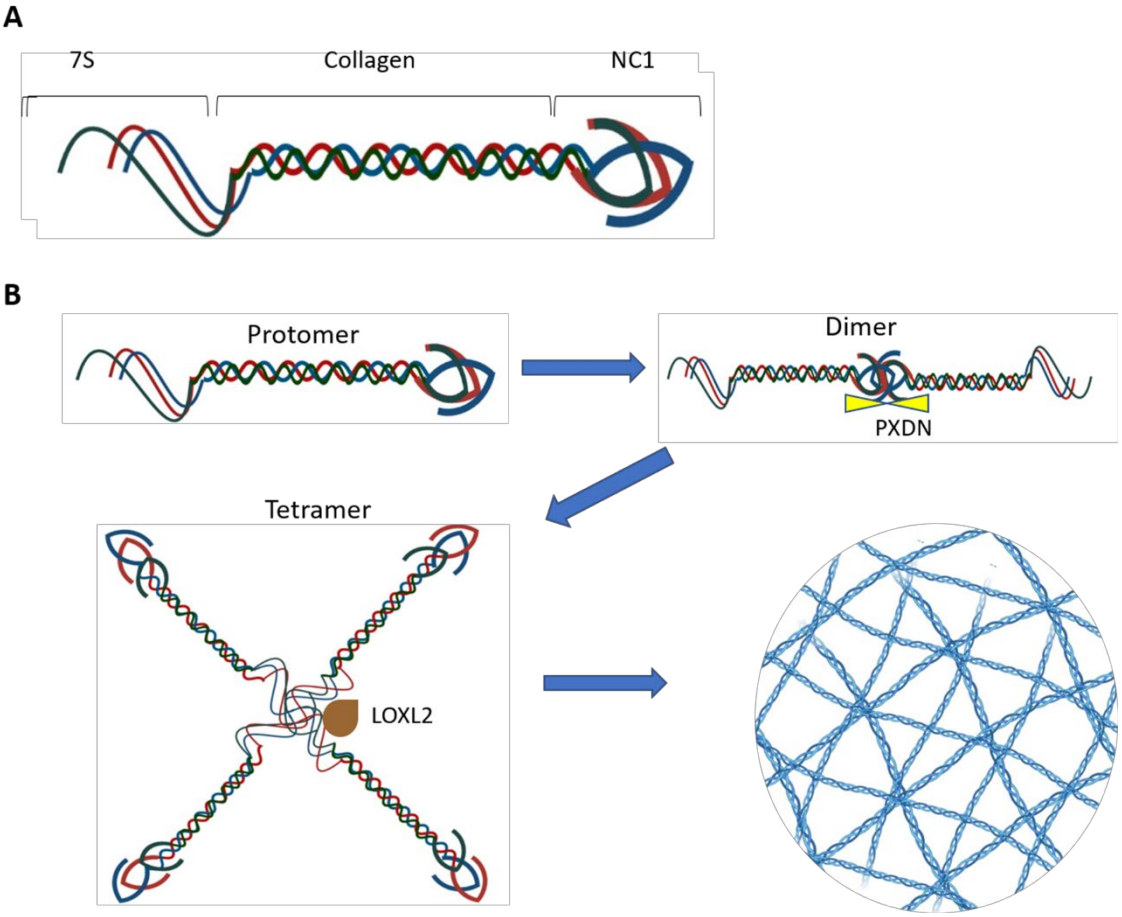

Figure 3: **Collagen IV protomer modification** (A) Co-translational and post-translational modification in the ER of single α-chains includes proline hydroxylation at the fourth or third carbon of the proline ring by the enzymes prolyl4-hydroxylase (P4H) and prolyl3-hydroxylase (P3H) respectively. Lysine residues at the Yaa position of the collagen triplet can also undergo hydroxylation by lysyl hydroxylases (LH1-3). (B) HSP47 binds and stabilises collagen IV protomers, in the ER and during transit to the cis-Golgi. (C) Schematic diagram of modified Collagen IV protomer. (D) HSP47 can act as anchor between TANGO1 to aid secretion of collagen IV from ER.

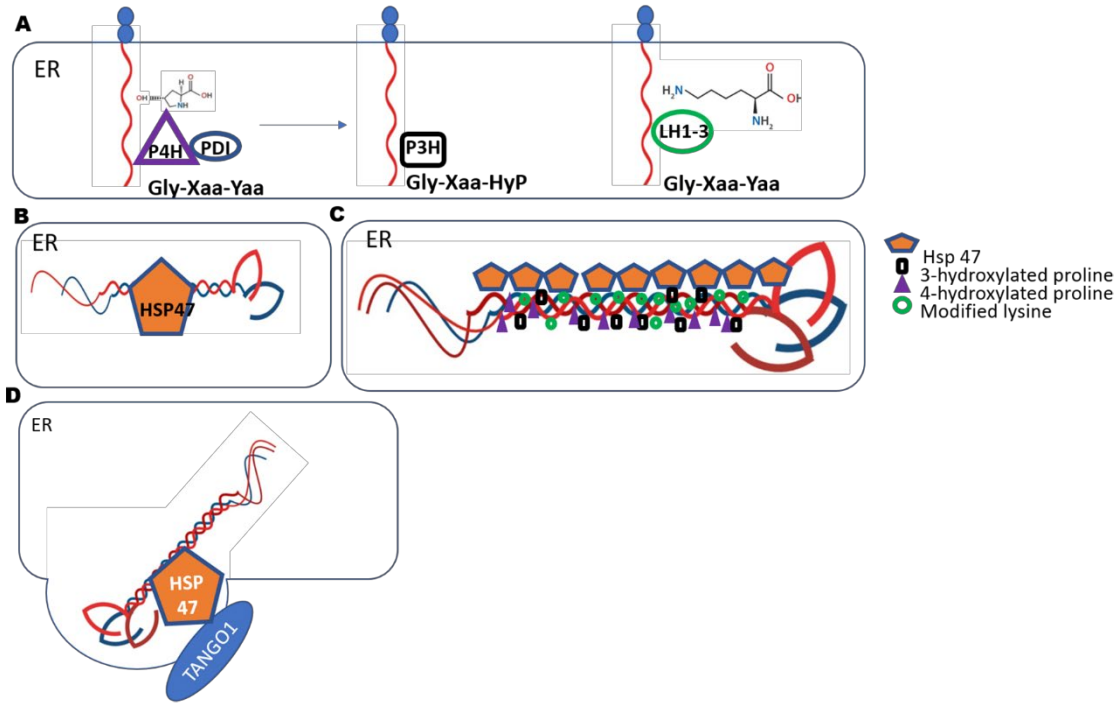

Figure 4 Collagen IV disease mechanisms (A) Under 'correct/normal' physiological conditions, collagen IV protomer is made in the ER, transported out of the cell to be incorporated into the ECM BM. (B) Non-sense mutations (star) reduce protein levels leading to reduced levels of collagen IV in the BM, causing BM defects. (C) Mis-sense mutations can cause mutant protein (yellow star) to be secreted from the cell but mutant protein may also accumulate in the ER. This can cause ER stress and reduced secretion, meaning ECM defects can be due to reduced protein levels and/or mutant protein incorporation.