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► **To cite this version:**

Claire Legay, Alexandre Dobbertin. Collagens at the vertebrate neuromuscular junction, from structure to pathologies. *Neuroscience Letters*, 2020, 735, pp.135155 -. <10.1016/j.neulet.2020.135155>. <hal-03490851>

HAL Id: hal-03490851

<https://hal.science/hal-03490851v1>

Submitted on 22 Aug 2022

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Collagens at the vertebrate neuromuscular junction, from structure to pathologies

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Abstract

The extracellular matrix at the neuromuscular junction is built upon components secreted by the motoneuron, the muscle cell and terminal Schwann cells, the cells constituting this specific synapse. This compartment contains glycoproteins, proteoglycans and collagens that form a dense and specialized layer, the synaptic basal lamina. A number of these molecules are known to play a crucial role in anterograde and retrograde signalings that are active in neuromuscular junction formation, maintenance and function. Here, we focus on the isoforms of collagens which are enriched at the synapse. We summarize what we know of their structure, their function and their interactions with transmembrane receptors and other components of the synaptic basal lamina. A number of neuromuscular diseases, congenital myasthenic syndromes and myasthenia gravis are caused by human mutations and autoantibodies against these proteins. Analysis of these diseases and of the specific collagen knock-out mice highlights the roles of some of these collagens in promoting a functional synapse.

Keywords

Collagens ; neuromuscular junction ; integrins ; perlecan ; congenital myasthenic syndromes ; myasthenia gravis

Introduction

The neuromuscular junction (NMJ) is a well-defined synapse formed between the axon terminal of a motoneuron (MN) and the skeletal muscle fiber. This contact is capped by one to three non-myelinating Schwann cells, called terminal Schwann cells. Neuromuscular transmission initiates muscle contraction and therefore is indispensable for many vital functions. In vertebrates, the neurotransmitter is acetylcholine and the postsynaptic receptors are ligand-gated ion channels of the nicotinic type. The duration of the synaptic transmission is largely controlled by the enzyme acetylcholinesterase (AChE) which hydrolyses acetylcholine.

The NMJ has been central to neurophysiology since the basic mechanisms of neurotransmitter release were elucidated in this synapse [1]. This synapse is characterized by a large synaptic cleft (50nm) compared to the same compartment in central synapses (20nm). The extent of the cleft between the presynaptic axonal membrane and the postsynaptic muscle membrane is considerably increased by the presence of muscle membrane folds. The cleft is filled by an extracellular matrix (ECM), composed of collagens, glycoproteins and proteoglycans. Collagens represent the major component. To date, 28 collagens designated by roman numbers (I-XXVIII) have been characterized on the basis of the repetition of a Gly-X-Y motif involved in the formation of the homomeric or heteromeric triple helix of α polypeptide chains. There are 2 classes of collagens, the non-fibrillar, including fibril-associated, collagens and fibrillar collagens (types I, II, III, V, XI, XXIV and XXVII).

Together, they form a network that ensures mechanical strength and plays a signaling role through membrane receptors. Mechanical strength is particularly important at the NMJ where the synaptic region, like the rest of the muscle fiber, is subjected to substantial stress resulting from muscle contraction. A major role of the collagens is illustrated by the regulation of acetylcholine receptors (AChR) on the postsynaptic membrane. Indeed, AChR aggregation is severely reduced in co-cultures of rat muscle and spinal cord explants treated by cis-hydroxyproline, an inhibitor of collagen formation [2].

At the NMJ, the ECM is organized into a highly structured basement membrane (BM) with a specific molecular composition. This dense BM is easily observable in electron micrographs and runs through the synaptic cleft, including the muscle folds. Collagen IV and laminins are the basic components of all BMs. They form 2 networks interconnected by nidogens and proteoglycans such as perlecan [3]. Both laminins and collagen IV are each encoded by multiple genes. Six different α polypeptide chains associate to form 3 distinct, heterotrimeric types of collagen IV ($\alpha 1$)₂($\alpha 2$), ($\alpha 3$, $\alpha 4$, $\alpha 5$) and ($\alpha 5$)₂($\alpha 6$) [4]. Among these chains, ($\alpha 1$) and ($\alpha 2$) are found in nearly all BMs whereas the other chains have a more specific localization. Laminins are heterotrimers consisting of 3 chains, α , β and γ . There are 5 isoforms of α chains ($\alpha 1$ -5), 3 isoforms of β chains ($\beta 1$ -3) and 3 isoforms of γ chains ($\gamma 1$ -3). 19 laminins result from the combination of the different chains. At the NMJ, 3 forms of laminins ($\alpha 4\beta 2\gamma 1$, $\alpha 5\beta 2\gamma 1$ and $\alpha 2\beta 2\gamma 1$) and the 2 Collagen IV heterotrimers, are only present in the synaptic BM [5, 6, 7].

While the different types of laminins and collagens underlie the variety of BMs through the body, this heterogeneity is increased by the expression of less abundant components that are tissue or/and region specific. In skeletal muscle, the BM covers each myofiber with a number of constituents that are significantly increased or decreased at the NMJ. Even for the same type of collagen, specific heterotrimers can be differentially distributed. For example, ColIV ($\alpha 3$, $\alpha 4$, $\alpha 5$) and ($\alpha 5$)₂($\alpha 6$) trimers are exclusively present at the NMJ whereas ColIV ($\alpha 1$)₂($\alpha 2$) trimers are less abundant in synaptic than extrasynaptic areas [5, 8, 9]. Besides ColIV, few collagens have been identified and studied at the NMJ in part due to the lack of specific antibodies. Perhaps the most documented of these is the collagen Q (ColQ), a specific non fibrillar collagen, that is associated with AChE. Here, we summarize the functions and dysfunctions in several diseases of ColQ and 5 other collagens (IV, VI, XIII, XXV, XXVIII) specifically expressed at the vertebrate NMJ.

I. Structures and functions of the NMJ collagens

Collagen IV (ColIV) is a non-fibrillar collagen composed of an N-terminal domain rich in cysteines followed by Gly-X-Y repeats that are interrupted by non collagenous regions and a globular C-terminal domain (NC1) containing inter-chain disulfide bounds. As mentioned above, ($\alpha 3$, $\alpha 4$, $\alpha 5$) and ($\alpha 5$)₂($\alpha 6$) trimers are the only trimers present at the synapse [5, 8]. The non-collagenous peptide NC1 of this collagen can be cleaved to stimulate a biological activity [10]. When motoneurons purified from chick embryos in culture were treated with the NC1 from α chains 2, 3 or 6, vesicular clustering was observed within axons and the effect was specific for these chains since it did not occur with the α chains 1, 4 or 5 [11]. In the same study, the authors generated and analyzed mutant mice for ($\alpha 3$), ($\alpha 5$) and ($\alpha 6$) chains. In agreement with the composition of the trimers, the ($\alpha 3$) and ($\alpha 6$) chain mutants affected the formation of one of the 2 synaptic trimers whereas ($\alpha 5$) mutant disorganized both trimers. No defects of the NMJ were observed in the absence of ($\alpha 3$) or ($\alpha 6$) chain whatever the age of the mutant mice, suggesting that these chains could have redundant functions. Defects due to the loss of ($\alpha 5$) chain were not present in embryonic NMJ but appeared later in adult mice. At that stage, NMJ were aberrant with fragmentation of NMJ and about 25% of postsynaptic domains

not contacted by nerve terminals. These late defects are consistent with $\alpha 5$ chain becoming detectable only from 3 weeks after birth [11]. In vitro, ColIV in combination with laminin-2 but not alone stabilizes AChR clusters in C2C12 myotubes [12].

Collagen VI (ColVI) α chains are encoded by 6 genes and are expressed in many tissues but the most abundant form corresponds to a triple helix of $\alpha 1/\alpha 2/\alpha 3$ chains [13]. It is a non-fibrillar collagen with a central collagen domain flanked by N- and C- terminal domains with homology to von Willebrand factor type A domains. Although collagen VI is not specifically enriched at the NMJ, adult mice deficient for ColVI $\alpha 1$ chain have abnormal NMJs with fragmented AChR clusters and some presynaptic areas not in contact with AChR clusters [14]. In addition, the lack of ColVI impairs neurotransmission at the NMJ as shown by the reduction in nerve evoked EPP and nerve independent mEPP. It should be noted that in this mutant mice, no triple helical ColVI could be detected [15].

Collagen XIII (ColXIII) belongs to the family of non-fibrillar collagens and is a class II transmembrane protein with an extracellular collagen domain [16, 17]. The protein contains 3 collagenous domains (COL1-3) interrupted by 4 non-collagenous domains (NC1-4). Two of the NC domains are located between the collagenous domains and the 2 others are at the N- and C-terminal ends of the protein. This collagen belongs to a sub-group of collagens called MACITs (for membrane-associated collagens with interrupted triple helices) and to a subclass of short-chain collagens ($Mr < 95000$). The collagen is formed by homotrimeric chains. Besides skeletal muscle, ColXIII is present in skin, bone, cartilage, lung and intestine. *Col13a1* mRNA expression tends to increase during mouse development [18]. In skeletal muscle, this collagen is relatively highly expressed but later than ColIV and is accumulated after birth in human and mouse NMJs [19, 20]. Mice lacking ColXIII have been analysed in detail by the group of Pihlajaniemi [19, 21]. They do not exhibit major defects except for tremors and worsened general appearance with age. However, there are pre- and postsynaptic abnormalities with small and sometimes fragmented AChR clusters, immature presynaptic terminals, unapposed pre- and postsynaptic sites and reduced adhesion between pre- and postsynaptic elements. These structural defects, together with results from electrophysiological studies, indicate that neurotransmission is affected by the absence of Col13. In addition, Schwann cell processes are observed in the synaptic cleft and around nerve terminals. Muscle analysis of *Col13A1* knock-out mice (*Col13a1*^{-/-} mice) revealed atrophy of slow fibers together with a shift from slow muscle fibers to fast fiber type in the soleus muscle as well as signs of regeneration. Like ColIV, ColXIII can be shed to yield a biologically active ectodomain at NMJs, though it is unknown what percentage of the full-length protein is cleaved. Nor do we know the respective roles of the full-length COL13A1 versus the truncated form when considering the complex phenotype observed in *Col13a1*^{-/-} mice. Comparison between the phenotypes of transgenic mice expressing a form of Col13 resistant to cleavage (*Col13a1*^{tm/tm} mice which express only the transmembrane form) and *Col13a1*^{-/-} mice or wt mice show increased fragmentation of AChR clusters and size of NMJs with postnatal age in *Col13a1*^{tm/tm} mice [21]. In *Col13a1*^{tm/tm} mice, pre- and postsynaptic elements are aligned and there are no Schwann cell processes intruding in the synaptic cleft. The number of active zones compared to wt mice is increased in *Col13a1*^{tm/tm} mice but decreased in *Col13a1*^{-/-} mice. So overall, most of the defects observed in *Col13a1*^{-/-} NMJs are absent in *Col13a1*^{tm/tm} NMJs although the non-cleavable COL13A1 form seems to lead to overgrowth of the NMJ and hyperexcitability suggesting a homeostatic role for the cleaved fragment.

Collagen XXV (ColXXV) was first identified as a collagenous component of amyloid plaques in Alzheimer's brains and named CLAC for collagenous alzheimer amyloid plaque component [22]. It is a triple helix of transmembrane collagen monomers with each monomer containing 3 collagenous domains flanked by 4 non-collagenous domains. Like ColXIII, this collagen is a member of the MACITs and can be shed by furin convertase. This, together with its high homology to ColXIII has prompted studies on its role at the NMJs. In contrast to most of the

other collagens discussed, ColXXV is expressed by motoneurons in addition to muscle cells although in the latter, it is only expressed in embryonic muscle after fusion of myoblasts in myotubes with a peak of expression around E12.5 in vivo [23]. Disrupting the *Col25* gene leads to birth lethality due to respiratory failure, a symptom that often reflects NMJ developmental defects [22]. **Analysis of the motor unit reveals that in the knock-out mice, AChR aneural clusters occur normally but the muscle seems immature with thinner muscles and smaller muscle fibers in diameter with central nuclei. This muscle phenotype was recently explained by the role of ColXXV in early myogenesis where it promotes fusion of myoblasts into myotubes [24].** The axon terminals do not branch within muscle and do not contact muscles causing the death of motoneurons, a phenotype consistent with the early expression of ColXXV [22].

Collagen XXVIII (ColXXVIII) is a rare collagen that consists of a homotrimer of α chains that contain a collagen domain flanked by 2 Von Willebrand Factor A (VWA) domains like in ColXIII [25]. It is specifically expressed in the peripheral nervous system and observed around terminal Schwann cells at the NMJ [26]. **A faint staining of this collagen has also been observed in zebrafish in structures that could correspond to nerves in adult muscle [27]. There are currently no knock-out mice that could give insights into its biological function.**

Collagen Q (ColQ) - where Q stands for tail in french - was cloned from *Torpedo marmorata* by the group of Massoulié in 1991 [28]. However, the existence of this collagen was suspected much earlier. It was shown on electron micrographs of AChE purified from eel that the enzyme is associated to a rod-like tail [29]. Silman and collaborators, using biochemical methods, demonstrated that some collagenous component was associated to AChE [30]. The biochemical and structural complexity of the AChE enzyme is reflected in the term « asymmetric ». The primary sequence of the collagen indicates that it is a non-fibrillar collagen in which the central collagen domain is flanked by N- and C-terminal peptides conserved in the mature protein [28, 31]. Each strand of ColQ can associate with a tetramer of AChE, generating more or less saturated forms corresponding to A4 (1 tetramer), A8 (2 tetramers) and A12 (3 tetramers). The last form is the main one present at the NMJ. Several functional domains have been identified. ColQ N-terminus contains 8 prolines (proline rich attachment domain; PRAD) that are involved in the association with AChE through the C-terminus of the catalytic subunit. Two heparan binding sites (HBS) are present in the collagenic domain. They are responsible for the interaction with perlecan, a heparan sulfate proteoglycan [32]. However, the 2 HBS have different physico-chemical properties, and it is not clear if perlecan is binding both sites and if another proteoglycan could as well be involved [33]. Two C-terminal subdomains have been characterized. The domain following the collagen region is necessary for initiating the formation of the triple helix and the very C-terminal domain interacts with a muscle signaling complex, MuSK (Muscle specific kinase)/LRP4 (Low-density lipoprotein receptor-related protein 4) that is essential for NMJ formation [34, 35, 36]. This interaction is supported by the work of K. Ohno's group showing that autoantibodies to MuSK inhibit the interaction between ColQ and MuSK and interfere with the agrin signaling pathway [37, 38]. It was shown that not all the C-terminal mutations tested in vitro impair MuSK/ColQ interaction, only the most C-terminal ones affect this interaction [36]. Since MuSK and LRP4 are strictly synaptic, this last interaction dictates AChE localization at NMJs, though both interactions with perlecan and MuSK are necessary to anchor and cluster AChE/ColQ hetero-oligomers at the synapse. Additional interactions between ColQ and proteins of the basal lamina have been suggested by the work of Maselli's group but remain to be precisely identified [36]. ColQ tissue distribution largely overlaps with that of AChE. As shown by Northern blot analysis, mammalian ColQ is expressed in skeletal muscle and brain but also in lung, kidney, testis, spleen and heart but not in liver [31]. **In most tissues explored, ColQ expression overlaps with that of AChE except for lung where AChE is not expressed [39].** There are 2 isoforms of ColQ, ColQ1 and ColQ1A that are generated by a

process of alternative splicing. The 2 transcripts encode different N-terminal sequences in the signal peptide. ColQ1A expression is restricted to the NMJ where it anchors AChE in the synaptic BM of the NMJ [40]. Since the discovery that ColQ also binds MuSK/LRP4, efforts have been made to parse this interaction distinct from its role in localizing the enzyme. MuSK is a transmembrane kinase receptor and LRP4 is its co-receptor. Both proteins are expressed by muscle cells before innervation and are crucial for NMJ formation including clustering of most of the synaptic proteins, axon positioning and maturation of the synapse [41, 42]. The analysis of a muscle cell line deficient for ColQ and of the *ColQ* knock-out mouse have provided insights into the functions of ColQ in this pathway. In absence of ColQ, AChE is not secreted and thus ACh levels are abnormally high in the synaptic cleft. Although muscle weakness is the major physiological hallmark of ColQ-deficient mice, the absence of ColQ does not affect muscle ultrastructure, although muscle fiber diameter is reduced and the fiber type composition is modified with a reduction in MHC type I fibers and an increase in MHC type 2A fibers [43, 44]. The largest impact concerns the structure of the NMJ [44, 45]. The postsynaptic element is fragmented with less dense AChR clusters. The diameter of the axon and the axon terminal area appear smaller and are abnormally embedded in a terminal Schwann cell. Processes of these glial cells sometimes extend into the synaptic cleft. The surface of contact between pre- and post-elements is reduced. Most of these defects in mice can be associated to the high concentration of ACh and excess Ca⁺⁺ influx in the postsynaptic element through AChR. However, the formation of the synapse occurs without ColQ and AChE and overall the structure of the NMJ remains rather normal, suggesting the existence of compensatory mechanisms in ColQ-deficient mice.

II. Collagen molecular partners at the NMJ: receptors and BM components

Receptors. Collagens are known to interact with each other but also to self-assemble like collagen IV. They also bind to membrane receptors, a characteristic that allows cell signaling. Integrins, dystroglycan and discoidin domain receptors (DDR1 and DDR2) are major receptors for basal lamina proteins [46].

Integrins are heterodimeric transmembrane receptors containing an α and a β subunit. There are 18 α isoforms and 8 β isoforms that can generate 24 heterodimers with differential affinity for their ligands. The main ligands of integrins are laminins and collagens. Among integrins, the $\alpha1\beta1$ and $\alpha2\beta1$ heterodimers are the major receptors for collagens and more specifically collagens I and IV [47]. At NMJ, the forms of integrins which are accumulated are the $\alpha3\beta1$ and $\alpha7\beta1$ heterodimers. Deficiency in $\beta1$ integrin subunit or $\alpha3$ integrin subunit, which would disrupt $\alpha3\beta1$ and $\alpha7\beta1$ forms, induce neuromuscular defects. The $\beta1$ subunit is expressed in muscles and motoneurons. The muscle specific $\beta1$ subunit gene (*Itgb1*) knock-out causes nerve defects with increased axonal length and arborization. The motoneuron knock-out has little effect with normal pre- and postsynaptic molecular structure suggesting that the phenotype is motoneuron cell autonomous [48]. Analysis of $\alpha3$ subunit gene (*Itga3*) knock-out mice reveals that the expression of active zone markers such as Bassoon and Piccolo is severely downregulated in embryos. However, active zone could still be visualized as puncta associated to vesicles in electron microscopy and, importantly, synaptic release was only mildly affected in $\alpha3$ null mice [49]. Thus, both knock-outs have little incidence on NMJ structure and function. In addition, colIV is normally localized in the $\alpha3$ subunit knock-out, suggesting that at least this collagen does not interact with heterodimers of integrins containing the $\alpha3$ isoform.

DDRs are transmembrane receptor tyrosine kinases and their activation by collagen binding regulates the expression of the collagen itself as well as the expression and activity of matrix metalloproteinases (MMPs) [50]. To date, there is no information available about DDR at the NMJ, although DDR binds ColIV and that ColIV is important for NMJ formation. In the context

of DDR functions, it is also interesting to note that MMP-3, a member of the MMP family, affects agrin levels and AChR clustering and that ColIV cleavage products are active in NMJ formation [11, 51]. It would thus be interesting to investigate a link between DDR, ColIV and MMP. It could provide new insights into basal lamina organization, mechanosensitivity and signaling at NMJ.

Besides these receptors, the type IIa receptor protein tyrosine phosphatase (RPTP σ/δ) has been identified as a receptor for ColXXV [52]. This transmembrane receptor contains in its extracellular domain 3 Immunoglobulin-like domains, fibronectin type III domains and 2 phosphatase domains in its cytoplasmic domain. It is expressed in motoneuron axons around E13.5 where it may impact NMJ formation via its interaction with collagen XV.

α Dystroglycan, a transmembrane protein that links the ECM to the cytoskeleton which is enriched at the NMJ binds the glycoprotein laminin as well as the proteoglycans perlecan and agrin but not collagens [46].

BM components. Laminins and ColIV form 2 independent sheets in the BM that are linked by nidogens. Nidogens, which are monomeric glycoproteins contain 3 globular domains (G1-G3) of which G1 and G2 bind ColIV, while G3 binds laminin [53]. Nidogen 1 is present all along muscle cells, whereas Nidogen 2 becomes accumulated at the NMJ by the 3rd week postnatal [54]. This late event may explain why the mice deficient for nidogen-2 do not exhibit structural defects before this stage but AChR clusters become subsequently fragmented. ColXIII is known to interact with α 1 β 1 integrin, nidogen-2, fibronectin, perlecan, heparin and ColIV [55, 56, 57]. In addition, in vitro assays suggest that ColXIII interacts with ColQ but not with acetylcholinesterase [21]. The same authors also show that ColQ binds COL3 domain in ColXIII. Absence of ColXIII leads to a mislocalization of AChE suggesting that ColXIII-ColQ interaction participates to the clustering of AChE at the synapse. However, it is unclear how ColXIII, which is expressed much later than perlecan and ColQ, would be part of this process. It remains that perlecan binds ColVI and ColQ which highlights the central role of perlecan in the collagen network.

III. Pathologies caused by mutations in synaptic collagens : from animal models to human pathologies

Neuromuscular disorders include 2 classes of pathologies. Myasthenia gravis is caused by autoantibodies against essential components of the neuromuscular junction. Congenital myasthenic syndromes (CMS) are inherited disorders that affect synaptic transmission at the neuromuscular junction. They result from mutations in genes coding for synaptic proteins or proteins enriched at the synapse. The mutated proteins can disrupt various functions including synapse formation, structure or activity. All the CMS are characterized by muscle weakness but other symptoms are specific to the mutated protein generating much clinical variability among the CMS. These disorders have been classified in pre-, synaptic (basal lamina compartment) or postsynaptic CMS, according to the normal site of protein expression [58]. According to this classification, mutations in collagens are causative for the synaptic type of CMS.

Mutations in *COL4A3/A5/A6* are causative for the Alport syndrome characterized by kidney failure, hearing loss and eye abnormalities [59]. Although models of knock-out mice support a role for this collagen in Alport Syndrome, no information is available as to the motor activity of patients bearing mutations in A chains.

Mutations in the 3 main chains of ColVI (1-3) have been found in patients and are causative for Ullrich congenital muscular dystrophy (UCMD) and Bethlem myopathy (BM) [60]. NMJ abnormalities are observed in these patients and are similar to the ones found in the *Col6a1* knock-out mice [15]. It remains to see if patients with UMD or BM present defects of neurotransmission. Similar to ColQ, an upregulation of synaptic mRNAs was observed in

Col6a1-deficient mice [14]. In addition, as stated above, in ColQ-deficient muscle cells in vitro, *Col6a3* mRNA levels are downregulated suggesting a possible interplay between both collagens [43].

Mutations in *COL13A1* were first identified by the group of Beeson in 3 patients [20]. These mutations cause a frameshift or alter the selection of a splice site in mRNA. Unlike in *Col13a1* *-/-* mice, human biopsies did not reveal any defect in muscle structure nor myopathic signs. The frameshift mutation was introduced into the genome of the murine muscle C2C12 cell line. Quantification of AChR in mutant C2C12 shows that the number, but not the size of AChR clusters, is severely reduced. In 2019, they were 26 cases of CMS type 19, which is the designated term for the form of CMS caused by mutations in *COL13A1* [61, 62, 63]. All patients present similar symptoms with an onset of the pathology at birth characterized by breathing and respiratory difficulties and a decrease in the severity of the symptoms with age. Salbutamol, an agonist of β 2 adrenergic receptors, but not AChE inhibitors, improves the symptoms [63]. Autoantibodies against collagen XIII have been found in biopsies of patients with myasthenia gravis but there is no information on the NMJ of these patients [64].

Congenital cranial dysinnervation disorder (CCDD) clusters a group of congenital pathologies sporadic or familial that are classified as neuromuscular disorders with a specificity for facial muscle or structure. In these pathologies, the development of innervation is abnormal. Among other genes, COL25A1 has been found to be involved in innervation defect but not at the level of the NMJ [65].

The first mutation in ColQ that was linked to a myasthenic syndrome was identified in 1977 by Engel and collaborators [66]. In this case, no AChE was observed at the NMJ. Subsequently, approximately 60 mutations have been identified in *COLQ* and are spread throughout the gene. Keeping in mind the cell biology and the functional domains of ColQ (see above), the consequences of each of the mutations reflect their localization in the gene and can be anticipated [67]. For example, mutations in the PRAD prevent the association of AChE with ColQ. Mutations in the collagen domain or in the trimerization domain impair or inhibit the formation of the collagen triple helix. Mutations in the ColQ C-terminus do not affect the formation of the triple helix nor the interaction with AChE as shown by the analysis of AChE/ColQ molecular forms. However, patients bearing these last mutations are also myasthenic. Studies on ColQ interactions reveal that ColQ is anchored and clustered in the synaptic basal lamina through interactions with perlecan and MuSK [34, 68, 69]. ColQ-deficient mice mimic rather faithfully CMS with AChE deficiency at the levels of the physiology and NMJ structure. Several observations in ColQ-deficient mice provide insights into the role of the ColQ C-terminus and suggest that this domain is responsible for upregulation of a subset of synaptic mRNAs expressed by the muscle cell [43, 70]. It suggests that compensatory mechanisms have evolved to preserve the function of this critical synapse as previously suggested for the *ColQ* knock-out mice [45]. In this context, it is also interesting to note that in *ColQ*-null mice, the mRNA levels coding for a large number of collagens including Col2, Col4, Col5, Col6, Col7, Col8 and Col11 are downregulated in vitro but not significantly modified in vivo, suggesting here too that compensatory mechanisms occur in the organization of the basal lamina. **No curative treatment is available for this CMS. However Salbutamol, a symptomatic treatment is highly efficient to improve motor skills in human and *ColQ*-deficient mice [71, 44]. In a screen of 415 serum samples from myasthenia gravis patients using a cell-based assay, the group of Beeson detected ColQ autoantibodies in 12 samples and among these samples, 5 were also positive for AChR and 2 for MuSK. These findings suggest that ColQ autoantibodies could be a cause for myasthenia gravis [72].**

Conclusions

This review summarizes the role of a number of collagens at the NMJ (see Table) but also emphasizes the necessity to obtain more details on the physiopathological mechanisms induced by mutations in human genes. A restricted number of collagens have been investigated at the NMJ. So far only mutations in *COL13A1* and *COLQ* have been causally linked to CMS. Autoantibodies to Col13 and ColQ have been found in patients with myasthenia gravis. However, there is no information on NMJ morphology from patients with Col13 autoantibodies and no causative link has been demonstrated for ColQ. **However, there is a good chance that mutations in other collagens could be responsible for CMS. Knowledge about the molecular partners of collagens at the synapse is rather limited and currently provides partial model of the molecular functional network. Obviously, this model evolves with age and suggests that some collagens are involved in NMJ formation while others rather play a role in NMJ maintenance. Most of the data regarding the function of synaptic collagens have been obtained in mice knock-out models. It raises several issues when extrapolating these results to human NMJ: (1) human mutations often correspond to small deletion or point mutations whereas in mice knock-out, the entire gene is invalidated eliminating all the functional domains and their interactions with other proteins (2) the morphology of NMJs in mice and human is quite different which could underlie some functional differences (3) some developmental studies in mice show that specific collagens are expressed at different stages which suggests, as mentioned above, that they play different roles in synapse formation or maintenance. For example, while ColQ is expressed soon after innervation, ColXIII and ColIV trimers are expressed much later, after birth. However since developmental data in human are rather sparse, it is hard to extrapolate the developmental profil of collagens observed in mice to human. Moreover, there is evidence for substantial compensations among collagens in mouse models which might not be the case in humans.** An important question would be to understand to what extent mutations in collagens disorganize the synaptic basal lamina. In that respect, some hints could be provided by electron microscopy in knock-out mice. In addition, for most collagens, pre- and postsynaptic signaling has not been explored in the knock-out mice raising the problem of identifying molecular markers in animal models for the pathologies. Thus, in-depth investigation is required to identify the origin of synaptic CMS and understand how collagens impact NMJ formation and activity.

Competing interests

The authors declare no competing interests

Acknowledgements

We would like to acknowledge supports from AFM-Téléthon, the Ministère de l'Enseignement Supérieur et de la Recherche and the CNRS for fundings.

References

- [1] R. Rudolf, M.M. Khan, V. Witzemann, Motor endplate-anatomical, functional, and molecular concepts in the historical perspective, *Cells* 8 (2019) 387-407.
- [2] C. Kalcheim, D. Duksin, Z. Vogel, Involvement of collagen in the aggregation of acetylcholine receptors on cultured muscle cells, *J Biol Chem.* 257 (1982) 12722-12727.
- [3] R. Jayadev, D.R. Sherwood, Basement membranes, *Curr Biol.* 27 (2017) R207-R211.
- [4] H. Colognato, P.D. Yurchenco, Form and function: the laminin family of heterotrimers, *Dev Dyn.* 218 (2000) 213-234.

- [5] J.H. Miner, J.R. Sanes, Collagen IV alpha 3, alpha 4, and alpha 5 chains in rodent basal laminae: sequence, distribution, association with laminins, and developmental switches, *J Cell Biol.* 127 (1994) 879–891.
- [6] B.L. Patton, Laminins of the neuromuscular system, *Microsc Res Tech.* 51 (2000) 247–261.
- [7] R.S. Rogers, H. Nishimune, The role of laminins in the organization and function of neuromuscular junctions, *Matrix Biol.* 57-58 (2017) 86–105.
- [8] J.R. Sanes, E. Engvall, R. Butkowski, D.D. Hunter, Molecular heterogeneity of basal laminae: isoforms of laminin and collagen IV at the neuromuscular junction and elsewhere, *J Cell Biol.* 111 (1990) 1685–1699.
- [9] J.R. Sanes, Laminin, fibronectin, and collagen in synaptic and extrasynaptic portions of muscle fiber basement membrane, *J Cell Biol.* 93 (1982) 442–451.
- [10] N. Ortega, Z. Werb, New functional roles for non-collagenous domains of basement membrane collagens, *J Cell Sci.* 115 (2002) 4201–4214.
- [11] M.A. Fox, J.R. Sanes, D.B. Borza, V.P. Eswarakumar, R. Fässler, B.G. Hudson, S.W. John, Y. Ninomiya, V. Pedchenko, S.L. Pfaff, M.N. Rheault, Y. Sado, Y. Segal, M.J. Werle, H. Umemori, Distinct target-derived signals organize formation, maturation, and maintenance of motor nerve terminals, *Cell* 129 (2007) 179–193.
- [12] S.P. Smirnov, P. Barzaghi, K.K. McKee, M.A. Ruegg, P.D. Yurchenco, Conjugation of LG domains of agrins and perlecan to polymerizing laminin-2 promotes acetylcholine receptor clustering, *J Biol Chem.* 280 (2005) 41449–41457.
- [13] M. Cescon, F. Gattazzo, P. Chen, P. Bonaldo, Collagen VI at a glance, *J Cell Sci.* 2015;128 (2015) 3525–3531.
- [14] M. Cescon, I. Gregorio, N. Eiber, D. Borgia, A. Fusto, P. Sabatelli, M. Scorzeto, A. Megighian, E. Pegoraro, S. Hashemolhosseini, P. Bonaldo, Collagen VI is required for the structural and functional integrity of the neuromuscular junction, *Acta Neuropathol.* 136 (2018) 483–499.
- [15] P. Bonaldo, P. Braghetta, M. Zanetti, S. Piccolo, D. Volpin, G.M. Bressan, Collagen VI deficiency induces early onset myopathy in the mouse: an animal model for Bethlem myopathy, *Hum Mol Genet.* 7 (1998) 2135–2140.
- [16] L. Tikka, T. Pihlajaniemi, P. Henttu, D.J. Prockop, K. Tryggvason, Gene structure for the alpha 1 chain of a human short-chain collagen (type XIII) with alternatively spliced transcripts and translation termination codon at the 5' end of the last exon, *Proc Natl Acad Sci U S A.* 85 (1988) 7491–7495.
- [17] P. Hägg, M. Rehn, P. Huhtala, T. Väisänen, M. Tamminen, T. Pihlajaniemi, Type XIII collagen is identified as a plasma membrane protein. *J Biol Chem.* 273 (1998) 15590–15597.
- [18] M. Sund, T. Väisänen, S. Kaukinen, M. Ilves, H. Tu, H. Autio-Harmainen, H. Rauvala, T. Pihlajaniemi, Distinct expression of type XIII collagen in neuronal structures and other tissues during mouse development. *Matrix Biol.* 20 (2001) 215–231.

- [19] A. Latvanlehto, M.A. Fox, R. Sormunen, H. Tu, T. Oikarainen, A. Koski, N. Naumenko, A. Shakirzyanova, M. Kallio, M. Ilves, R. Giniatullin, J.R. Sanes, T. Pihlajaniemi, Muscle-derived collagen XIII regulates maturation of the skeletal neuromuscular junction, *J Neurosci.* 30 (2010) 12230–12241.
- [20] Logan CV, Cossins J, Rodríguez Cruz PM, D.A. Parry, S. Maxwell, P. Martinez-Martinez, J. Riepsaame, Z.A. Abdelhamed, A.V.R. Lake, M. Moran, S. Robb, G. Chow, C. Sewry, P.M. Hopkins, E. Sheridan, S. Jayawant, J. Palace, C.A. Johnson, D. Beeson, Congenital myasthenic syndrome type 19 is caused by mutations in COL13A1, encoding the atypical non-fibrillar collagen type XIII α 1 chain, *Am J Hum Genet.* 97 (2015) 878–885.
- [21] H. Härönen, Z. Zainul, H. Tu, N. Naumenko, R. Sormunen, I. Miinalainen, A. Shakirzyanova, T. Oikarainen, A. Abdullin, P. Martin, S. Santoleri, J. Koistinaho, I. Silman, R. Giniatullin, M.A. Fox, A. Heikkinen, T. Pihlajaniemi, Collagen XIII secures pre- and postsynaptic integrity of the neuromuscular synapse, *Hum Mol Genet.* 26 (2017) 2076-2090.
- [22] T. Hashimoto, T. Wakabayashi, A. Watanabe, H. Kowa, R. Hosoda, A. Nakamura, I. Kanasawa, T. Arai, K. TAakio, D.M.A Mann, T. Iwatsubo, CLAC: a novel Alzheimer amyloid plaque component derived from a transmembrane precursor, CLAC-P/collagen type XXV, *EMBO J.* 21 (2002) 1524-1534.
- [23] T. Tanaka, T. Wakabayashi, H. Oizumi, N. Shu, S. Takashi, H. Akihiro, F. Daisuke, M. Yuko, H. Tadafumi, I. Takeshi, CLAC-P/collagen type XXV is required for the intramuscular innervation of motoneurons during neuromuscular development, *J Neurosci.* 34 (2014) 1370–1379.
- [24] T.J.M. Gonçalves, F. Boutillon, S. Lefebvre, V. Goffin, T. Iwatsubo, T. Wakabayashi, F. Oury, A-S. Armand, Collagen XXV promotes myoblast fusion during myogenic differentiation and muscle formation, *Sci. Rep.* 9 (2019) 5878-5890.
- [25] G. Veit, B. Kobbe, D.R. Keene, M. Paulsson, M. Koch, R. Wagener, Collagen XXVIII, a novel von Willebrand factor A domain-containing protein with many imperfections in the collagenous domain, *J Biol Chem.* 281 (2006) 3494–3504.
- [26] S. Grimal, S. Puech, R. Wagener, S. Ventéo, P. Carroll, A. Fichard-Carroll, Collagen XXVIII is a distinctive component of the peripheral nervous system nodes of Ranvier and surrounds nonmyelinating glial cells, *Glia* 58 (2010) 1977–1987.
- [27] J.M. Gebauer, B. Kobbe, M. Paulsson, R. Wagener, Structure, evolution and expression of collagen XXVIII: lessons from the zebrafish, *Matrix Biol.* 49 (2016) 106-119.
- [28] E. Krejci, F. Coussen, N. Duval, J.M. Chatel, C. Legay, M. Puype, J. Vandekerckhove, J. Cartaud, S. Bon, J. Massoulié, Primary structure of a collagenic tail peptide of Torpedo acetylcholinesterase: co-expression with catalytic subunit induces the production of collagen-tailed forms in transfected cells, *EMBO J.* 10 (1991) 1285–1293.
- [29] J. Cartaud, F. Rieger, S. Bon, J. Massoulié, Fine structure of electric ell acetylcholinesterase, *Brain Res.* 88 (1975) 127–130.
- [30] Y. Dudai, I. Silman, The effects of solubilization procedures on the release and molecular state of acetylcholinesterase from electric organ tissue, *J Neurochem.* 23 (1974) 1177-1187.

- [31] E. Krejci, S. Thomine, N. Boschetti, C. Legay, J. Sketelj, J. Massoulié, The mammalian gene of acetylcholinesterase-associated collagen, *J Biol Chem* 272 (1997) 22840-22847.
- [32] R.L. Rotundo, S.G. Rossi, L.M. Kimbell, C. Ruiz, E. Marrero, Targeting acetylcholinesterase to the neuromuscular synapse, *Chem Biol Interact.* 157-158 (2005) 15-21.
- [33] P. Deprez, N.C. Inestrosa, E. Krejci, Two different heparin-binding domains in the triple helical domain of ColQ, the collagen tail subunit of synaptic acetylcholinesterases, *J Biol Chem.* 278 (2003) 23233-23242.
- [34] A. Cartaud, L. Strohlic, M. Guerra, B. Blanchard, M. Lambergeon, E. Krejci, J. Cartaud, C. Legay, MuSK is required for anchoring acetylcholinesterase at the neuromuscular junction, *J Cell Biol.*, 165 (2004) 505-515.
- [35] T. Nakata, M. Ito, Y. Azuma, K. Otsuka, Y. Noguchi, H. Komaki, A. Okumura, K. Shiraishi, A. Masuda, J. Natsume, S. Kojima, K. Ohno, Mutations in the C-terminal domain of ColQ in endplate acetylcholinesterase deficiency compromise ColQ-MuSK interaction, *Hum Mutat.* 34 (2013) 997-1004.
- [36] J. Arredondo, M. Lara, F. Ng, D.A. Gochez, D.G. Lee, S.P. Logla, J. Nguyen, R.A. Maselli, COOH-terminal collagen Q (COLQ) mutants causing human deficiency of endplate acetylcholinesterase impair the interaction of ColQ with proteins of the basal lamina, *Hum Genet.* 133 (2014) 599-616.
- [37] Y. Kawakami, M. Ito, M. Hirayama, K. Sahashi, B. Ohkawara, A. Masuda, H. Nishida, N. Mabuchi, A.G. Engel, K. Ohno, Anti-MuSK autoantibodies block binding of collagen Q to MuSK, *Neurology* 77 (2011) 1819-1826.
- [38] K. Otsuka, M. Ito, B. Ohkawara, A. Masuda, Y. Kawakami, K. Sahashi, H. Nishida, N. Mabuchi, A. Takano, A.G. Engel, K. Ohno, Collagen Q and anti-MuSK autoantibody competitively suppress agrin/LRP4/MuSK signaling. *Sci Rep.* 5 (2015) 13928-13941.
- [39] C. Legay, S. Bon, P. Vernier, F. Coussen, J. Massoulié, Cloning and expression of a rat acetylcholinesterase subunit: generation of multiple molecular forms and complementarity with a Torpedo collagenic subunit, *J Neurochem.* 60 (1993) 337-346.
- [40] E. Krejci, C. Legay, S. Thomine, J. Sketelj, J. Massoulié, Differences in expression of acetylcholinesterase and collagen Q control the distribution and oligomerization of the collagen-tailed forms in fast and slow muscles, *J Neurosci* 19 (1999) 10672-10679.
- [41] S.J. Burden, N. Yumoto, W. Zhang, The role of MuSK in synapse formation and neuromuscular disease, *Cold Spring Harb Perspect Biol.* 5 (2013) a009167-a009178.
- [42] R. Herbst, MuSK function during health and disease, *Neurosci Lett.* 716 (2020) 134676-134684.
- [43] S.M. Sigoillot, F. Bourgeois, J. Karmouch, J. Molgó, A. Dobbertin, C. Chevalier, R. Houlgatte, J. Léger, C. Legay, Neuromuscular junction immaturity and muscle atrophy are hallmarks of the ColQ-deficient mouse, a model of congenital myasthenic syndrome with acetylcholinesterase deficiency, *FASEB J.* 30 (2016) 2382-2399.

- [44] G.M. McMacken, S. Spendiff, R.G. Whittaker, E. O'Connor, R.M. Howarth, V. Boczonadi, R. Horvath, C.R. Slater, H. Lochmüller, Salbutamol modifies the neuromuscular junction in a mouse model of ColQ myasthenic syndrome, *Hum Mol Genet.* 28 (2019) 2339–2351.
- [45] G. Feng, E. Krejci, J. Molgo, JM Cunningham, J. Massoulié, J.R. Sanes, Genetic analysis of collagen Q: roles in acetylcholinesterase and butyrylcholinesterase assembly and in synaptic structure and function, *J Cell Biol.* 144 (1999) 1349–1360.
- [46] P.D. Yurchenco, B.L. Patton, Developmental and pathogenic mechanisms of basement membrane assembly, *Curr Pharm Des.* 15 (2009) 1277-1294.
- [47] J. Khoshnoodi, V. Pedchenko, B.G. Hudson, Mammalian collagen IV, *Microsc Res Tech.* 71 (2008) 357–370.
- [48] M. Schwander, R. Shirasaki, S.L. Pfaff, U. Müller, Beta1 integrins in muscle, but not in motor neurons, are required for skeletal muscle innervation, *J Neurosci.* 24 (2004) 8181–8191.
- [49] J.A. Ross, R.G. Webster, T. Lechertier, L.E. Reynolds, M. Turnaine, M. Bencze, Y. Jamshidi, H Cetin, F. Muntoni, D. Beeson, K. Hodilvala-Dilke, F.J. Conti, Multiple roles of integrin- α 3 at the neuromuscular junction, *J Cell Sci.* 130 (2017) 1772–1784.
- [50] C.M. Borza, Y. Su, T.L. Tran, L. Yu, N. Steyns, K.J. Temple, M.J. Skwark, J. Meiler, C. W. Lindsley, B.R. Hicks, B. Leitinger, R. Zent, A. Pozzi, Discoidin domain receptor 1 kinase activity is required for regulating collagen IV synthesis, *Matrix Biol.* 57-58 (2017) 258–271.
- [51] Z.C. Chan, M.J. Oentaryo, C.W. Lee, MMP-mediated modulation of ECM environment, during axonal growth and NMJ development, *Neurosci. Lett.* 724 (2020) 134822.
- [52] H. Munezane, H. Oizumi, T. Wakabayashi, S. Nishio, T. Hirasawa, T. Sato, A. Harada, T. Yoshida, T. Eguchi, Y. Yamanashi, T. Hashimoto, T. Iwatsubo, Roles of Collagen XXV and its putative receptors PTP σ / δ in intramuscular motor innervation and congenital cranial dysinnervation disorder, *Cell Rep.* 29 (2019) 4362–4376.
- [53] J.W. Fox, U. Mayer, R. Nischt, M Aumailley, D Reinhardt, H Wiedemann, K Mann, R Timpl, T. Krieg, J. Engel, Recombinant nidogen consists of three globular domains and mediates binding of laminin to collagen type IV, *EMBO J.* 10 (1991) 3137–3146.
- [54] M.A. Fox, M.S. Ho, N. Smyth, J.R. Sanes, A synaptic nidogen: developmental regulation and role of nidogen-2 at the neuromuscular junction, *Neural Dev.* 3 (2008) 24.
- [55] P. Nykvist, H. Tu, J. Ivaska, J. Käpylä, T. Pihlajaniemi, J. Heino, Distinct recognition of collagen subtypes by alpha(1)beta(1) and alpha(2)beta(1) integrins. Alpha(1)beta(1) mediates cell adhesion to type XIII collagen, *J Biol Chem.* 275 (2000) 8255–8261.
- [56] Tu H., T. Sasaki, A. Snellman, W. Göhring, P. Pirilä, R. Timpl, T. Pihlajaniemi, The type XIII collagen ectodomain is a 150-nm rod and capable of binding to fibronectin, nidogen-2, perlecan, and heparin, *J Biol Chem.* 277 (2002) 23092-23099.

- [57] A. Heikkinen, H. Härönen, O. Norman, T. Pihlajaniemi, Collagen XIII and other ECM components in the assembly and disease of the neuromuscular junction, *Anat Rec.* 303 (2020) 1653-1663.
- [58] A.E. Vanhaesebrouck, D. Beeson, The congenital myasthenic syndromes: expanding genetic and phenotypic spectrums and refining treatment strategies, *Curr Opin Neurol.* 32 (2019) 696–703.
- [59] S.D. Funk, M.H. Lin, J.H. Miner, Alport syndrome and Pierson syndrome: diseases of the glomerular basement membrane, *Matrix Biol.* 71-72 (2018) 250–261.
- [60] C.G. Bönnemann, The collagen VI-related myopathies: muscle meets its matrix, *Nat Rev Neurol.* 7 (2011) 379-390.
- [61] M. Dusl, T. Moreno, F. Munell, A. Macaya, M. Gratacòs, A. Abicht, T.M. Strom, H. Lochmüller, J. Senderek, Congenital myasthenic syndrome caused by novel COL13A1 mutations, *J Neurol.* 266 (2019) 1107–1112.
- [62] R.J. Marquardt, Y Li, Congenital myasthenic syndrome type 19 due to a novel mutation in the COL13A1 GENE, *Muscle Nerve* 60 (2019) E3–E4.
- [63] P.M. Rodríguez Cruz, J. Cossins, E.P. Estephan, F. Munell, K. Selby, M. Hirano, R. Maroofin, M.Y.V Mehrjardi, G. Chow, A. Carr, A. Manzur, S. Robb, P. Munot, W. Wei Liu, S. Banka, H. Fraser, C. De Goede, E. Zanolati, U. Conti Reed, A. Sage, M. Gratacos, A. Macaya, M. Dusl, J. Senderek, A. Töpf, M. Hofer, R. Knight, S. Ramdas, S. Jayawant, H. Lochmüller, J. Palace, D. Beeson, The clinical spectrum of the congenital myasthenic syndrome resulting from COL13A1 mutations, *Brain* 142 (2019) 1547–1560.
- [64] H. Tu, R. Pirskanen-Matell, A. Heikkinen, T. Oikarainen, J. Risteli, T. Pihlajaniemi, Autoimmune antibodies to collagen XIII in myasthenia gravis patients, *Muscle Nerve* 57 (2018) 506–510.
- [65] J.M. Shinwari, A. Khan, S. Awad, Z. Shinwari, A. Alaiya, M. Alanazi, A. Tahir, C. Poizat, N. Al Tassan, Recessive mutations in COL25A1 are a cause of congenital cranial dysinnervation disorder, *Am J Hum Genet.* 96 (2015) 147–152.
- [66] A.G. Engel, E.H. Lambert, M.R. Gomez, A new myasthenic syndrome with end-plate acetylcholinesterase deficiency, small nerve terminals, and reduced acetylcholine release, *Ann. Neurol.* 1 (1977) 315-330.
- [67] C. Legay, Congenital myasthenic syndromes with acetylcholinesterase deficiency, the pathophysiological mechanisms, *Ann N Y Acad Sci.* 1413 (2018) 104-110.
- [68] C. Jacobson, P.D. Cote, S.G. Rossi, R.L. Rotundo, S. Carbonetto, The dystroglycan complex is necessary for stabilization of acetylcholine receptor clusters at neuromuscular junctions and formation of the synaptic basement membrane, *J Cell Bio.* 152 (2001) 435-450.
- [69] L.M. Kimbell, K. Ohno, A.G. Engel, R.L. Rotundo, C-terminal and heparin-binding domains of collagenic tail subunit are both essential for anchoring acetylcholinesterase at the synapse, *J Biol Chem.* 279 (2004) 10997-11005.

[70] S.M. Sigoillot, F. Bourgeois, M. Lambergeon, L. Stochlic, C. Legay, ColQ controls postsynaptic differentiation at the neuromuscular junction, *J Neurosci* 30 (2010) 13-23.

[71] M. Lee, D. Beeson, J. Palace, Therapeutic strategies for congenital myasthenic syndromes, *Ann N Y Acad Sci.* 1412 (2018) 129-136.

[72] M. Zoltowska Katarzyna, K. Belaya, M. Leite, W. Patrick, A. Vincent, D. Beeson, Collagen Q- a potential target for autoantibodies in myasthenia gravis, *J Neurol Sci.* 348 (2015) 241-244.

Collagens at the neuromuscular junction

Collagens	Interactors	Mice knock-out phenotype	Human pathologies
ColIV	ColIV Fibronectin Integrin Laminins Nidogens	COL4A5 : late NMJ defects	Alport syndrome Goodpasture syndrome (autoimmune disease) Myopathies
ColVI	Biglycan ColIV Decorin Perlecan	COL6A1 : NMJ defects	Ullrich congenital dystrophy Bethlem Myopathy NMJ defects similar to mutant mice
ColXIII	ColIV ColQ Fibronectin Heparin $\alpha 1\beta 1$ integrin Nidogen-2 Perlecan	COL13A1 : NMJ and muscle defects	Congenital Myasthenic Syndrome
ColXXV	RPTP σ/δ	NMJ and muscle defects	Congenital cranial dysinnervation disorders
ColXXVIII	-	No knock-out mice available	-
ColQ	ColXIII Heparin MuSK Perlecan	NMJ defects	Congenital Myasthenic Syndrome with AChE deficiency