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A Review of the Type-1 Fibrillinopathies: Pathophysiology, Diagnosis and Novel Therapeutic Strategies

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Abstract

Type-1 fibrillinopathies are a family of connective tissue disorders with major clinical manifestations in the skeletal, ocular and cardiovascular systems. The type-1 fibrillinopathies are caused by mutations in the fibrillin-1 gene (FBN1), which encodes fibrillin-1, a large glycoprotein and a major component of the extracellular matrix microfibrils, providing both structural and regulatory support to connective tissues. The type-1 fibrillinopathies have been associated with over 1800 unique mutations within the FBN1 and demonstrate a wide range of phenotypic variability. This, in conjunction with a number of other factors has impacted on the identification of genotype-phenotype correlations, pathogenesis and diagnostic tests for this family of diseases, leaving many open-ended theories.

Current standard of care relies heavily on surgical intervention and lifelong use of β-blockers to slow disease progression, with research focused heavily on antagonism of transforming growth factor β, which is known to be dysregulated in patients with FBN1 mutations. Antisense oligonucleotides present a novel therapeutic strategy for the type-1 fibrillinopathies, by mediating the alteration of exon arrangement of both the normal and disease-causing mRNA transcripts, to re-establish the periodicity of fibrillin-1. The induced proteins, while internally truncated, should be homologous and thus be able to form multimer units. This treatment alone or in association with isoform switching, TGF-β antagonism or enhanced/inhibited protein degradation could facilitate the assembly of fibrillin-1 monomers into multimers and consequently a decrease in phenotypic severity.

This review presents a basic overview of the past and current knowledge about the spectrum of type-1 fibrillinopathies with a particular focus on Marfan syndrome, as well as presenting novel potential therapeutic strategies.

Keywords: Type-1 fibrillinopathies; Marfan syndrome; Fibrillin-1; Genetic therapy

Introduction to the Type-1 fibrillinopathies

The type-1 fibrillinopathies are a family of heritable connective tissue disorders characterised by skeletal, ocular and cardiovascular abnormalities. These diseases are caused by mutations in the fibrillin-1 gene (FBN1) [1], with over 1800 unique mutations, spread throughout the FBN1 sequence, described in the universal mutation database (UMD) [2]. The majority of mutations described are missense mutations, however, insertions, deletions and splice site mutations have also been described [2,3].

FBN1 is one of three distinct genes in the fibrillin family, along with fibrillin-2 and fibrillin-3, all of which share sequence similarities [4]. FBN1 is a large gene consisting of 66 exons spanning over 200 Kb [5]. While exon 1 of FBN1 does not directly contribute to the translated product, the exon numbering system used in this review is based on the full 66 exon transcript (GenBank reference sequence NM_000138.4). The remaining 65 exons encode a 2871 amino acid preproprotein, which is cleaved, by the protease furin, into the large glycoprotein fibrillin-1 and the protein hormone asprosin [6,7].

Fibrillin-1 is present in the majority of connective tissues and has both structural and regulatory roles. As a major structural element of microfibrils fibrillin-1 acts as a backbone to which other microfibril associated proteins bind [8,9], while also being essential for the stability of elastic fibres [10,11]. The assembly of fibrillin into microfibrils is initiated immediately after synthesis and secretion when fibrillin-1 monomers aggregate into multimer units, bound by disulphide bonds between the first 4 cysteine residues at the N-terminus [12]. Heterodimers between fibrillin-1 and the other fibrillin monomers have not been observed, suggesting that the proline-rich sequence at the N-terminus (Figure 1), unique to fibrillin-1, provides the specificity responsible for this binding [13].

Figure 1: The domain structure of the fibrillin-1 preproprotein.

Spectrum of Type-1 Fibrillinopathies

Made up of four EGF-like domains and 43 calcium binding EGF-like (cbEGF-like) domains interspaced with seven TGF-β binding protein-like (TB) domains and two hybrid domains. A proline rich region toward the N-terminus has been implicated in the assembly and specificity of fibrillin-1 mutimers [13].

The structure of fibrillin-1 is complex and highly repetitive, consisting of a number of cysteine-rich repeats (Figure 1). These include 47 elongation growth factor (EFG)-like repeats, seven transforming growth factor β binding protein-like (TB) domains and two domains that share similarities with both EGF-like and TB domains known as hybrid domains [14,15]. Of the 47 EGF-Like repeats, 43 contain a consensus sequence for calcium binding, which is essential for protein stability and protection from proteolysis [16], these repeats are therefore further denoted cbEFG-like domains [15]. Each EGF-like, TB and hybrid domains has 6-8 highly conserved cysteines [14,17] that form disulphide bonds in specific patterns that assist in protein folding and enhance protein function. Mutations that disrupt these bonds are the most common cause of the type-1 fibrillinopathy, Marfan syndrome [18].

Marfan syndrome

Marfan syndrome (MFS, MIM 154700) is the most common of the type-1 fibrillinopathies [19], with a consistent prevalence of 2-3 in 10,000 individuals across gender, ethnicity and geographical distribution [20,21]. MFS is inherited in an autosomal dominant manner with approximately 25% to 30% of mutations arising de novo [22,23]. However, despite consistently being referred to as an autosomal dominant condition, as of 2017, twelve cases of homozygous mutations have been recorded in the UMD-FBN1 database [2]. A number of these cases have an unequivocal autosomal recessive inheritance pattern, with relatives of the proband being asymptomatic heterozygous carriers [24,25]. This suggests that the inheritance pattern of MFS is complex and still not fully understood.

Clinical features

MFS is a multisystem disorder characterised by skeletal, cardiovascular and ocular abnormalities [26,27]. The most noticeable features include increased height with dolichostenomelia and arachnodactyly; the disproportionate overgrowth of long bones and digits respectively, as well as joint hypermobility [26]. Spinal deformities such as scoliosis and dural ectasia, and chest wall deformities are also common features [26,27].

Ocular manifestations include myopia or near sightedness, and ectopia lentis, which is the displacement of the crystalline lens from its natural location [27]. Such features generally present early in disease progression and are therefore important diagnostic indicators, especially for children. However, these features are also common to a multitude of other diseases including a number of other type-1 fibrillinopathies [26].

Cardiovascular abnormalities are the most common cause of death of MFS patients especially in the most severe form, neonatal Marfan syndrome (nMFS), which is characterised by the early onset of cardiovascular manifestations [28,29]. These deaths are typically the result of progressive aortic root enlargement and aortic aneurysm, that can eventuate into aortic regurgitation, dissection or rupture [30]. Other cardiovascular features include mitral valve prolapse and mitral regurgitation [27]. Due to their late onset and progressive nature, key cardiovascular features are often not present or noticeable in younger patients. However, with advances in technology, features such as aortic enlargement can now be readily detected in suspected MFS patients using echocardiography, allowing for much needed early intervention [20].

Diagnostic odyssey

The diagnosis of MFS and delineation from other type-1 fibrillinopathies is challenging for a number of reasons, including the large size of FBN1, number of unique mutations and the lack of defined mutation hotspots [23,31]. These characteristics mean that, despite progress in understanding the genetic basis of MFS, as well as advances in genetic testing techniques, there is still no efficient, time and cost effective molecular test for Marfan syndrome [19,32]. Molecular diagnosis is most often reserved for patients who either have a clinical diagnosis or a diagnosed relative [33].

There are also diagnostic issues that arise from the extensive phenotypic overlap between MFS and the other type-1 fibrillinopathies, as well as the phenotypic variability observed both between and within affected families [23,31]. The progressive nature of MFS, in particular the late onset of cardiovascular features also adds to the challenge, especially in the diagnosis of children in which the symptoms have not fully developed [34].

To overcome these limitations, the diagnosis of MFS is based on a well-defined set of criteria, known as the Ghent nosology and supplemented with molecular testing when appropriate [20,33]. These criteria were first described by Beighton et al. [35] under the umbrella of the Berlin nosology, which encompassed the diagnosis of a number of connective tissue disorders. This document outlines features considered major or minor in the MFS phenotype, organised according to the organ system involved. The requirements of diagnosis varied, depending on the presence of an affected relative and were based on the involvement of at least 2 organ systems with a number of major and minor manifestations [35].

These diagnostic criteria were subsequently updated in 1996 and reworked into the Ghent nosology that is more specific to the diagnosis of Marfan syndrome. These updated criteria provide more stringent diagnosis for relatives of MFS individuals, revised skeletal involvement and delineation of MFS and MFS-like disorders [36]. In 2010, the Ghent nosology was revised again due to concerns about the sensitivity of diagnosis, especially in regard to age-dependant manifestations and the resulting potential for misdiagnosis of children [27]. The revised criteria place more emphasis on aortic root aneurysm and ectopia lentis, with less emphasis on features such as flat feet and pulmonary artery dilation that are common to other type-1 fibrillinopathies [27]. Current diagnosis of a patient without a diagnosed relative requires the major involvement of at least two organ systems with minor involvement of a third. Individuals with known FBN1 mutations or a first degree relative with a MFS diagnosis, are diagnosed based on the presence of one major and one minor manifestation in different organ systems [27].

Due to the considerable phenotypic variability amongst individuals with type-1 fibrillinopathies, affected individuals are often classified according to where they sit on the 'Marfan spectrum' [3]. At one end are those diagnosed with neonatal Marfan syndrome, the most severe form of MFS, characterised by its early onset and life expectancy of less than 24 months [37]. At the other end of the spectrum are those who

do not fully meet the Ghent nosology or have additional features not observed in the Marfan phenotype. Such patients are most often diagnosed with other type-1 fibrillinopathies, as described below.

Marfan lipodystrophy syndrome

Marfan lipodystrophy syndrome (MFLS, MIM 616914) is an extremely rare autosomal dominant disease, with only 7 known cases globally [38]. MFLS is characterised by congenital lipodystrophy, the severe lack of fat in the subcutaneous tissues, as well as premature birth and disproportionate growth to weight gain [39]. Affected individuals also have distinctive facial features, including protruding eyes, down slanting palpebral fissures and a posteriorly positioned lower jaw resulting in a severe overbite [39]. Other features overlap with Marfan syndrome including long limbs and digits, hyper extensible joints and myopia [39,40]. Due to these similarities there are cases in which individuals fulfil the Ghent nosology, however due to the characteristic lack of subcutaneous fat tissue are diagnosed with MFLS [40].

MASS syndrome

MASS syndrome (MIM 604308) is the diagnosis given to individuals who have phenotypes involving the mitral valve, aorta, skeleton and skin, but do not fulfil the Ghent nosology [41]. Despite not meeting the diagnostic criteria of MFS, the MASS phenotype shares a number of features with MFS, including disproportionately long limbs, chest deformities, mitral valve prolapse and aortic root dilation [36,41]. Loeys et al. [27] suggests caution in the diagnosis of MASS syndrome due to its ambiguity, the lack of understanding of the underlying mutations and the potential for disease progression into classic MFS.

Ectopia lentis syndrome

While ectopia lentis is a key feature of the MFS phenotype, ectopia lentis syndrome (ELS, MIM 129600) describes patients who have ectopia lentis but lack the cardiovascular involvement typical of MFS [27]. ELS has an autosomal dominant inheritance pattern and affected individuals present with dislocation of the lens from abnormal stretching of the zonular fibres and this can in turn result in acute or chronic impaired vision [42]. Much like MFS, ELS is caused by numerous mutations throughout the FBN1, with around 38% of mutations that result in ELS also identified in MFS patients [43].

Stiff skin syndrome

Stiff skin syndrome (SSKS, MIM 184900) is another rare autosomal dominant disorder characterised by thick and hardened skin that leads to reduced joint mobility [44]. Due to its rarity, the exact cause and pathogenesis of SSKS remains unknown, however using pulse chase analysis Loeys et al. [44] determined that while SSKS patients have normal levels of fibrillin-1 secretion, they have increased deposition of fibrillin-1; and elastin, in the dermis. The group also observed that patient microfibrils were noticeably shorter than those seen in control samples, suggesting that FBN1 mutations are implicated in this syndrome [44].

Other type-1 fibrillinopathies

Weill-Marchesani syndrome 2 (WMS2, MIM 608328), acromicric dysplasia (ACMID, MIM 102370) and geleophysic dysplasia 2 (GPHYSD2, MIM 614185) are allelic autosomal dominant disorders characterised by severe short stature, brachydactyly and limited joint movement [45,46]. While all three disorders share skeletal phenotypes, WMS2 patients also present with lens abnormalities including glaucoma and ectopia lentis [46]. GPHYSD2 differs from ACMID as affected patients have additional cardiovascular abnormalities that lower life expectancy [45]. ACMID also has unique craniofacial feature including rounded face, with distinctive well-defined eyebrows and eyelashes, bulbous nose and small mouth with thick lips [45].

Genotype-Phenotype Correlations

Several studies have attempted to correlate mutations in particular regions of FBN1 with specific phenotypes. However, this has proved difficult due to the high frequency of unique mutations, approximately 60% [2] and the extensive variability in phenotype between and within families [3,47]. Despite these challenges, one trend that is well accepted is the association of neonatal MFS with mutations within exons 25-33 (Figure 2) [18]. However, while the entire region most often quoted as associated with nMFS, Booms et al. [37] reported that evidence actually supports the presence of two nMFS hotspots. The first encompasses exons 25-28 and mainly consists of missense mutations and in-frame insertions [37]. The second hotspot spans exon 32 and 33, in which splice site mutations resulting in exon skipping most often lead to nMFS [37].

Several of the studies attempting to unravel genotypes-phenotypes associations have noted that the type of mutation, rather than its location, influences the resulting phenotype [18,37,48,49]. A good illustration of this trend is that missense mutations within exons 32 and 33 are most often associated with the classic MFS phenotype [2], while donor or acceptor splice site mutations within this region lead to the severe and early onset phenotype of nMFS [37]. Other examples are the association of premature protein truncation mutations with severe skeletal phenotypes, and cysteine substitutions with ectopia lentis [18,50].

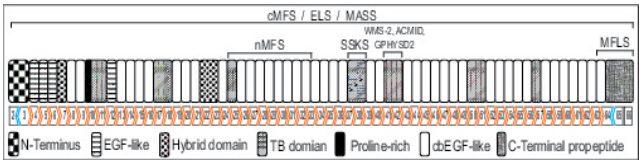

Figure 2: The type-1 fibrillinopathies and associated regions in FBN1.

In particular, the 'neonatal' (nMFS) region that spans exons 25-33, which is associated with the most severe form of Marfan syndrome. cMFS: classic Marfan syndrome, ELS: ectopia lentis syndrome, SSKS: stiff skin syndrome, WMS-2: Weill-Marchesani syndrome 2, ACMID: acromicric dysplasia, GPHYSD2: geleophysic dysplasia 2 and MFLS: Marfan lipodystrophy syndrome.

While mutations causing MFS and ELS are found throughout the FBN1 sequence, many of the mutations associated with other type-1 fibrillinopathies are clustered within specific regions of FBN1 (Figure 2). For example, MFLS is associated with mutations that affect the region of FBN1 that encodes asprosin. The mutations identified to date include 2 bp, 8 bp and 20 bp deletions in the 65th exon [39,51] as well as mutations resulting in early protein truncation and the loss of the C-terminus [52].

The majority of mutations that have been associated with WMS2, ACMID and GPHYSD2 are within the 42nd and 43rd exons of FBN1 (Figure 2) [45,53]. It is the effect of these mutations that is thought to result in the phenotypic differences between these diseases. For example, mutations leading to GPHYSD2 have been shown to affect residues with structural roles, such as the cysteines involved in disulphide bond formation, while ACMID mutations are distributed throughout exons 42 and 43 [45]. Le Goff et al. also suggest that short stature and digits are associated with the disruption of the 5th TB domain specifically, while mutations in the other TB domains lead to other phenotypes [45]. For example mutations within the 4th TB domain are associated with SSKS, which shares phenotypic similarity with WMS2, ACMID and GPHYSD2 but lacks the short stature and digits [44].

Models of Pathogenesis

The Marfan phenotype, particularly the cardiovascular manifestations have been observed to progressively worsen with age. The reason for this remains unknown, as the mechanism behind the pathogenesis of MFS, and the other type-1 fibrillinopathies, is still not fully understood. Based on current knowledge, this progression has been attributed to both the compounding weakness of microfibrils and continuing dysregulation of transforming growth factor beta (TGF-β) [54].

Four models of MFS pathogenesis have been proposed to date. The first is known as the dominant negative model, which describes mutations resulting in an altered protein that acts antagonistically against the normal protein [55]. In the case of MFS, this model suggests that aberrant fibrillin-1 monomers bind incorrectly with normal monomers, forming semi- or non-functional multimers and/or prevent the normal assembly of microfibrils. This in turn would lead to the disorganisation of the extracellular matrix and the observed disease phenotype [55]. Therefore, based on this model, the severity of disease is dependent on the level of fibrillin-1 expression [55].

The dominant negative model began to be questioned after the identification of homozygous mutations and an autosomal recessive form of MFS. de Vries et al. [24] studied two related individuals who harboured homozygous c.1453C>T mutation (p.Arg485Cys), and presented with classic MFS. Hilhorst-Hofstee et al. [25] similarly identified a homozygous c.7454A>T mutation (p.Asp2485Val) in three related individuals diagnosed with MFS. Both groups observed that in the heterozygous state these mutations did not have a dominant negative effect, conflicting with the dominant negative model [24,25]. de Vries et al. [24] suggested that in such cases the pathogenesis is more in line with a haploinsufficiency model. That is a lack of microfibrils or fibrillin-1 resulting from protein degradation, intermolecular cross-linking or reduced fibrillin-1 synthesis [24,56].

The second model suggests that mutations in FBN1 increase the sensitivity of fibrillin-1 to proteolysis, resulting in a steady decline in microfibrils, parallel to the progression of disease severity [16]. This model is particularly relevant to mutations affecting cbEGF-like repeats, as calcium has been shown to be involved in the formation of microfibrils, and specially in their stabilisation and protection from proteolysis [11].

The third model suggests that the major roles of fibrillin-1 is to maintain tissue homeostasis and therefore MFS is a result of a loss of homeostasis [11]. This model was based on findings from two mouse models showing that MFS which caused a typical phenotype in the vesicular tissue and resulted in death, did not affect elastic fibres in other tissues [11]. Therefore, the authors came to the conclusion that the primary role of fibrillin-1 was not in the assembly of elastic fibres, rather in maintaining homeostasis of existing elastic fibres [11]. The model also suggests, a critical threshold of functional microfibrils required for tissue homeostasis, therefore mutations in FBN1 result in a decrease in microfibril abundance resulting in MFS [11]. This hypothesis was based on observations of the two mouse models showing that the ultimate outcome of both dominant negative and hypomorphic mutations is a similar decrease in the abundance of functional microfibrils [57].

The fourth model was proposed in the early 2000s, in light of more recent research that linked decreased fibrillin-1 with the dysregulation of TGF-β, a multifunctional cytokine with a role in cell signalling and survival and subsequently with the development of phenotypic features associated with MFS [58]. The study by Neptune et al. [58] identified that the dysregulation of TGF-β lead to apoptosis in the lung during development. However, when TGF-β activation was neutralised lung apoptosis was reduced and alveolar development was rescued [58]. A number of other studies have now supported these finding, providing more evidence that TGF-β dysregulation is the main cause of pathogenesis in Marfan syndrome, favouring the fourth model and directing research focus [8,59].

Mutations in FBN1 lead to an increase in active TGF-β by disrupting the interaction between latent TGF-β binding protein (LTBP) and fibrillin-1 [60]. In the absence of organised microfibril lattices, the large latent complex (LLC), made up of TGF-β, latency-associated protein and LTBP, is unable to anchor to microfibrils and as a result the components of the LLC remain uncomplexed [60]. This leaves the free TGF-β to bind to its receptor, activating a phosphorylation cascade and a number of downstream effects [60,61]. One such effect is increased expression of matrix metalloproteinases leading to the degradation of elastin and the resulting loss of extracellular matrix stability [62].

The initial disruption of LTBP-fibrillin 1 interaction, could be the result of a number of different factors and is likely dependant on the type and position of a mutation. Aoyama et al. [56] suggested that the majority of FBN1 mutations could be categorised into 5 groups depending on their effects on the synthesis and/or deposition of fibrillin 1. The group also suggested that FBN1 mutations result in reduced synthesis and/or deposition in different ways, supporting dominant negative effects, haploinsufficiency and protein degradation, all of which are likely to result in the dysregulation of TGF β [56].

Life Expectancy and Current Treatments

The mean age of death for Marfan patients was predicted in 1972 to be 32 years, with cardiovascular complications associated with aortic dilation the main cause [28]. A continuation of this study in 1995 found that the mean age of death had increased significantly to 41 years, with the average life expectancy increasing several decades [63]. The increased survival was attributed to an overall increase in life expectancy for the general population, an increase in the proportion of individuals diagnosed with milder phenotypes due to increased molecular genetic testing, and significant advances in medical intervention, specifically cardiovascular surgery [63]. There is, however, still a significant burden on the livelihood and quality of life of MFS patients and currently no cure [21].

The management of Marfan syndrome is multidisciplinary, most often involving geneticists, ophthalmologists, orthopaedists and cardiologists [26]. Current standard of care consists of lifelong use of β-adrenergic receptor blockade or β-blockers [26], which have been shown to slow progressive aortic dilation and reduce the associated complications [64,65]. This is coupled with numerous surgical interventions aimed at correcting major abnormalities in the chest, eye, spine and cardiovascular system [20,26].

The progressive nature of type-1 fibrillinopathies means that constant re-evaluation is required throughout life. For example, ocular features such as lens dislocation are most often managed with corrective lenses, however with increasing severity surgical intervention such as aphakia, removal of the lens, may be required [66]. Similarly, progressive scoliosis is initially managed with bracing, however, patients are monitored throughout development and surgical stabilisation is often required [67].

Following the implication of TGF-β dysregulation in the pathogenesis of MFS, research into potential ways to antagonise TGF-β has been the main research focus for potential therapeutics. The most notable outcome of which is trials into the use of Losartan, a drug that is currently used to treat hypertension [59]. Studies in mouse models have shown that treatment with Losartan can prevent aortic root aneurysm, as well as partially rescue lung structure [68]. However, clinical trials comparing Losartan with β-blockers have shown that while treatment with Losartan significantly reduces aortic dilation, there was no significant difference in the outcome between the two treatment groups [69,70].

Novel Therapies for Type-1 Fibrillinopathies

Due to the nature of MFS as a progressive multisystem disease, with a dominant genetic basis, conventional therapeutic techniques such as cell or gene replacement are unlikely to be applicable. The wide range of causes of MFS also reduce the applicability of such techniques, for example, while gene replacement has potential for cases of haploinsufficiency, it would not be appropriate for FBN1 mutations resulting in dominant negative effects. Similarly, techniques such as siRNA induced allele specific silencing has potential for patients with dominant negative mutations, however, MFS is also known to be caused by insufficiency of FBN1 expression, therefore reduction in FBN1 expression is likely to result in disease. For these reasons, the majority of current treatment options specifically target particular clinical features. Antisense oligonucleotides provide a novel therapeutic approach with the potential to treat all mutation types by targeting the pre-mRNA directly.

The primary gene transcript (pre-mRNA) of a gene must be processed in a number of ways, before the mature mRNA can be translated into a protein. These include 5' capping, splicing of exons and removal of intervening sequences, cleavage, polyadenylation of the 3' end and finally export to the cytoplasm [71]. The splicing of non-coding regions (introns) and the subsequent joining of coding regions (exons) is a highly complex and coordinated process that must take place for most human gene transcripts. It is estimated that 95% of multi-exon genes also undergo an additional process called alternative splicing [72]. This process allows for further diversification of gene expression in a highly regulated tissue or development specific manner.

Antisense oligonucleotides (AOs) are single stranded nucleic acid analogues that can be used to achieve a number of outcomes to modify gene expression, including exon skipping for reading frame restoration, isoform switching and gene transcript knockdown. AOs are typically 20-25 bp long and may be designed to bind specifically to a targeted motif within the pre-mRNA of a gene of interest. There are two broad classes of AOs; those that promote the degradation of targeted mRNA, such as RNase H-dependent oligonucleotides or siRNAs and those that physically block or inhibit the splicing or translational machinery, steric-blocker oligonucleotides [73].

Antisense oligonucleotides, in particular steric blockers, have therapeutic applications for a number of diseases. One notable case is a splice-switching phosphorodiamidate morpholino oligomer, now called Exondys51, that was granted accelerated approval by the Food and Drug Administration as a treatment for Duchenne muscular dystrophy [74]. Exondys51 was designed to induce skipping of dystrophin exon 51 to restore the mRNA reading frame around frame-shifting deletions that flank exon 51. Removal of dystrophin exon 51 from these amenable deletions allows translation of an internally truncated dystrophin isoform, similar to that observed in patients with the phenotypically milder Becker's muscular dystrophy [75,76].

Targeted switch splicing could also have potential therapeutic applications for the type-1 fibrillinopathies. We hypothesise that the skipping of exons harbouring disease-causing mutations from FBN1, along with the corresponding exon from the normal transcript, could re-establish periodicity of fibrillin-1 monomers. Due to their homology, monomers from all transcripts should therefore be able to correctly aggregate into multimer units forming an organised and functional microfibril backbone.

There is also potential for therapeutic strategies by designing AOs against associated targets. For example, an AO that inhibits the activation of TGF-β could have therapeutic potential because while Losartan proved no more effective than β-blockers, the antagonistic effect of Losartan on TGF-β is effective at preventing aortic root aneurysm and decreasing aortic dilation [68-70]. In particular, TGF-β antagonism works efficiently in patients with FBN1 mutations that result in haploinsufficiency [77].

Another example is isoform switching, which is a potential way of increasing the expression of fibrillin-1. Burchett et al. [78] identified two alternative isoforms, 54A-FBN1 and 57A-FBN1, that arise from the incorporation of cryptic exons from introns 54 and 57 respectively. 57A-FBN1 in particular was observed to make up a significant portion of the total number of FBN1 transcripts, approximately 10-40% depending on the tissue and developmental stage [78]. Antisense oligonucleotides can be designed to block inclusion of these cryptic exons, pushing expression toward the normal transcript thus increasing the abundance of normal fibrillin protein.

Lastly components of the protein degradation pathway could be targeted in two ways depending on the pathogenesis of a mutation. For patients who harbour dominant negative mutations, enhanced proteolysis of aberrant fibrillin-1 would theoretically lead to a higher portion of normal protein that could assemble into functional multimers, as long as there was an increase in expression. Conversely for mutations that result in increased proteolytic sensitivity, inhibiting protein degradation could lead to increased fibrillin-1 abundance and thus a decrease in disease severity. Along with targets within fibrillin-1 itself these alternative targets mean there are numerous ways in which AOs could be used as a treatment for individuals suffering from MFS and the other type-1 fibrillinopathies.

Final Remarks

Type-1 fibrillinopathies are a family of connective tissue disorders, of which Marfan syndrome is the most common, with a prevalence of 2-3 in 10,000 individuals. These diseases are caused by mutations in FBN1, which encodes fibrillin-1, a major component of the extracellular matrix microfibrils providing both structural and regulatory support. The type-1 fibrillinopathies have variable ages of onset and are progressive in nature, affecting multiple body systems with major clinical manifestations in the skeletal, ocular and cardiovascular systems.

Current standard of care relies heavily on surgical intervention and lifelong use of β-blockers to slow disease progression. Antisense oligonucleotides present a novel therapeutic strategy for the type-1 fibrillinopathies, by mediating the alteration of exon structure of both the normal and disease-causing mRNA transcripts to re-establish the periodicity of fibrillin-1. Resulting proteins, while internally truncated, would be homologous thus able to form multimer units. This treatment alone or in association with isoform switching, TGF-β antagonism or enhanced/inhibited protein degradation could facilitate the assembly of fibrillin-1 monomers into multimers increasing the abundance of microfibrils and decreasing phenotypic severity.

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