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Precise Therapy for Thoracic Aortic Aneurysm in Marfan Syndrome: A Puzzle Nearing Its Solution☆

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ABSTRACT

Marfan Syndrome (MFS) is a rare connective tissue disorder, resulting from mutations in the fibrillin-1 gene, characterized by pathologic phenotypes in multiple organs, the most detrimental of which affects the thoracic aorta. Indeed, thoracic aortic aneurysms (TAA), leading to acute dissection and rupture, are today the major cause of morbidity and mortality in adult MFS patients. Therefore, there is a compelling need for novel therapeutic strategies to delay TAA progression and counteract aortic dissection occurrence. Unfortunately, the wide phenotypic variability of MFS patients, together with the lack of a complete genotype-phenotype correlation, have represented until now a barrier hampering the conduction of translational studies aimed to predict disease prognosis and drug discovery. In this review, we will illustrate available therapeutic strategies to improve the health of MFS patients. Starting from gold standard surgical overtures and the description of the main pharmacological approaches, we will comprehensively review the state-of-the-art of *in vivo* MFS models and discuss recent clinical pharmacogenetic results. Finally, we will focus on induced pluripotent stem cells (iPSC) as a technology that, if integrated with preclinical research and pharmacogenetics, could contribute in determining the best therapeutic approach for each MFS patient on the base of individual differences. Finally, we will suggest the integration of preclinical studies, pharmacogenetics and iPSC technology as the most likely strategy to help solve the composite puzzle of precise medicine in this condition.

Contents

Abbreviations and Acronyms: ACEi, angiotensin converting enzyme inhibitors; ARBs, angiotensin II receptor type 1 blockers; βBs, beta blockers; BP, blood pressure; CCB, calcium channel blockers; CV, cardiovascular; CVD, cardiovascular disease; DN, dominant negative; ECM, extracellular matrix; ESC, embryonic stem cells; FBN1, fibrillin 1; HI, haploinsufficient; iPSC, induced pluripotent stem cell; MFS, Marfan Syndrome; MMPs, matrix metalloproteinases; NC, neural crest; PEARS, personalized external aortic root support; SMC, smooth muscle cells; TAA, thoracic aortic aneurysm; TGF-β, transforming growth factor β; TRR, total root replacement; VSRR, valve-sparing root replacement.

☆ Statement of Conflict of Interest: see page 333.

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Background

Marfan Syndrome (MFS) is a rare autosomal Mendelian disease (http://www.omim.org/; OMIM#154700), with a reported prevalence from 4 to 20/100000 individuals, depending on diagnostic criteria and ethnicity.[1, 2] This monogenic connective tissue disorder, caused by a mutation in the gene encoding for fibrillin-1 (*FBN1*), segregates as a dominant trait in families. Notably, 20–25% of cases are nevertheless reported to be due to de novo mutations.[3]

The cardinal disease manifestations in adults with MFS include alterations in the skeletal (long bones overgrowth, long slender digits, anterior chest deformity, scoliosis and flatfoot), ocular (eye lens dislocation, abnormal flat cornea and severe myopia) and cardiovascular (CV; aortic root, proximal ascending aorta and pulmonary artery aneurysms, mitral and aortic valve calcifications, dilated cardiomyopathy, arrhythmias and myxomatous thickening of the mitral valve with prolapse and regurgitation) systems.[3–6] Although the clinical manifestations at skeletal and ocular levels in MFS patients are prominent, the most detrimental clinical phenotypes affect the CV system.[4] In particular, thoracic aortic aneurysms (TAA) are observed in the vast majority of MFS patients and aneurysm dissection is the main cause of mortality in adult patients.[7]

To date the main therapy for TAA in MFS remains surgery, as an etiology-based pharmacological strategy is still lacking. Thus, the scientific community is directing its efforts in finding novel therapeutic approaches to limit aortic dilatation. Here, we describe the genetic background of MFS and outline the current and emerging strategies to provide a comprehensive overview of the plethora of opportunities that physicians and scientists hold to find the best path to counteract the development of TAA in MFS patients. Although several reviews have been previously published on this topic, we have chosen a unique approach by providing a broad overview of all the available weapons that have been gained from the most advanced discoveries in order to integrate the best and most precise therapy for each MFS patient.

MFS Genetic Background

In 1991 Dietz et al. identified a recurrent mutation in the *FBN1* gene as the genetic cause of MFS.[8] Since that time, more than 3000 *FBN1* mutations have been described (http://www.umd.be/FBN1/).[9] Interestingly, the majority of these mutations are carried by the components of single families.[10] MFS patients carry *FBN1* mutations mainly in heterozygosis, although in very few probands homozygous or compound heterozygous mutations have been found.[11–15]

FBN1 mutations basically affect the production and function of the encoded fibrillin-1, a glycoprotein of the extracellular matrix (ECM) that through polymerization participates in microfibril network formation.[16] The loss of functional fibrillin-1 results in impairment of tissue elasticity and structural support due to microfibrillar architecture degeneration and ECM destruction. The molecular mechanisms underlying MFS have been extensively studied.[17] In particular, transforming growth factor β (TGF-β) has emerged as the major culprit in the development of TAA and other clinical manifestations of MFS. This is likely due to the inability of mutated fibrillin-1 to sequester the inactive form of TGF-β resulting in its enhanced release and activation into the extracellular environment.[18–23]

The relationship between *FBN1* mutations and MFS patient phenotypes has been thoroughly investigated, although patient stratification is difficult because of the low number of MFS patients and the high number of different *FBN1* mutations. Particularly, differences in phenotypic severity have been associated with different localization and type of *FBN1* mutations. For instance, a *mutational hot spot* in *FBN1* has been identified that leads to the most detrimental MFS phenotype.[24, 25] Specifically, patients carrying *FBN1* mutations in exons 24–32 usually present a complete phenotype, earlier disease exacerbation, higher probability to develop ectopia lentis, scoliosis, aortic dilatation, mitral valve abnormalities and consequently demonstrating a poorer prognosis.[24, 25] Moreover, it was observed that missense mutations are associated with ectopia lentis, while mutations determining truncated protein synthesis lead to more severe systemic phenotype.[24, 26]

Interestingly, a classification based on the final effect that mutations have on fibrillin-1 has been devised subdividing mutations into haploinsufficient (HI) and dominant negative (DN). The HI mutations are usually due to insertions or deletions in *FBN1* that can cause premature stop-codon formation with consequent prevention of protein translation or degradation.[27, 28] Thus, patients carrying HI *FBN1* mutations in heterozygosis only express the wild-type *FBN1* allele and are theoretically characterized by a reduced amount of normal fibrillin-1 protein production, resulting in a weaker aortic wall more susceptible to high shear stress damage.[27] On the other hand, DN mutations (usually missense or exon skipping mutations) mostly determine the formation of a fibrillin-1 with abnormal functionality due to disturbed protein folding or defective activity. Patients carrying a heterozygous DN mutation should express a normal amount of fibrillin-1; however, it is likely that only half of the produced protein (that derived from the wild-type allele) is active. Theoretically, the DN condition should be characterized by a discrete phenotypic variability due to the differing type and location of the mutations that determine it.[29] Functionally, it has been postulated that the abnormal activity of DN mutated fibrillin-1 could negatively impact the subtle organization of fibrillar structure in the ECM, particularly its strength and its interaction with TGF-β.[27, 28]

Interestingly, on the basis of this classification, Franken et al. recently observed that patients carrying HI mutations, when compared with patients carrying DN mutations, have a 2.5-fold increased risk for CV disease (CVD) death, a 2.4-fold increased risk for combined CVD mortality and dissection, and a 1.6-fold increased risk for any aortic complication.[30] The association of the MFS aortic phenotype with mutations resulting in truncated FBN1 has also been observed by other groups. In one cohort of 80 MFS patients, those carrying truncating mutations were reported to be more prone to develop aortic events, including both TAA and type A dissections, than patients carrying missense mutations (57.1% vs. 13.6%).[26]

Some critical points about the HI-DN classification have recently been raised due to the overlap between phenotypes induced by different types of mutations.[28] Precisely, Dietz reported that DN mutations are frequently, but are not always associated with severe pediatric presentation and that HI mutations are associated with phenotypes ranging from mild presentations without cardiovascular involvement to the classic MFS presentation.[31] To overcome these crucial points, it has been also postulated that the phenotypic variability among MFS patients carrying the same type of mutation could be due to alteration in the expression of the normal *FBN1* allele,[32] a hypothesis that might also explain the strong intra-familial clinical variability recorded in MFS.

Furthermore, since DN mutations are more associated with the development of ectopia lentis, it should be more simple for clinicians to diagnose MFS in these patients early in life and, thus, begin prophylactic treatment for aortic disease determining a slower progression of aortic dilatation.[29]

Based on these controversial and complex scenarios, we believe that, to date, a clear and complete genotype-phenotype correlation in MFS is still missing, and further studies will be needed to unravel this intricate

question.[10] However, we strongly feel that an effective *FBN1* mutation classification, together with a more precise genotype-phenotype correlation, would be useful to develop an appropriate instrument to predict disease progression, prognosis and response to drugs for each patient.

Current Therapeutic Strategies

Surgical Approaches

Since TAA is the MFS clinical feature most heavily impacting patient prognosis, many efforts have been focused on strategies that limit aortic enlargement and dissection occurrence. In the early 1970s, prior to the recent advances in diagnostic and prophylactic surgical approaches for TAA, MFS patients' lifespan was about 2/3 of that of healthy subjects. During the last four decades, however, life expectancy of these patients has dramatically increased to now near normal levels.[3, 33, 34]

To avoid a detrimental dissection event, the 2010 ACC/AHA/AATS guidelines suggested that the TAA surgical correction for MFS patients is necessary when the ratio between the maximal cross sectional area of the ascending aorta or root (expressed in cm^2) and patient's height (expressed in meters) exceeds the value of 10.[35] Afterwards, the 2014 ESC guidelines indicated that the elective surgical therapy should be recommended when: 1) the aortic diameter exceeds 45 or 50 mm (depending on the presence of family history of aortic dissection), 2) the aneurysm is rapidly dilating (at a rate higher than 3 mm/year), 3) in case of severe valve regurgitation or 4) before a planned pregnancy.[36]

The surgical strategy basically consists of total aortic root replacement with a vascular graft including coronary reimplantation, that can be performed with a prosthetic aortic valve implant (TRR, *Bentall procedure*)[37] or a valve-sparing procedure (VSRR, *David procedure*, Fig 1).[38] Although the Bentall operation is considered the gold standard for TAA surgery, the David procedure has become increasingly popular whenever the patient presents with a morphologically and functionally normal aortic valve. The latter option is particularly advantageous as it does not require a prosthetic valve, thus avoiding life-long anticoagulant therapy, which is mandatory when a mechanical valve is utilized, or durability issues that are common when a bio-prosthesis is implanted in younger patients.[39–41] Both these approaches are valuable options for treating TAA in MFS patients; however they are not always definitive. Indeed, after a TRR the re-intervention rate is ~0.3%/year and the thromboembolic event rate is ~0.7%/year. Conversely, the VSRR has shown increased need for re-intervention (~1.3%/year) but less thromboembolic events (~0.3%/year).[39] As for long-term patient outcome, Price et al. reported a 10-year survival rate of 90.5% after TRR and 96.3% following VSRR.[42]

Interestingly, a more recent procedure based on the implantation of a personalized external aortic root support (PEARS) is emerging for the surgical management of TAA in patients at an early stage of aortic dilatation (Fig 1).[43, 44] Briefly, a macroporous fabric sleeve, modeled on the patient's aorta, is created by 'computer aided design' and '3D printing' for implantation around aneurysmal aorta, with the aim to halt aortic root expansion, thus limiting the risk of acute dissection.[44, 45] Interestingly, it has been observed that, over time, the macroporous mesh completely integrates with the aortic adventitia.[46] Despite only a hundred patients currently being followed after PEARS implantation, the functional positive effects of this innovative technique on longitudinal ascending aortic wall stress due to the axial downward reduction have already been reported, together with aortic valve competence restoration incidentally being obtained in some patients.[47–50]

Life Style and Pharmacological Approaches

Once the diagnosis of MFS has been confirmed, precautions regarding behavioral and lifestyle measures should immediately be discussed with the patient and their family. Although there are no clear reported data regarding the dangers of physical activity (PA) in patients with TAA, it is generally suggested that MFS patients avoid weightlifting as well as intensive, contact and competitive sports, due to their potential risk in aorta rupture. However, regular low-intensity and low-impact PA is permitted in most people with MFS, after taking into consideration their personal PA limitations.[51]

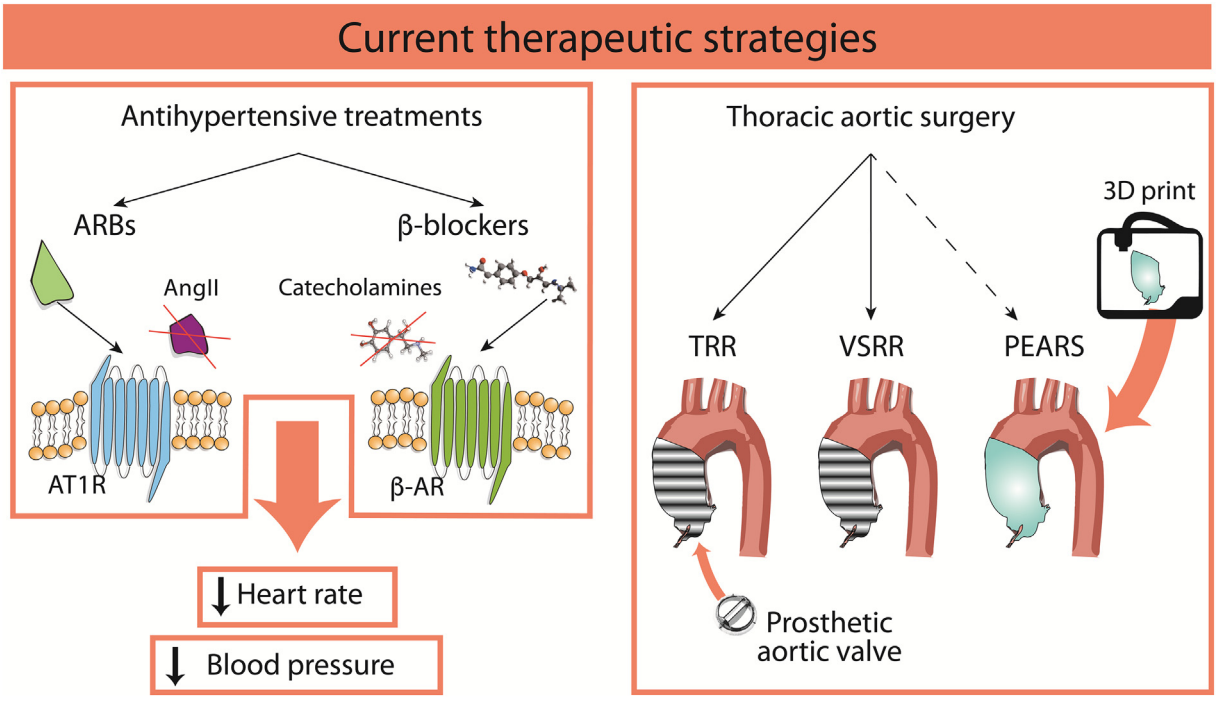

Fig 1. Current therapeutic strategies for MFS-TAA. In the left panel are presented the more commonly prescribed pharmacological treatments to reduce heart rate and blood pressure that basically consist of ARBs and/or β-blockers therapy. In the right panel are illustrated the current overtures for the surgical repair of the thoracic aorta dilated tract: the TRR, VSRR or PEARS techniques. *ARBs: angiotensin II receptor blockers, AngII: angiotensin II, AT1R: angiotensin II receptor type 1, β-AR: β-adrenergic receptor, TRR: total root replacement, VSRR: valve-sparing root replacement, PEARS: personalized external aortic root support.*

In parallel the most frequent pharmacological approach reserved to MFS patients is aimed at decreasing heart rate and lowering blood pressure (BP) average and amplitude to reduce the hemodynamic stress on the proximal aorta.[40] Specifically, the drugs commonly prescribed are the β-adrenergic receptor antagonists (βBs) that can be administered alone or in combination with angiotensin II (AngII) receptor type 1 (AT1R) blockers (ARBs, Fig 1).[52,53] The β-blockers strategy was initiated in 1971, when Halpern et al. suggested that the control of BP could be fundamental to decrease haemodynamic stress on the proximal aorta in MFS patients.[54] Since that time several studies have been conducted, but the efficacy of βBs in these patients remains controversial.[55] Interestingly, a recent meta-analysis revealed that only one low quality, open-label, randomized clinical trial has been performed to assess the efficacy of βBs (e.g. propranolol) in 70 MFS patients. It reported a decrease in aortic diameter growth rate, but not mortality reduction.[55,56] Additional small studies have reported that βBs can limit aortic root expansion,[57–59] while others have shown no difference in aortic measurements in patients receiving the drug.[60] Dean et al. ascribed the heterogeneous efficacy of βB therapy in adult patients to the timing of therapy, with greater efficacy seen when treatment was started in younger patients with smaller aortas.[61] Despite these variable results, βBs have become the most prescribed therapy for MFS patients.[62] Atenolol is often utilized because of longer half-life, greater cardiac selectivity and fewer side effects.[63] Recently, the third-generation βB nebivolol has emerged as a possible valuable alternative in MFS treatment due to its effect on heart rate in association with anti-stiffness properties. In this regard, a clinical trial to test nebivolol efficacy in MFS patients has been designed[64] and is currently recruiting (NCT00683124; http://clinicaltrials.gov).

The recently unraveled role of AngII in MFS pathophysiology has identified ARBs as a potentially promising pharmacologic strategy.[17] Indeed, the pivotal role of the AngII/AT1R axis in MFS aneurysm development was confirmed by the effectiveness of ARB therapy in reducing the rate of aortic root dilatation in MFS mice,[20,65–67] as well as in adult patients co-treated with βBs.[52,68–71] Despite this finding, MARFANSARTAN, a recently published placebo-controlled randomized clinical trial, did not demonstrate the efficacy of ARBs in MFS patients.[72] However, it is noteworthy that results from smaller studies evaluating ARB therapy in MFS children and young adults[73–76] have shown impressive outcomes, both in severely affected children who did not previously respond to other conventional therapies and in children with milder MFS phenotypes. Overall, these patients showed a marked decrease in aortic root dilatation rate.[73–75] In another cohort of 608 children and young adult MFS patients, Lacro et al. reported no significant difference between patients allocated to ARB or βB treatment groups over a 3-year follow-up, and that younger age was associated with a greater decrease in aortic root dilatation in both groups.[76]

In 2015, two additional studies were published evaluating short-term vascular and haemodynamic effects (e.g. pulse way velocity, arterial stiffness, left ventricular function) in MFS patients treated with ARBs or βBs alone. The results of both studies suggest that either of these classes of medication may be effective by improving vascular function via their distinct mechanisms.[77,78] To further clarify the ability of ARBs to interfere with MFS pathology and their mechanism of action when administered alone, more controlled clinical studies are ongoing (The Oxford Marfan Trial [NCT01949233], NCT00723801; http://clinicaltrials.gov).

In case of intolerance to βBs, angiotensin converting enzyme inhibitors (ACEi) or calcium channel blockers (CCB) may also be utilized.[79] ACEi act by blocking the abnormal activation of the renin-angiotensin system, a known cause of aortic dilatation, while CCB prevent the influx of calcium from the extracellular space into cells and have a negative ionotropic action.[62,80] The efficacy of CCB is currently under debate, both in humans and in MFS mice, in which it has been recently shown that the CV phenotype may worsen with CCB administration.[55,62,80]

In summary, since published clinical studies have involved drugs that i) do not specifically target MFS-related mechanisms, and ii) are variably effective in these patients, it appears difficult to define which pharmacological therapy is most favorable for delaying TAA dilatation and dissection occurrence in MFS. Thus, to date the gold standard therapy for significant TAA in MFS remains surgery.

Novel Therapeutic Perspectives

The search for potential MFS targets for novel classes of molecules is ongoing, with the dual purpose of better understanding TAA pathological mechanism and being able to slow aortic disease progression. To pursue these aims, the combination of different tools such as MFS transgenic mice, pharmacogenetics and induced pluripotent stem cell (iPSC) is the most complete approach to reach the personalized medicine goal (Fig 2).

Mouse Models

During the last two decades, several different transgenic mice models of MFS have been developed and are currently being used to investigate pathogenic mechanisms and validate novel treatment approaches. The most promising animal models developed to study *fibrillin 1* function are the hypomorphic model (mgR), the *Fbn1*-null (mgN), and the C1039G mouse model (Table 1).[21,81,82] In particular, the $Fbn1^{mgR}$ and the $Fbn1^{mgN}$ mice show fibrillin1 under-expression, recapitulating the HI condition and MFS manifestations, while the $Fbn1^{C1039G}$ mice harbour a *FBN1* mutation similar to that more frequently carried by MFS patients and express a normal level of FBN1 recapitulating the DN condition (Fig 2). The most attractive novel approaches are reported in $Fbn1^{mgR/mgR}$ mice and concern i) the management of TGF-β signalling for controlling its dimorphic effect on aortic aneurysm progression[83] and ii) the inhibition of matrix metalloproteinases (MMPs) obtained with doxycycline.[84] Briefly, Cook et al. reported that TGF-β neutralization could exacerbate TAA progression in MFS mice when the treatment was initiated in the perinatal period (before aneurysm formation), while it determined TAA mitigation when the treatment was administered in the postnatal life (after aneurysm formation).[83] In addition, Baxter and collaborators showed that doxycycline administration significantly prolonged MFS mice lifespan delaying aneurysm rupture, probably through the inhibition of the MMP-2 and MMP-9 production and the consequent decrease in aortic elastic fibre fragmentation.[84,85] Interestingly, both these treatments were more effective in diminishing aortic aneurysm progression when combined with losartan administration.[83,86] Another emerging approach for MFS aortic aneurysm treatment came from preclinical studies based on the use of statins in $Fbn1^{C1039G}$ mice. Specifically, a positive role of pravastatin in attenuating aortic root dilatation and preserving elastin within the aortic wall was observed.[87,88]

Future studies are needed to evaluate whether the preclinical results obtained in MFS mouse models after TGF-β neutralization, doxycycline and pravastatin administration will be clinically applicable.

Pharmacogenetics

In the context of personalized medicine, the study of the genetic differences that may affect specific drug responses appears promising in understanding how to most effectively treat MFS patients. To this purpose the classification approach based on the final effect of the genetic mutation on the protein is a novel concept aimed to overcome the high number of different disease-causing mutations already described (Fig 2).

Franken et al. showed that ARB losartan was effective in reducing arterial BP, as well as in slowing aortic root dilatation in MFS patients carrying HI *FBN1* mutations, but not in those carrying the DN mutations.[27] In these patients the mean arterial BP did not correlate with aortic root dilatation rate, suggesting that the impact of losartan could not simply be ascribed to its BP lowering action.[27] It was also speculated that

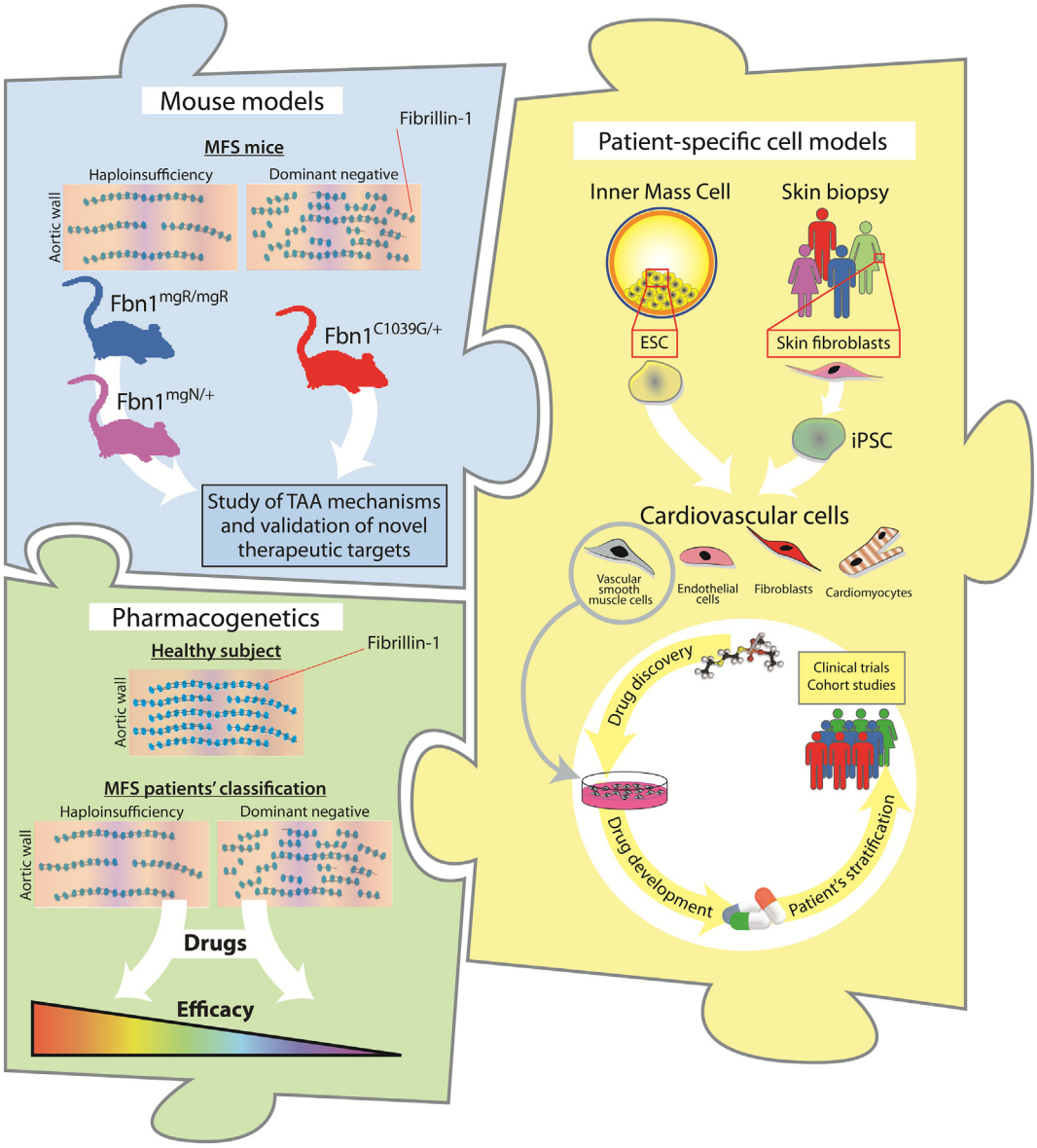

Fig 2. Available tools to solve the puzzle of precise therapy for TAA in MFS. In the upper-left panel are represented the three more commonly used mouse models for preclinical studies: the *Fbn1*$^{mgR/mgR}$ and the *Fbn1*$^{mgN/+}$ presenting a haploinsufficient fibrillar phenotype, and the *Fbn1*$^{C1039G/+}$ presenting a dominant negative phenotype. In the lower-left panel is illustrated the pharmacogenetic tool which is the study of the drug response in patients classified on the basis of their genotype. Specifically, in respect to healthy subjects that show a normal fibrillar structure, patients carrying haploinsufficient mutations have theoretically less fibrillin-1, while patients carrying dominant negative mutations are supposed to present a dysfunctional fibrillar structure. Different ECM pattern could determine various response to pharmacological treatment. In the right panel are illustrated the potentialities of the personalized medicine for MFS based on patient-specific cell models. Starting from the ESC of the inner mass cell or from the iPSC obtained from patient skin biopsy, several cardiovascular cells characterized by the patient genotype and phenotype can be obtained to test and develop drugs, and to stratify patients for clinical trials and cohort studies. *MFS: Marfan syndrome, Fbn1: fibrillin-1, TAA: thoracic aortic aneurysm, ESC: embryonic stem cells, iPSC: induced pluripotent stem cells.*

patients carrying an HI *FBN1* mutation have enhanced beneficial effects from ARB therapy in comparison to patients carrying DN mutations because of higher activation of AngII signalling. However, it is worth highlighting that patients in this study were adults that had not required aortic root surgery and did not stop previously prescribed medications before enrollment (βBs use in 70–75% of the cohort).[69] Therefore, it is possible that the study population was enriched with milder cases (also evidenced by the low aortic dilation rate during the study) and that the βB therapy, administrated at different dosages, influenced the effect of losartan.[28] To confirm these results, it will be important to evaluate the impact of ARB therapy alone on a more heterogeneous population of MFS patients considering their underlying *FBN1* mutations. Although this study is a milestone in a pharmacogenetic perspective, further investigations are needed to confirm the validity of this classification, considering the discordant opinions reported in the literature about the phenotype heterogeneity of patients carrying DN or HI mutations and also among family members presenting the same mutation.[27,28]

Patient-Specific Cell Models

A new tool in the field of personalized medicine concerns MFS patient-specific cell models, useful to advance mechanistic knowledge and assist in drug discovery. Among new patient-based cell platforms, those involving human embryonic stem cells (ESC) and iPSC appear particularly promising. The ESC have an unlimited capacity to proliferate and are able to differentiate in all the three embryonic germ layer derivatives. Notably, the iPSC hold the same proliferative and differentiating

Table 1
Common MFS mouse models.

MFS mouse model	Main cardiovascular phenotypic manifestation	References
Fbn1$^{mgR/+}$ *Fbn1*$^{mgR/mgR}$	- Normal phenotype throughout life - No phenotypic abnormalities at birth - Medial calcification and aortic aneurysm formation - Death for aortic aneurysm dissection during early adulthood	81
Fbn1$^{mgN/+}$	- Half amount of functional fibrillin 1 - Normal phenotype throughout life	82
Fbn1$^{mgN/mgN}$	- Total lack of fibrillin 1 - Neonatal death due to rupture of aortic aneurysm	
Fbn1$^{C1039G/+}$	- Half amount of dysfunctional fibrillin 1 - Development of proximal aortic aneurysms and mitral valve thickening - Normal lifespan	21
Fbn1$^{C1039G/C1039G}$	- Total amount of dysfunctional fibrillin 1 - Death during perinatal period	

capacities of ESC, without the ethical issues of embryo employment (Fig 2).[89]

Since ESC and iPSC have the same genotype and phenotype of the donor, they offer a valid tool for disease modelling. This aspect is of paramount importance for translational purposes, since many drugs reported to be efficient in animal models have been found not to be effective in humans. This gap in translation highlights the necessity to move from studies grounded on animal models to patient-specific strategies. In this regard, patient-derived iPSC in particular open up new perspectives in patient-centered care, as they could be used to uncover key patho-mechanistic insights and in preclinical trials to assess for individual toxicity and drug responsiveness.[90] For all these features iPSC are now in the limelight.

Concerning the MFS pathological scenario, patient-derived iPSC recently showed similarities to patient-derived ESC, especially in terms of skeletogenic phenotype, thus they could be used indiscriminately in disease modelling.[91,92] In particular, it was observed in both cell lines an enhanced activation of TGF-β signalling, a classical feature of MFS, causing a strong inhibition of osteogenesis and promotion of chondrogenic differentiation without TGF-β supplementation.[91]

However, in MFS the most detrimental alteration concerns the aortic wall, in which smooth muscle cells (SMC) were ascribed as the major cell type involved in the disease pathogenesis.[17] In this regard, a study by Saito et al. reveals that the MFS iPSC-derived SMC (MFS iPSC-SMC) had characteristics similar to those of SMC isolated from MFS patients' aneurysmal aortic wall. Moreover, MFS iPSC-SMC showed augmented TGF-β signalling and expressed more mature contractile proteins and transcription regulator of smooth muscle genes in comparison to iPSC-SMC derived from healthy subjects.[93]

More recently, Granata et al. developed a MFS vascular model starting from dermal fibroblasts of patients and generating MFS iPSC-SMC that were then differentiated into the three embryonic origin-specific SMC lineages.[94] The lineage that best recapitulated the MFS aortic phenotype was derived from neural crest (NC-SMC), which showed hyperactivation of both the TGF-β canonical and non-canonical pathway in respect to healthy subject NC-SMC at early developmental stages.[94] Interestingly, three different drugs (i.e. losartan, doxycycline and anti-TGF-β treatment) were tested on this model and results indicated that losartan was the most effective drug in reducing ECM degradation in MFS NC-SMC. Losartan, however, only showed a partial rescue in impaired proliferation and had no effect on cell death.[94] In light of these results, it appears hopeful that iPSC will provide an optimal platform for the process of drug development and patient stratification (i.e. responder selection) for clinical trials or cohort studies in MFS (Fig 2).[90]

Conclusions

Several novel therapeutic strategies are under investigation aimed at ameliorating MFS patient outcomes and lifespan. Since a given molecule efficacy may not be equivalent for all MFS patients due to their intrinsic genomic differences, the goal of precise medicine is laborious to achieve. In this regard, we firmly believe that the choice of a pertinent modelling strategy is fundamental to discover the effective treatment for limiting TAA development and progression in MFS. To this end it is essential to consider together i) mouse models to study TAA pathological mechanism and to assess potential efficient drugs, ii) iPSC technology to test on patient-specific cells the *in vivo* identified compounds and to individualize the best responders and stratify patients for future clinical trial, and iii) pharmacogenetics to treat MFS patients with different medications based on their inherent characteristics. Only such an integrated approach involving all the presented tools will be the most likely solution for the composite puzzle of precise medicine for TAA in MFS.

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Statement of Conflict of Interest

There is no conflict of interest of any of the listed authors.

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