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Understanding Marfan Syndrome, or “How not invent the light bulb”

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In this issue of *Trends in Cardiovascular Medicine*, Pyeritz [1] presents a concise review of the past and recent progress made in understanding the cause, pathogenesis, and clinical management of Marfan syndrome (MFS). From the time of its initial description in 1886, through key discoveries in the 1970's and 1990's, culminating in the initiation and completion of a series of mechanistically based clinical trials in recent years, our understanding of the development and treatment of MFS has truly seen remarkable progress. While many questions remain about the molecular pathogenesis, and how to best manage the cardiovascular-related manifestations, as Pyeritz points out, life expectancy has steadily improved over the last four decades, approaching normal for most patients. Future efforts must continue to focus on defining the molecular and cellular response to fibrillin-1 (FBN-1) mutations, and how these basic mechanisms can be used for clinical advantage.

Work by Dietz and Pyeritz in the early nineties identified key mutations in the fibrillin-1 (*FBN-1*) gene that were associated with Marfan syndrome and its related disorders.[2] Fibrillin-1 is a 350kDa glycoprotein comprised of a series of epidermal growth factor-like motifs, many of which contain calcium binding sequences, termed calcium-binding EGF (cbEGF) repeats [3]. These cbEGF modules are arranged in tandem, separated by cysteine-rich motifs (8-cys/TB) that have high homology to the family of latent transforming growth factor-beta (TGF- β) binding proteins (LTBPs)[3, 4]. Fibrillin monomers self-assemble into macroaggregates forming the basic structures on which mature elastin fibers are synthesized from tropoelastin subunits. Accordingly, it was postulated that *FBN-1* mutations within the aorta result in a weakened and disordered microfibril network connecting the elastic lamellae to the adjacent interstitial cells, and that this weakening predisposed patients to ascending aortic aneurysm, the primary cardiovascular manifestation of MFS and the primary cause of mortality in MFS patients.[5] Dietz and Pyeritz however, suggested that MFS syndrome may be caused by more than just a disordered microfibril matrix, suggesting that the inability of fibrillin to sequester latent TGF- β may play a prominent role in its many pathological manifestations.[2] This was followed by a series of seminal studies in which a TGF- β neutralizing antibody was used to demonstrate that antagonizing the activation of the TGF- β signaling pathway (using phosphorylated Smad-2 as the primary indicator) could

account for the lung, skeletal muscle, mitral valve, and aortic dysfunction associated with MFS.[6-8]

At this time, β -blockers were used as the primary medical therapy offered to patients with aortic root dilatation. As a class, β -blockers (e.g. atenolol) could regulate systemic blood pressure as well as dP/dt (*rate-rise time*), by attenuating the force of the cardiac pulse wave as it moved through the aorta. This was all done under the premise that regulating systemic blood pressure must be advantageous towards the prevention of aneurysm rupture and continued aortic dilatation. With this in mind, Habashi and coworkers tried another class antihypertensives; the angiotensin-II receptor blockers (ARBs), specifically losartan.[6] Losartan was interesting for many reasons. First, Daugherty and colleagues, who studied the role of angiotensin II (AngII) in abdominal aortic aneurysm development, discovered that treatment with an Ang-II type-I specific receptor (AT1R) blocker (losartan) could attenuate AngII-induced abdominal aortic aneurysm formation, while treatment with an Ang-II type-II specific receptor (AT2R) inhibitor (PD123319) enhanced aortic pathology and aneurysm development. This work suggested that activation of the AT1R pathway had deleterious effects on aneurysm development, while signaling through the AT2R pathway might prove to be beneficial; even arguing that AT1R antagonism may be better than the combined antagonism achieved with angiotensin converting enzyme (ACE) inhibitors.[9] Secondly, many studies have demonstrated that losartan can attenuate TGF- β signaling by inhibiting AngII-dependent expression of TGF- β ligands and receptors.[10-13] This was an important discovery that led Habashi et al. to hypothesize that losartan treatment may be advantageous both for its ability to lower systemic blood pressure and its ability to attenuate TGF- β signaling. Indeed, treating MFS mice (C1039G/+) with losartan, similar to TGF- β antagonism, was shown to attenuate aortic root dilatation.[6] Accordingly, these results indirectly implicated enhanced TGF- β signaling as the primary mechanism underlying *FBN-1* mutations. Yet, paradoxical findings in other disorders closely related to MFS, but characterized by mutations in the TGF- β receptors including the ligand binding and kinase domains, also demonstrated enhanced TGF- β signaling as determined by elevated Smad-2 phosphorylation.[14-17] Illuminating studies by Carta [18] and Rodriguez-Vita [19] and coworkers demonstrated that AngII could stimulate the phosphorylation of Smad-2, independent of signaling through the type-I TGF- β receptor (ALK-5), resulting in enhanced production of connective tissue growth factor (CTGF). Importantly, losartan treatment was able to attenuate both the phosphorylation of Smad-2 and the accumulation of CTGF, implicating a role for an unknown downstream mediator in the AT1R pathway. Interestingly, when mice or cells were treated with a p38 MAPK inhibitor (SB203580), AngII stimulation of Smad-2 phosphorylation was attenuated. Thus, these results suggested that AngII induced Smad-2 phosphorylation by a non-canonical pathway involving p38 MAPK that was independent of TGF- β receptor signaling. The importance of non-canonical TGF- β signaling in MFS has gained further support by a subsequent series of studies by Habashi [20] and Holm [21] demonstrating both ERK1/2 and JNK1 could be activated by AngII, and inhibition of either pathway could attenuate aortic disease in MFS (C1039G/+) mice. These results further implicated the importance of these other pathways in the pathogenesis of MFS aortopathy, and have identified key areas where future study is needed to identify the therapeutic targets and potential clinical benefits.

The preclinical results with losartan fed a crescendo of excitement leading into clinical trials, spurred on by results from a small clinical study in which pediatric MFS patients with severe aortic root enlargement, having failed β -blocker therapy alone, received losartan and experienced a delay in aortic root dilatation.[22] This early indication of success led to the initiation of multiple clinical trials world-wide, with one of the largest studies comparing losartan to high-dose atenolol sponsored by the Pediatric Heart Network here in the US. Unfortunately, as detailed by Pyeritz, results after three years have shown no significant benefit of losartan over atenolol.[23] This was followed by mixed results from several other clinical studies as well. While this left both clinicians and researchers perplexed, Pyeritz points out that these studies are hard to compare because of differences in design (inclusion/exclusion, dosing) and lack of a proper placebo control group. At minimum, these results demonstrate that losartan is no worse than atenolol, or as some critics have pointed out, serves as an equally ineffective treatment for MFS patients. In time, these results will be further dissected, and will most definitely stimulate investigation into the genotype-phenotype association, trying to determine whether specific subsets of patients respond better than others. For example, in a study by Franken and coworkers, investigators found that losartan was more effective at attenuating aortic root dilatation in MFS patients that are haploinsufficient for FBN1 protein (reduced protein content, normal protein function; similar to the C1039G/+ MFS mouse model), versus those who carried dominant negative *FBN1* mutations (normal protein content, aberrant protein function).[24] The authors hypothesized that haploinsufficient patients may have a weaker aortic wall due to reduced FBN1 content, leading to enhanced mechanotransduction as a result of aortic dilatation and enhanced activation of the angiotensin pathway. Clearly further studies are warranted to delineate this and other genotype-phenotype associations, and may suggest that additional treatment strategies are needed for MFS patients with dominant negative *FBN1* mutations.

There are several key areas of interest that are ripe for further development as alternate therapeutic strategies. First, previous studies have explored the use of doxycycline, an FDA approved tetracycline antibiotic with non-specific MMP inhibitory activity, as a potential treatment for MFS aortopathy. Doxycycline was found to delay rupture in fibrillin-1 under expressing mice (mgR/mgR mouse model), associated with its ability to inhibit MMP-2 and MMP-9.[25] Furthermore, it was found to be more effective at preserving elastic fiber integrity, normalizing aortic stiffness, and preventing vessel wall weakening when compared to atenolol.[26] Interestingly, in the same model when compared to losartan, doxycycline was able to both inhibit MMP-2, limiting latent TGF- β release, as well as prevent the phosphorylation of ERK1/2.[27] Accordingly, further studies of doxycycline alone and in combination with β -blockers or ARBs, should be considered. Second, understanding the role of elevated wall stress and mechanotransduction may also shed light on mechanisms involving the activation of angiotensin receptors.[28-30] Further elucidating pathways that regulate mechanical sensing in cells within the aortic wall may lead to the identification of other potential therapeutic targets, such as ERK1/2, which has garnered great interest as a focal point in non-canonical TGF- β and AngII signaling pathways. Finally, the role of integrin-mediated release/activation of latent TGF- β must be further explored in relation to MFS.[31] Recent reports have detailed potential roles for integrin $\alpha\beta1$ and $\alpha\beta3$ in TGF- β activation/release and the development of fibrosis. Importantly, newly developed integrin-

specific inhibitors such as C8 ($\alpha\beta 1$ specific)[32] or Cilengitide ($\alpha\beta 3/\alpha\beta 5$ specific)[33-35] have been used to attenuate TGF- β activation and its downstream signaling in the fibrotic response. In patients with dominant-negative *FBN1* mutations, which prevent LTBP sequestration, TGF-beta may be more readily activated by integrin-mediated mechanisms. Thus, reducing integrin signaling may therefore have some added benefit for these patients.

In this outstanding review by Pyeritz, he describes the truly remarkable progress that has been made in understanding the pathogenesis and clinical management of MFS patients. At the highest level, this review details the process and progress of the bedside-to-bench-to-bedside model of clinical translational research, and the importance of rational experimental design directed toward a unified goal of changing clinical practice. While many may feel, in light of the recent equivocal clinical trial results with losartan, that we have discovered yet another way “how not to invent a light bulb”, reality is, in the words of Thomas Edison, “When you have exhausted all possibilities, remember this – you haven’t.”

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