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Pathogenesis of Aortic Root Aneurysm in Marfan Syndrome

Marfan syndrome is a heritable genetic disease with pleiotrophic manifestations revolving around a systematic disorder of connective tissue caused by mutations to the *FBN1* gene. Globally, Marfan syndrome occurs in 1 in 5,000 births, and the incidence of the disease in United States ranges from 1 in 5,000 to 1 in 10,000 births[16]. Marfan syndrome is an autosomal dominant disease with a reported penetrance of 100%. About 75% of individuals with Marfan syndrome inherited the mutation from an affected parent, while 25% of Marfan syndrome individuals acquired the disease from a *de novo* mutation. Due to the broad continuum of clinical presentations associated with the disease, the severity of the Marfan syndrome symptoms cannot be predicted[16].

The disease is caused by an array of mutations to the *FBN1* gene[11]. The *FBN1* gene codes for the fibrillin-1 protein, which is an extracellular matrix (ECM) protein essential for microfibril formation. The fibrillin-1 protein and microfibrils participate in homeostasis of the elastic matrix, matrix-cell attachments, and regulating specific growth factors[5]. Scientists have linked the disease to more than 1,000 different *FBN1* mutations[19]. Some mutant forms of the fibrillin-1 protein have a dominant negative effect, which the mutant proteins forms interfere with the body's ability to utilize the wild type protein. The disease is also caused by haploinsufficiency of the fibirllin-1 protein due to nonsense mutations of the *FBN1* gene[9].

Marfan syndrome is commonly diagnosed through clinical methods based on family history and observed physical findings in multiple organ systems according to the Ghent nosology[10]. The diagnostic criteria consists of several tests including an echocardiogram, electrocardiogram, slit lamp eye exam, as well as other imaging tests such as a CT scan or MRI. These test allow doctors to evaluate the structural development and functionality of the person's heart, the location of the lenses in their eyes, as well as the presence of skeletal deformations[14]. In cases which Marfan syndrome is suspected in an individual without a family history of the disease, protein-based tests and genetic sequence of the *FBN1* gene are the preferred diagnostic methods[16].

The most common clinical manifestations of Marfan syndrome affect the ocular, musculoskeletal, and cardiovascular systems[12]. The most obvious clinical features are due to bone over growth and joint laxity which lead to increased height, disproportionately long extremities, scoliosis, anterior chest deformity and pes planus as well as pes cavus. Individuals with Marfan syndrome have a long, narrow face with characteristic features including enopthalmos, malar hypoplasia, micrognathia, and palpebral fissures that are directed downward[16]. Individuals may have a highly arched and narrow palate that causes dental crowding[11]. Marfan syndrome individuals have an increased risk for retinal detachment, glaucoma, and early cataract formation. Myopia is the most common ocular symptom, and 60% of affected individuals experience ectopia lentis. The major debilitating and potentially fatal conditions of Marfan syndrome concern the cardiovascular system. Affected individuals exhibit aortic dilations, aortic

regurgitation, mitral valve prolapse often constitutive of mitral regurgitation, tricuspid valve prolapse, and enlargement of the proximal pulmonary artery [11,12,16].

The primary cause of premature mortality among Marfan syndrome individuals is due an aortic root aneurysm and subsequence aortic dissection[12]. Surgical intervention via a Bentall operation to replace the ascending aorta and aortic valve with a Dacron graft and a prosthetic valve is the sole medical therapy for the condition[2]. Due to the limited treatment options for the cardiovascular symptoms, doctors and scientists have collaborated to research the pathogenesis of aortic root aneurisms with the intention of developing alternative, less invasive therapies.

Recent research on the underlying *FBN1* genetic mutations have elucidated several potential biological explanations for the degeneration of aortic root walls that predispose the development of aneurysms and potential dissections. Mutations that alter protein conformation and reduce the calcium binding affinity along the calcium binding sequence region of the fibrillin-1 protein increase subjection to protease degradation. The biochemical characteristics of the mutated fibrillin-1 protein result in a reduced steric hindrance against the ECM proteases[17]. These observations pose the notion that increased susceptibility to protease either reduces the amount of the fibrillin-1 present for microfibril organization or reduces the amount of microfibrils available if the protease attacks the mutated protein that has incorporated in the microfibrils[18]. The mutated fibrillin-1 proteins are also known to have impaired incorporation into the ECM, which inhibit the ECM assembly mechanisms and may weaken the aortic wall[1].

Fibrillin-1 proteins within microfibrils are integral to regulating the function of transforming growth factor-β (TGF-β), which is a cytokine responsible for proliferation, differentiation, apoptosis, and the formation of the ECM[12]. Fibrillin microfibrils are targets for the large latent complex (LLC), which contains mature TGF-β, in order to transport the cytokine to the ECM. However, mutated fibrillin-1 proteins are not recognized by the LLC causing an increase in TGF-β available for activation and signaling different pathways[4]. Experiments have identified a correlation of deficient fibrillin-1 protein with enhanced TGF-β signaling[15].

Sequestering TGF-β to the ECM is critical to regulating the cytokine activity in order to prevent superfluous pathway signaling that induce Marfan syndrome symptoms. When TGF-β cytokines fail to be delivered to the ECM due to lack of recognition of mutated fibrillin-1 proteins, active TGF-β accumulates. Studies link excess TGF-β signaling with an increase in the expression of matrix metalloproteinases (MMPs) and ECM degradation that may elicit aortic root aneurysm development [8]. The MMPs are capable of degrading all components of the aortic ECM[3]. Aortic dilation has also been associated with the apoptosis of smooth muscle cells (SMC), and studies have colocalized the SMC apoptosis with high levels of active TGF-β[6]. Increased levels of the cytokine have been observed to promote the differentiation of fibroblasts to myofibroblasts through TGF-β mediated pathways. The increased levels of TGF-β activity instigate the remodeling of the aortic root cellular components such as the destruction of the ECM and the alteration of cells composing the aortic walls.

Recent research at Stanford University's Department of Cardiothoracic Surgery has discovered that an increased expression of microRNA-29b (miR-29b) mediated the decomposition of the ECM and the apoptosis of the smooth muscle cells that contribute to initiation of aortic root aneurysms within their murine Marfan model. miR-29b regulates apoptosis and ECM synthesis, and initial studies have shown elevated levels of miR-29b in the Marfan model, which evidence suggest that the miRNA plays an active role in cardiovascular manifestations of Marfan syndrome. The two week and four week time point data has expressed high levels of miR-29b with increased levels of proapoptotic factors including caspase-3 and caspase-9 activity as well as reduction of the Bcl-2 and Mcl-1 antiapoptotic protein levels. The two and four week time points also show increased fragmentation of elastin suggesting heightened proteolytic activity that might be expressed by the abundance of miR-29b. The increased levels of the miR-29b were correlated with pathological causes of aortic aneurysms[12].

The other component of the miR-29b research conducted by the Stanford researchers revolved around identifying the biological cause of the excess miR-29b expression in the Marfan model. miR-29b is regulated by c-Myc and NF-κB transcription factors[13]. The data revealed that during the two and four week time points when there is a heightened level of miR-29b, there was also significantly reduced levels of c-Myc and NF-κB factors necessary for NF-κB activity as well as a decreased nuclear staining of the NF-κB p64 subunit. TGF-β is known to inhibit NF-κB activation and several tests determined that increased levels of TGF-β reduced NF-κB expression

as well as increased miR-29b expression, suggesting that excessive levels of TGF-β

instigates aortic aneurysm development though miR-29b expression. To confirm that

miR-29b participates in aortic root aneurysm in Marfan syndrome, the researchers

investigated the effect of the locked nucleic acid-antimiR-29 inhibitor, which contains an

antisense oligonucleotide that silences miR-29b. The results from this test revealed a

significant reduction of the miR-29b levels as well as completely blocked aortic root

dilations at four weeks in the treated groups. The treated groups exhibited no

difference in aortic root dilation compared to the wild type controls. The treated

Marfan group also exhibited reduced aortic wall apoptosis by a reduction in caspase-3

and caspase-9 activity and an increase in Mcl-1 and Bcl-2 activity. The treated mice

also had increased elastin protein levels. The presence of excess miR-29b expression is

critical to initiating early aortic root aneurysm development[12].

Currently, the only potential treatment undergoing clinical studies to attenuate and

prevent aortic root aneurysms is the angiotension II receptor 1 blocker Losartan[7]. The

drug is currently used to treat hypertension and prevent strokes in both children and

adults. Marfan studies of Losartan have shown to antagonize excess TGF-β signaling

and protect against aortic root aneurysm development[7]. This drug seems promising,

however there is still a need for more alternative drug therapies to treat the potentially

fatal cardiovascular manifestations of Marfan syndrome. Research on miR-29b is still in

its primary stages, but a potential therapy that targets the microRNA may be an

auspicious treatment for Marfan syndrome which proper management and treatment of

cardiovascular manifestations can prolong the life of individuals with the disease to have

a life expectancy of that of the general population.

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