Exploring novel treatments to prevent aortic aneurysm growth in Marfan syndrome

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Chapter 8

Summary and General discussion

Nederlandse samenvatting

Curriculum Vitae

Portfolio

Dankwoord/Acknowledgements
**Summary**

Excessive widening of the aorta is called ‘aneurysm formation’ and is a life-threatening characteristic of the genetic connective tissue disease Marfan Syndrome (MFS). In this thesis, different drugs were explored for their capacity to inhibit aortic aneurysm growth and thus prevent rupture of the vessel wall. We tested anti-inflammatory medication in mouse models for abdominal aortic aneurysm formation (Chapter 2) and MFS (Chapter 3) to study their effect on aortic dilatation. Next, the anti-aging compound resveratrol, which is a component of red wine, was administered to MFS mice (Chapter 4), and as a third treatment option, complement activation was inhibited in MFS mice (Chapter 5). In addition to these studies, I observed two phenomena in MFS mice that were further characterized and described; microcalcification in the aorta (Chapter 6) and cystic kidney disease in a MFS mouse with excessive aneurysm formation (Chapter 7).

The immunosuppressive drug azathioprine is effective to prevent organ rejection after transplantation and is also applied to reduce inflammatory bowel disease. Given that aneurysm formation in the abdominal aorta of man and in the entire aorta of the angiotensin-II (AngII) aneurysm mouse model involves severe inflammation, we tested whether azathioprine could prevent initiation of the disease. Indeed, initiation of aneurysm formation is inhibited by azathioprine. In addition, this drug affects aneurysm progression in ongoing disease by reducing the aneurysm severity score. However, the diameter of the existing aneurysms is not decreased and based on our data in this mouse model we conclude that azathioprine should probably not be considered as treatment strategy in human patients (Chapter 2).

Inflammation occurs in the aneurysmal vessel wall and the influx and activation of inflammatory cells can be inhibited in different ways, which motivated us to also test other anti-inflammatory drugs such as; methylprednisolone, an activator of the glucocorticoid receptor with strong anti-inflammatory capacity, and abatacept, which inhibits T-cell activation and is used to treat the chronic inflammatory disease rheumatoid arthritis. We observed reduced influx of macrophages in the aortic wall in MFS mice, however, these drugs do not delimit aortic dilatation. Methylprednisolone even increases aortic wall damage, which is not desired in aortic disease in MFS patients. In these studies we applied the AngII-antagonist losartan as a positive control, because this drug consistently reduces aortic widening in MFS mice and is also effective in MFS patients in certain clinical trials (Chapter 3).

Complement activation is another aspect of the immune response and activation of this system can be observed in MFS patient aortic specimen. We rationalized that inhibition of the complement system by the C1-esterase inhibitor ‘cetor’ may reduce aneurysm growth. We show effectiveness of the drug in reducing aortic macrophage influx and lowering circulating cytokine TNF-α levels, however, cetor does not decrease aortic root growth in MFS mice (Chapter 5).

The major conclusion from the studies in Chapters 2, 3 and 5 is that the mere inhibition of inflammation does not prevent aneurysm formation (in MFS).

As cellular senescence, a sign of ageing or distress, correlates with aortic root dilatation in MFS, we hypothesized that the anti-ageing polyphenol resveratrol might be effective to inhibit senescence and aortic root growth. Treatment of MFS mice with resveratrol inhibits aortic root growth by another mechanism than losartan as resveratrol does not
inhibit inflammation and TGF-β signaling in the vessel wall. Aortic root dilatation is not be influenced by specific modulation of sirtuin-1, which is known to be activated by resveratrol and influences senescence. We demonstrated that resveratrol acts by protecting the elastic laminae and smooth muscle cells in the aortic vessel wall through downregulation of the aneurysm-related micro-RNA-29b in an endothelial cell-dependent manner (Chapter 4).

Calcification is an important component in cardiovascular disease and is also observed in the aorta of MFS patients and mice. In MFS mice, microcalcification is especially increased in the ascending aorta and correlates to aortic root dilatation, distensibility and elastin fragmentation. To understand the underlying mechanism, we established that elastin-derived peptides induce microcalcification in human and murine smooth muscle cells via the signaling cascade of the kinase ERK1/2. Based on our findings I propose that visualization of aortic microcalcification by imaging with specific probes may be considered as a marker for local decrease of aortic integrity in MFS patients, thereby being an indicator for rupture risk (Chapter 6).

Cystic kidneys are relatively common and have an even higher prevalence in MFS patients. In a case of renal cystic disease in a MFS mouse, aortic aneurysm growth is tremendously accelerated. In addition, this mouse shows features of aortitis, a saccular aneurysm, and increased circulating inflammatory markers and blood pressure regulators. Monitoring of cystic kidney disease in MFS patients may provide more insight in the clinical relevance of this finding, to reveal if cystic kidneys could serve as a marker for aneurysm severity (Chapter 7).

Altogether, new insights in the pathology of aneurysm development are generated, especially in MFS. Even though we had a good rationale to perform the presented experiments, not all treatment strategies were equally effective. It has become clear that different types of anti-inflammatory drugs are not the solution in MFS. Yet, the compound resveratrol has proven to be a successful treatment option in MFS mice, acting via a different mechanism than the currently prescribed drug losartan. Lastly, I described microcalcification and renal cystic disease as potential markers for aneurysm severity. Extensive conclusions and future lines of research will be discussed below (Chapter 8).
General discussion
The aim of this thesis is to explore novel non-invasive treatment strategies to prevent aortic aneurysm growth, in particular in Marfan syndrome (MFS). Currently, MFS patients are primarily treated with β-blockers and angiotensin-II (AngII) receptor blockers (ARBs) to lower systemic blood pressure, but this provides only limited protection against aneurysm growth and rupture. So far, no clear pharmacological aneurysm treatment strategy has surfaced from multiple clinical studies, in part because most studies were performed in relatively small cohorts. Combining the clinical data from all the ARB trials in MFS is necessary and will result in novel insight in MFS and categorization of patient groups. We rationalized that the exact underlying mechanism of aneurysm formation is still unknown and that such basic knowledge is essential to design novel therapeutic strategies. Therefore, we performed extensive studies in mouse models and in cultured vascular cells to delineate key pathways in aneurysm formation and growth.

The role of inflammation in aneurysm formation and growth
The known risk factors associated with aneurysm development are increased blood pressure, atherosclerosis and aortic inflammation. In animal models for abdominal aortic aneurysm (AAA) formation, especially these risk factors were targeted with therapeutics. Angiotensin-II (AngII), a key player in the renin angiotensin aldosterone system (RAAS), induces these three processes and causes thoracic and abdominal aortic aneurysm formation in mice. MFS mice develop spontaneous thoracic aortic aneurysms (TAA) due to a mutation in the fibrillin-1 (Fbn1) gene. Aneurysms in MFS show infiltration of inflammatory cells, but to a lesser extent than in AAA. To assess the relative contribution of inflammatory cells in aneurysm growth, we applied a number of anti-inflammatory drugs in the AngII-induced aneurysm model and in the Fbn1C1039G/+ MFS mice. Next to a prevention study in the AngII-induced model, we also aimed to mimic the human situation. Therefore, we started treatment of the mice once aneurysms were already formed; ApoE-deficient mice were infused with AngII for 10 days before initiation of drug-treatment. In the MFS model, MFS mice were aged 8 weeks before treatment started, to assure that significantly enhanced aorta dilatation was already present. In the AngII-infusion model we demonstrated that the immunosuppressive drug azathioprine inhibits aortic inflammation and endothelial cell activation, resulting in effective prevention of AAA (Chapter 2). After late onset of treatment, there is reduced aortic pathology throughout the whole aorta trajectory, while the aortic diameter of the existing AAA is not reduced by azathioprine (Chapter 2). Based on this observation, we conclude that inhibition of inflammation is not key to stabilize AAA growth. Possibly, the cytostatic effect of azathioprine on vascular smooth muscle cells (SMCs) plays a detrimental role, by interfering with both purine biosynthesis and nucleotide incorporation in proliferating cells, by inhibition of the small GTPase Rac and by activation of the cytostatic transcription factor Nur77 in SMCs, limiting aortic repair.

Next, we used other types of anti-inflammatory drugs, namely the corticosteroid methylprednisolone and the T-cell inhibitor abatacept in MFS mice (Chapter 3). These drugs also reduce aortic inflammation, however, aneurysm progression remains unaltered. Moreover, methylprednisolone enhances aorta pathology with increased glucosaminoglycan
deposition at sites of SMC loss. These data may explain the findings that transplant patients, who often use azathioprine and/or corticosteroids, show enhanced aneurysm growth14,15.

Complement activation is yet another aspect of inflammation and is observed both in the aneurysmal aorta in AAA16–18 and in MFS aorta (Chapter 5), which may contribute to inflammation and targeted SMC death. We applied the complement inhibitor Cetor against C1 activation in MFS mice to study the contribution of the complement system in aortic dilatation (Chapter 5). Cetor has been shown to effectively inhibit inflammation and SMC proliferation in a mouse model of vascular restenosis19. In line with other anti-inflammatory approaches taken, we showed that Cetor inhibits aortic complement deposition and inflammation, but does not reduce aneurysm growth (Chapter 5).

Consistent with our observations, inhibition of the pro-inflammatory chemokine receptor CCR2 in mice, by overexpression of a dominant-negative mutant of its ligand CCL2/MCP1, enhances aneurysm growth20. Finally, inhibition of the pro-inflammatory p55 TNF-α receptor results in reduced arterial disease of atherosclerosis, but shows a trend towards enhanced aortic dissections in mice21. Taking all these data together, I conclude that complete inhibition of inflammatory processes is not sufficient to protect against aneurysm progression. Rather, a controlled, mild pro-inflammatory status of the vessel wall seems to be necessary to promote injury healing in the aorta to restrain aortic dilatation or aortic dissection.

(Dis)similarities in AAA versus TAA
As mentioned above, inflammation of the vessel wall is more prominent in AAA than in the dilated MFS aorta, however, blocking inflammation did not reduce the aortic diameter in existing AAA in the AngII-induced model, nor TAA progression in the MFS mice. Thus the strategy to bluntly inhibit inflammation in the aortic wall does not seem to be essential to combat AAA or TAA.

Activation of the AngII receptor type-1 (AT1R) by AngII infusion is the most widely used aneurysm model for AAA in mice (also generating TAA)7. Specific inhibition of AT1R-mediated signaling by the ARB losartan has been shown to decrease aortic inflammation, TGF-β signaling and micro-RNA (miR)-29b expression in mice. In addition, losartan reduces aortic dilatation (TAA) in MFS mice and patients22,23. Since we can now rule out that losartan has its beneficial effect by blocking aortic inflammation in MFS, it leaves a role for this drug in TGF-β signaling or miR-29b down-stream effects. Interestingly, the role of TGF-β in AAA and TAA is contradictory, as TGF-β neutralization results in accelerated progression of AAA and dissections in the AngII-induced mouse model24, while the same neutralizing TGF-β antibody approach protects against aneurysm growth in MFS mice22.

Although miR-29b is found to be downregulated in AAA and upregulated in TAA in both mice and patients25, miR-29b antagonism decreased aneurysm growth in both the AAA and TAA mouse models26,27. Therefore, miR-29b is considered a detrimental miR in aneurysmal disease25–27. A beneficial effect of resveratrol on aneurysm formation was reported in a rat and a mouse AAA study28,29 and in a mouse MFS study (as miR-29b modulator; Chapter 4), thus it seems to address a process universal in both AAA and TAA progression. The above-described data strengthen the concept that a common pathway to tackle aneurysm growth is feasible for AAA and TAA, and involves miR-29b inhibition to reduce SMC apoptosis.
Endothelial cell - SMC communication in aneurysms

Crosstalk between SMCs and endothelial cells is instrumental to maintain vascular homeostasis and protect against the detrimental process of aneurysm formation\(^{10-32}\). The importance of endothelial cell dysfunction in TAA is demonstrated by reduced ascending aorta aneurysms after AngII-infusion in endothelial cell-specific AT1R-deficient mice\(^{33}\). Moreover, in an intracranial aneurysm model, deficiency of an endothelial-specific transcription factor, enhanced intracranial aneurysms formation by promoting endothelial dysfunction\(^{34}\). Endothelial cell dysfunction is also observed in MFS mice and patients\(^{35-38}\). We showed that the resveratrol-mediated reduction in miR-29b expression in the MFS aorta is caused by an effect of resveratrol on endothelial cells; these cells produce a so far unknown factor that inhibits miR-29b expression in SMCs (Chapter 4). Since endothelial cells are the cell type easiest accessible in the aorta, influencing this cell type with drugs that alleviate endothelial cell dysfunction has potential to achieve a beneficial effect in the underlying SMCs. This is shown by the anti-apoptotic/pro-survival effect of resveratrol on miR-29b reduction in MFS mice, which prevents SMC death (Chapter 4). I propose that enhancing SMC survival/proliferation may be a more successful approach in novel pharmacological studies to stabilize the aneurysmal vessel wall. This concept should be further explored, because promoting SMC proliferation may cause other problems such as in-stent restenosis in patients with atherosclerosis.

Endothelial cells obtain cues from the bloodstream by mechanosensing machinery or by circulating molecules, such as miRs, transferring the message to the SMC\(^{30,39,40}\). Defects in the mechanosensing, for example by mutations in the polycystin (PKD) genes, necessary to develop flow sensing cilia (hairs) on the cell surface, lead to intracranial aneurysms in humans and aortic aneurysms in mice, in addition to cystic kidney disease in humans and mice\(^{41-45}\). Interestingly, the miR-143/145 cluster is secreted by endothelial cells and essential for proper SMC function\(^{10}\), and deficiency of this cluster leads to hydronephrosis in mice\(^{44}\). Since we observed cystic kidney disease in two MFS mice (Chapter 7), and cystic kidneys are described to be more apparent in aneurysm patients\(^{45,46}\), these data may indicate that abnormal communication between endothelial cells and SMCs is a potential mechanism causing aneurysmal disease, which requires further investigation.

MiR-containing vesicles in blood can travel over long distances and are relatively stable, therefore, targeting endothelial cells and/or SMCs via a miR-based approach as therapeutic strategy to influence aortic dilatation would be feasible. Actually, the company MiRagen (http://miragentherapeutics.com) is generating such type of tools. Interestingly, it has generated a synthetic miR mimic (pro-miR) to miR-29b, which is being tested for adverse effects in healthy volunteers in a phase 1 trial (ClinicalTrials.gov Identifier: NCT02603224). Since miR-29b expression is involved in different fibrotic diseases, the use of this pro-miR should be carefully followed in future patients in relation to aneurysm development.

Resveratrol and miR-21a/miR-29b balance in MFS

The anti-ageing polyphenol resveratrol is known to inhibit inflammation and oxidative stress in AAA\(^{28,29}\). In a non-human primate study, involving a high fat/sucrose diet, resveratrol treatment also prevented arterial wall inflammation and in addition revealed an increase in the pulse wave velocity\(^{47}\), thus a decrease in arterial wall stiffening. Interestingly, we have observed enhanced aortic stiffness in the dilated ascending aorta of 8-month old MFS mice
by measuring a decrease in aortic distensibility (Chapter 6). We show a correlation between the amount of elastin degradation and distensibility, and between aortic calcification and distensibility. However, I did not measure the effect of resveratrol on aortic stiffness in the MFS mice, which would still be interesting, to value the relevance of aortic stiffness in aneurysm formation.

Yet, we did observe that resveratrol inhibits aortic aneurysm progression effectively in MFS mice, unexpectedly, without a reduction in aortic inflammation (Chapter 4). Resveratrol changes metabolic pathways in the cell by activation of SIRT1 and AMP-kinase (AMPK)48–50. I demonstrated that the SIRT1-dependent pathway does not contribute to reduced aneurysm growth (Chapter 4). Therefore, activation of the AMPK-pathway may be relevant. Mirroring SIRT1, AMPK serves as a metabolic sensor, when ATP levels decrease, more ADP/AMP is present, which will cause AMPK phosphorylation and thus activation of the AMPK pathway to generate ATP51. AMPK activation in MFS mice could be addressed, for example by the widely used anti-diabetic drug metformin, to study if this pathway is responsible for the decrease in miR-29b expression in SMCs and/or reduced aortic dilatation.

Resveratrol-treated aortae show a gene transcription signature of enhanced pro-inflammatory nuclear factor κB (NF-κB) activity (Chapter 4). The expression of miR-29b is detrimental in aneurysm development and is downregulated by NF-κB57. We propose that NF-κB-mediated regulation of miR-29b is (part of) the mechanism by which resveratrol exerts its beneficial effect on the murine MFS aorta (Chapter 4). AAA progression is inhibited by miR-21a in both the murine elastase-induced and the AngII-infusion AAA model52. In our experiments, we observed enhanced miR-21a expression in the aorta of MFS mice after resveratrol treatment, which may also contribute to its beneficial effect. MiR-21a has been found to promote SMC proliferation and inhibit apoptosis55. Degradation of the phosphatase PTEN is promoted by miR-21a, thereby enhancing activation of the pro-survival Akt signaling cascade. The Akt pathway regulates SMC proliferation and migration, thus contributing to SMC survival54. It is known that NF-κB is activated via the Akt pathway55 and, as mentioned earlier, this may subsequently result in reduced miR-29b expression56. Collectively, I propose that increasing miR-21a may be considered a pharmacological treatment strategy in MFS mice and at a later stage in MFS patients, to delimit aneurysm formation by promoting aortic repair via Akt-dependent stimulation of SMC proliferation and inhibition of SMC apoptosis (in a miR-29b-dependent fashion) (Figure 1).

In conclusion, resveratrol is readily available and no contra-indications have been observed in different patient studies so far57,58. This provides the opportunity for a resveratrol clinical trial in MFS patients, to study the effect of resveratrol on top of the existing medicinal regime on aortic growth rate and complications.

**Importance of ERK1/2 activation in aortic dilatation in MFS**

Excessive TGF-β-mediated signaling, as present in MFS mice and patients, activates extracellular signal-regulated kinase (ERK1/2) via the non-classical TGF-β cascade. Inhibition of ERK1/2 in MFS mice reduces aortic dilatation59–61. ERK1/2 activation is thus detrimental in the pathology of MFS. ERK1/2 is induced by multiple stimuli, such as AngII and reactive oxygen species (ROS) and not just by TGF-β62,63. In cultured SMCs, I observed that the elastin peptide-driven calcification process is dependent on activation of ERK1/2
(Chapter 6), which may contribute to locally enhanced ERK1/2 activity at sites of elastin degradation, calcification and aortic dilatation in MFS mice.

While the blood pressure lowering ARB losartan has been shown to reduce ERK1/2 in MFS mice, and thereby inhibits aneurysm formation\(^6\) (Chapter 3), the blood pressure lowering calcium channel blockers enhance aneurysm development in MFS mice by increasing ERK1/2-mediated signaling\(^6\). These data nicely illustrate that blood pressure lowering per se is not instrumental, but inhibition of specific underlying signaling cascades is. Indeed, in a large cohort of MFS patients (n=531), the patients receiving calcium channel blockers were at 12-fold increased risk of development of an aortic dissection and had a 5-fold higher chance of meeting aortic surgery criteria (enhanced growth)\(^6\). A similar trend was already observed in a Dutch MFS patient cohort (n=600), where ARBs protected against aortic type B dissection, while calcium channel blockers and ACE-inhibitors showed a trend of increased risk\(^6\). More intense worldwide collaborations are essential to validate these data to be able to potentially change treatment guidelines for MFS patients concerning beneficial blood pressure lowering medication.

In MFS mice, statins have been proven to reduce aortic dilatation\(^6\). In heart failure, the lipid lowering statins improve endothelial function by upregulating the pro-survival Akt pathway and downregulating the detrimental ERK1/2 pathway\(^4\), emphasizing their opposing role. To follow up on that concept, it is of interest that the AngII-mediated AT1R signaling pathway has an opposing axis via the AngII receptor type-2 (AT2R) and via the Ang(1-7) receptor Mas\(^6\,7\). Ang(1-7) is formed from angiotensin I or AngII by angiotensin converting enzyme 2, also known as ACE2. ACE2 is a homologue of ACE, that is responsible for generation of AngII\(^6\). Ang(1-7) can improve fibrosis (in the lungs, heart and kidneys), reduce vascular inflammation, inhibit endothelial dysfunction and modulate metabolism, by positively regulating the Akt and negatively regulating the ERK1/2 pathways\(^4\). ACE2 activation, in AngII-infused hyperlipidemic mice, decreased the formation and severity of AAAs\(^7\). In addition, in the intracranial aneurysm model, mortality by intracranial aneurysm rupture is decreased by Ang(1-7)\(^7\). Both studies show no effect of Ang(1-7) on blood pressure, which would make ACE2/Ang(1-7)/Mas activation a treatment option for MFS patients as they often experience side-effects of blood pressure lowering agents, since they do not have elevated blood pressure. It would be interesting to activate the ACE2/Ang(1-7)/Mas axis via treatment strategies in MFS patients.

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**Figure 1.** Schematic illustration of the potential protective mechanism of miR-21a, by regulation of Akt signaling and the unfavorable miR-29b, in aneurysm formation.
7)/Mas axis in MFS mice to study if aortic dilatation can also be inhibited, similar to what is observed with ARB losartan, and to assess whether reduced ERK1/2 and increased Akt is essential in this effect. Signaling pathways involved in ERK1/2 signaling in the arterial vessel wall, are summarized in Figure 2.

In conclusion, the data as presented in this thesis make a strong case against inhibition of inflammation to delimit aneurysm growth. My thesis provides important clues to initiate a number of novel research lines to further explore potential beneficial mechanisms focusing on tipping the balance of counteracting forces of miR-21a/miR-29b and Akt/ERK pathway activities to preserve endothelial and SMC health.

**Figure 2.** Summary of regulators of ERK1/2 signaling in the arterial vessel wall.
References


Nederlandse samenvatting

Overmatige verwijding van de aorta wordt ‘aneurysma vorming’ genoemd en is een levensbedreigend aspect van de genetische bindweefselziekte Marfan Syndroom (MFS). In deze thesis werden verschillende medicijnen onderzocht op de mogelijkheid om de groei van een aneurysma in de aorta te remmen en daardoor het scheuren van het vat te voorkomen. We hebben anti-inflammatoire middelen toegepast in muismodellen voor abdominale aorta aneurysma vorming (Hoofdstuk 2) en MFS (Hoofdstuk 3) om het effect op aorta dilatatie te onderzoeken. Daarnaast werd het anti-verouderingsmiddel resveratrol, wat zich in rode wijn bevindt, toegepast aan MFS muizen (Hoofdstuk 4), en als derde behandelingsoptie werd complement activatie geremd in MFS muizen (Hoofdstuk 5). Tevens heb ik twee fenomenen gekarakteriseerd en beschreven in MFS muizen; namelijk microcalcificatie in de aorta (Hoofdstuk 6) en cystenieren in relatie tot aneurysma vorming (Hoofdstuk 7).

Het immunsuppressivum azathioprine wordt gebruikt om organaafstoting te voorkomen na transplantatie en wordt ook gebruikt om inflammatoire darmziekten te behandelen. Aangezien ontsteking van de vaatwand sterk aanwezig is in aneurysmata in de abdominale aorta van de mens en het de initiator is van aneurysma vorming in het angiotensine-II aneurysma muismodel, hebben we getoetst of azathioprine het ontstaan van aneurysmata kan voorkomen in de muis. Zoals verwacht werd aneurysma vorming geremd door azathioprine. Wanneer azathioprine werd toegediend als er al aneurysma vorming was opgetreden, remde het wel de algehele aorta schade, maar het had geen invloed op de diameter van het reeds gevormde abdominale aneurysma. Uit onze data in dit muismodel concluderen we dat azathioprine waarschijnlijk geen effectieve behandelmethode zal zijn voor patiënten met een buik aneurysma (Hoofdstuk 2).

Ontsteking komt ook voor in de aorta van MFS patiënten. Influx en activering van inflammatoire cellen kan worden geremd op verschillende manieren, wat ons motiveerde om ook andere anti-inflammatoire middelen toe te dienen in het MFS muismodel; namelijk methylprednisolon, een activator van de glucocorticoïd receptor, dat bijvoorbeeld wordt voorgeschreven om afstoting van getransplanteerde organente voorkomen en abatacept, een T-cel activatie remmer, dat gebruikt wordt om de chronische ontstekingsziekte reumaatide artritis te behandelen. We hebben verminderde influx van macrofagen geobserveerd in de aortawand van MFS muizen, echter, deze middelen remmen de aortaverwijding niet. In deze studies hebben we angiotensine-II remmer losartan als positieve controle gebruikt, omdat het de aortaverwijding in MFS muizen consistent verminderd en ook effectief is gebleken in MFS patiënten in bepaalde klinische studies (Hoofdstuk 3).

Complement activatie is een ander aspect van het immuunsysteem, waarvan ik activatie heb aangetoond in aortaweefsel van MFS patiënten. De hypothese was dat de aneurysma groei verminderd zou kunnen worden door remming van complement activatie met de C1-esterase remmer ‘cetor’. We hebben laten zien dat cetor de macrofaag influx in de aorta en circulerend TNF-α verminderde, echter, cetor remt de aortaverwijding in MFS muizen niet (Hoofdstuk 5).

De conclusie die we kunnen trekken uit de studies in Hoofdstukken 2, 3 en 5, is dat louter remming van ontsteking de aneurysma groei niet afremt.

Aangezien cellulaire senescence, een teken van veroudering en stress, correleert met aortaverwijding in MFS, was het aannemelijk dat het anti-verouderingsmiddel resveratrol
Effectief zou kunnen zijn om de senescence en aortaverwijding te verminderen. Behandeling van MFS muizen met resveratrol resulteerde inderdaad in verminderde aneurysma groei, via een ander werkingsmechanisme dan bekend is voor losartan. Aneurysma vorming werd niet beïnvloed door specifieke modulatie van sirtuin-1, waarvan bekend is dat het door resveratrol geactiveerd wordt en senescence kan verminderen. We laten zien dat resveratrol werkt via bescherming van de elastische vezels en gladde spiercellen in de vaatwand door de aneurysma-gerelateerde micro-RNA-29b te verlagen op een endotheelcel-afhankelijke manier (Hoofdstuk 4).

Calcificatie is een belangrijk component in hart- en vaatziekten en is ook aanwezig in de aorta van MFS patiënten en muizen. In MFS muizen is microcalcificatie vooral verhoogd in de aorta ascendens en het correleert met aortadilatatie, distensibiliteit en fragmentatie van elastine. Om het onderliggende mechanisme te begrijpen, hebben we laten zien dat elastine fragmenten de microcalcificatie verhoogt in humane en muis gladde spiercellen, wat afhankelijk blijkt van de kinase ERK1/2. Gebaseerd op onze bevindingen, meen ik dat visualisatie van microcalcificatie met specifieke probes overwogen kan worden als marker voor verminderde aorta integriteit in MFS patiënten en daarmee een indicatie geeft van het risico op aorta rupturen (Hoofdstuk 6).

Cystenieren komen vrij veel voor in de algemene bevolking en zelfs frequenter bij MFS patiënten. In een geval van een MFS muis met cystenieren is de aneurysmagroei sterk versneld. Daarnaast vertoont deze muis tekenen van aortitis, een sacculair aneurysma, en verhoogde markers voor ontsteking en bloeddruk regulatoren in het bloed. Wanneer bij de aorta controle in MFS patiënten tevens gekeken zou worden naar cystenieren, dan zou dat inzicht kunnen geven in de klinische relevantie van onze bevinding in de MFS muizen, om te bepalen of cystenieren als marker voor de ernst van aneurysma complicaties kunnen fungeren (Hoofdstuk 7).

Samenvattend, nieuwe inzichten zijn gegenereerd in de pathologie van de ontwikkeling van aneurysmata, vooral op het gebied van MFS. We hadden gegronde argumenten om bovengenoemde experimenten uit te voeren, en kunnen nu een aantal hypotheses verwerpen, aangezien niet alle behandelmethoden de aneurysma groei effectief konden remmen. Het is duidelijk geworden dat anti-inflammatoire middelen niet de oplossing zijn tegen aneurysma groei. Het zogenaamde anti-verouderingsmiddel resveratrol vermindere wel aneurysma groei in MFS muizen, en functioneert via een ander mechanisme dan het medicijn losartan dat nu vaak wordt voorgeschreven in MFS patiënten. Tenslotte vond ik een relatie tussen microcalcificatie en cystenieren met de ernst van aneurysma pathologie, waardoor ze mogelijk als markers kunnen dienen in de kliniek. Uitgebreide conclusies en ideeën voor toekomstig onderzoek worden hieronder bediscussieerd (Hoofdstuk 8)
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EDUCATION
2011-2016  PhD Candidate at the Academic Medical Center (AMC)
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Subject: “How is the isoprenoid biosynthesis pathway flux regulated during an immune response?”.
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**AWARDS & SCHOLARSHIPS**

2015  **ESC Best Poster Presentation**, European Society of Cardiology Congress
2015  **ZonMw Scholarship**, ZonMw, The Netherlands.
A personal scholarship to publish negative data from mouse experiments.

2014  **SSBN Travel grant**, KNCV, The Netherlands.
A travel grant for researchers with a biochemistry background to visit an international scientific meeting.

2011  **AMC PhD Scholarship**, AMC Graduate School, The Netherlands.
A personal scholarship for a four year PhD project.

2009  **STUNT scholarship**, University of Amsterdam, The Netherlands.
A scholarship for students to study abroad.

**SCIENTIFIC PUBLICATIONS**


*shared first author

## PhD portfolio

Name: Stijntje Hibender  
PhD period: November 2011 – December 2015  
PhD supervisors: Prof. Dr. C.J.M. de Vries, Prof. Dr. B.J.M. Mulder and Dr. V. de Waard

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