Genetics of Aortic Diseases

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AbbreviationsAneurysm Types

TAA	Thoracic aortic aneurysm
AAA	Abdominal aortic aneurysm
FTAAD	Familial thoracic aortic aneurysm and dissection
LDS	Loeys–Dietz syndrome
MFS	Marfan syndrome
vEDS	Vascular Ehlers–Danlos syndrome
BAV	Bicuspid aortic valve syndrome
ATS	Arterial tortuosity syndrome

Terms Related to Pathology of the Aortic Wall

ECM	Extracellular matrix
VSMC	Vascular smooth muscle cell
AngII	Angiotensin II
NAD+	Nicotinamide adenine dinucleotide

Terms Related to Heart Disease

MI	Myocardial infarction
CAD	Coronary artery disease

Terms Related to Genetic Analysis

SNP	Single nucleotide polymorphism (the associated rs number unambiguously identifies both the SNP's exact genetic location and specific nucleotide change)
NGS	Next-generation sequencing
GWAS	Genome-wide association study
WES	Whole exome sequencing
Exon	Protein coding DNA sequences of a gene
Intron	Non-protein coding DNA sequences of a gene, usually larger than exons
Promoter	Short DNA sequence located upstream of the transcription start site of a gene that induces production of the mRNA and protein encoded by the gene
Enhancer	Short DNA sequence located up to 100kB away from a gene; interacts with a gene's promoter to enhance protein production of the gene
OMIM	Online Mendelian Inheritance in Man database

Terms Characterizing Genes, RNAs, and Proteins

kB	Kilobases of DNA
kD	Kilodalton of protein
miRNA	microRNA, short ~20 nucleotide long RNA produced by a short gene, it regulates activity of other genes
lncRNA	Long non-coding RNA, a long RNA (produced from a long gene) that cannot be translated into a protein but nevertheless regulates the activity of other genes

Genes Involved in FTAAD and AAA

FBN1	Fibrillin 1
TGFbeta	Transforming growth factor beta

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TGFBR1	Transforming growth factor beta receptor 1
TGFBR2	Transforming growth factor beta receptor 2
TGFB2	Transforming growth factor beta 2
TGFB3	Transforming growth factor beta 3
MYH11	Myosin heavy chain 11
ACTA2	Alpha 2 actin, smooth muscle cell specific
MYLK	Myosin light chain kinase
PRKG1	cGMP-dependent protein kinase 1
MFAP5	Microfibril-associated protein 5
LOX	Lysil oxidase
FOXE3	Forkhead box E3 gene
NOTCH1	NOTCH1 gene
SMAD2	SMAD family member 2 gene
SMAD3	SMAD family member 3 gene
SKI	SKI proto oncogene
LDLR	Low-density lipoprotein receptor
SORT1	sortilin 1
IL6R	Interleukin 6 receptor
MMP9	Metalloproteinase 9
9p21	Chromosome 9 locus 21
ANRIL	Long non-coding RNA ANRIL
SMYD2	SET and MYND Domain containing 2 (protein lysine N methyl transferase)
ERG	ETS-related gene
DAB2IP	DAB2 interacting protein
LINC00540	Long non-coding RNA LINC00540

Introduction

The clinically most important aortic diseases are those that cause aneurysms and dissections in the aortic wall. Aortic aneurysms and/or dissections are sometimes subject to sudden rupture, which often causes sudden death. Currently the biggest problem in addressing sudden rupture is, that early stage aneurysm, which can be successfully treated by surgery, remains usually undetected because it is not associated with clearly detectable symptoms.

Aortic aneurysmal diseases include thoracic aortic aneurysms (TAAs) and abdominal aortic aneurysms (AAAs). TAAs have a strong and well-characterized genetic component. In the western world, TAA occurs with an incidence of about 12 per 100,000 per year in all age groups, shows little gender bias, and does not show strict association with cardiovascular risk factors [1]. In contrast, AAA is generally diagnosed in people over the age of 65, has a prevalence rate of about 8% for men and 1% for women [2] and shows strong association with male gender, smoking and cardiovascular disease. The genetics of TAA is well defined, and more than 20 genes have been found that, when mutated, directly cause TAA in an autosomal dominant Mendelian manner. The genetics of AAA is less well understood, mostly because all genes that have so far been associated with AAA seem to cause a phenotype in concert with environmental factors. In general, the observed prevalence of abdominal aortic aneurysm is likely below its actual occurrence because many cases may go undiagnosed. Combined, all aortic diseases, including inherited and non-inherited, are the 18th most common cause of death and are responsible for 1–2% of all deaths in industrialized nations [3].

At least 20% of all TAA are familial and directly caused by well-characterized mutations which are inherited in an autosomal dominant manner with high penetrance [4–8]. Familial TAAs are abbreviated often as TAAD or FTAAD where F stands for familial and D for dissections.

AAA differs from TAA as it does not usually feature strict Mendelian inheritance due to directly causative mutations. However, in the past several years, it became abundantly clear that AAA is indeed strongly associated with known genetic loci, although in all cases mutations in these loci predispose to AAA and cause AAA only in the context of additional environmental factors [1, 2, 9–11].

With the advent of gene targeting in mice more than 20 years ago that most recently was dramatically facilitated by the new CRISPR technology, it became possible to introduce any type of precise amino acid change into any endogenous gene of the mouse (e.g. [12–14]), and to date, several mutations found in genes of patients with aortic disease have been introduced by gene targeting into the mouse homologues of these genes. This has allowed the generation of experimental mouse models for several genetic types of TAAD and AAA, and these will be discussed below in detail together with the corresponding specific diseases.

The most recent discoveries of genes causing both TAAD and AAA suggest that any patient with TAA or with AAA should undergo genetic screening. If a known aneurysm-associated mutation is found – for TAA, usually a mutation that changes amino acid(s) of a protein coded for by a single gene and for AAA a single nucleotide polymorphism (SNP) usually located in intergenic regions – blood relatives should also be screened for such mutations. Without a doubt this will aid in early detection and therapy of aortic aneurysms and should significantly reduce ruptured TAA and AAA in family members.

Developmental biology has firmly established that the aortic root develops from the secondary heart field, the ascending aorta from the neural crest, and the descending aorta from the paraxial/somitic mesoderm and that the corresponding vascular smooth muscle cells (VSMCs) feature different proliferative and secretory responses to cytokines and produce different types of extra cellular matrix (ECM) in the aortic media [15]. This is consistent with the fact that generation of TAADs and AAAs is mechanistically clearly distinct and, as we describe in the paragraphs below, many of the genes involved in their generation are different. In addition, the cellular pathologies of the dilated aortic wall in TAA and AAA patients are distinct, although they share

some features [16]. A prominent difference is the paucity of atherosclerotic plaques and calcification found in the dilated thoracic aortic wall compared to the undilated and dilated abdominal aortic wall, as well as the distinct Th1-specific immune response for TAA compared to the predominantly observed Th2 immune response for AAA [16]. In addition, intraluminal thrombus is common in AAA but not TAA [17]. On the other hand, for both thoracic and abdominal aortic aneurysms, the aortic media is the predominant location of the causative injury which consists of infiltration of multiple types of inflammatory cells, destruction of the elastic fibers, and loss of VSMC which together lead to structural weakness. The exact proportion of the contribution of inflammatory cells, elastic fiber remodeling, and VSMC metabolism in Mendelian and non-Mendelian forms of TAA as well as AAA is subject of intense research.

Importantly, the knowledge gained from the molecular pathology of inherited aneurysms may also inform how to prevent aneurysms that are not genetically determined, which would be significant as these are the most frequent aneurysms: Indeed several important pathological pathways have already been discovered by genetic linkage analysis and extensively confirmed by animal studies – the TGF-beta pathway was discovered in 2006 to be involved in TAA [18], and this was confirmed extensively [7, 10, 19, 20]. Importantly, this pathway has originally been known to play important roles in the embryonal development of many types of tissues, including the thoracic and abdominal aorta and in the occurrence of many types of cancer – more recently it has been found that it may also be involved in AAA formation [20]. In the past several years, multiple genes regulating the lipid metabolism were found to be involved in AAA [1]. In addition, several genes regulating infiltration of immune cells into the aortic media or affecting extracellular matrix remodeling or proliferation of vascular smooth muscle cells in the media were found to be crucial for development of AAA [1, 21].

The currently known contributions of specific genetic loci to the two main classes of inherited aortic aneurysms, familial thoracic aortic aneurysms and dissections (FTAADs) and abdominal aortic aneurysms (AAAs), are described below.

Familial Thoracic Aortic Aneurysms and Dissections (FTAADs)

The first genetic condition and first familial disease observed to cause TAA is Marfan syndrome, a disease characterized by early TAA-induced cardiac death and characteristically long limbs. Marfan syndrome was also the first familial TAA syndrome shown to be caused by mutations in a specific gene, the fibrillin 1 (FBN1) gene [22]. Later, additional mutations in more than 20 genes causing FTAAD were discovered, and these cause Loeys–Dietz syndrome, vascular Ehlers–Danlos syndrome, Arterial Tortuosity Syndrome, as well as multiple types of non-syndromic familial TAA (FTAAD).

Indeed, familial thoracic aortic diseases are usually classified as either syndromic FTAADs or non-syndromic FTAADs, respectively. Syndromic FTAADs are also referred sometimes simply as syndromic TAADs. Non-syndromic FTAADs are often known simply as FTAADs or even just as TAADs. D for "dissections" is sometimes omitted for brevity. However, if the letter F for "familiar" is not included, the abbreviations can be mixed up with sporadic TAA/TAAD that may lack a genetic cause but indeed comprise the majority (~70+%) of all TAADs. To what degree their molecular pathology is different from that of FTAADs is subject to intense research. For clarity, we will here always include the F for all familial diseases, both syndromic and non-syndromic.

Syndromic FTAADs are characterized by inherited thoracic aortic disease with additional extensive non-aortic-related symptoms, such as long limbs for Marfan disease, while non-syndromic FTAADs have been defined by a lack of additional symptoms. In some cases FTAADs can present as either non-syndromic or syndromic, depending on the age of the patient or the exact position and type of mutation within a given gene, such as for FTAAD caused by mutations in the ACTA2 gene (see below). Each of these inherited aortic diseases is caused by a single mutation in a single different and characteristic genetic locus, and remarkably, at the time of submission of this article, both syndromic and non-syndromic FTAADs are associated with a growing list of genetic mutations in more than 20 different genes. The majority of these mutations are already known to be functionally responsible for FTAAD, based on a combination of family studies and genetic mouse models. Importantly, most of these genetic loci are not involved in AAA, strongly underscoring the functionally distinct pathology of FTAADs.

Among all thoracic aortic aneurysms (inherited plus non-inherited), about 20% are familial and are usually inherited in a classic autosomal dominant manner with high or complete penetrance [4–8]. Due to major recent advances in next-generation DNA sequencing (NGS), new inherited mutations associated with FTAADs are found continuously, and it is likely that the real percentage of FTAADs among all TAAs significantly exceeds 20%. Similar to most forms of AAA, FTAADs typically show distinct and extensive remodeling of the aortic media, characterized by significantly reduced numbers of vascular smooth muscle cells (VSMCs), fragmentation of the elastic fibers in the extracellular matrix (ECM), and inflammatory infiltration of lymphocytes that usually invade from the micro vessels of the adventitia. However, a major difference to AAA is the relative absence of atherosclerotic plaques in the aortic wall of TAA patients [23] (see also Table 5.1).

Since the 1990s, preventative genetic screening of blood relatives of familial syndromic or non-syndromic FTAAD

Table 5.1 Characteristic features of familial TAAD and familial AAA

	FTAAD	Familial AAA
Prevalence of familial disease	At least 20% of all cases	15–20% of all cases
Developmental origin of aortic section	Aortic root: Secondary heart field Ascending aorta: Neural Crest Descending aorta: Somatic mesoderm	Somatic mesoderm
Pathological features as reviewed in [16], for more details on inflammatory cells see [171–174]	Somewhat influenced by non-genetic factors Little or no atherosclerosis Intraluminal thrombus usually absent Extensive fragmentation of elastic fibers in the media - the normal thoracic media is significantly thicker and contains many more elastic lamina than the abdominal media Loss/apoptosis of vascular smooth muscle cells in the media accumulation of proteoglycans, no cyst formation, or overt necrosis (the original diagnosis of cystic medial necrosis is a misnomer) Infiltration of Th-1 inflammatory cells and macrophages through the adventitia into the media. The T-cells have an unusual flattened appearance and thus were not recognized for decades. elevated INFγ, IL2, IL12 and IL18 elevated MMP2 and MMP9	Strongly influenced by non-genetic factors, especially atherosclerosis, smoking and hypertension Frequent/extensive atherosclerosis Intraluminal thrombus is common Fragmentation of elastic fibers in the media Loss/apoptosis of vascular smooth muscle cells in the media Infiltration of Th-2 inflammatory cells through the adventitia into the media as well as NK and NKT cells and macrophages elevated IL4, IL5, IL10, IL12, IL18 and INFγ elevated MMP1, MMP2, MMP3 and MMP9
Types of Syndromic FTAAD all caused by mutations in exons	Caused be mutations in exons of the indicated genes: Marfan syndrome (MFS) FBN1 Loeys-Dietz syndrome (LDS) TGFBR1,TGFBR2,SMAD3,TGFB2, TGFB3 Vascular Ehlers-Danlos Syndrome (vEDS) Col3A1 Bicuspid valve syndrome SMAD6 Arterial tortuosity syndrome (ATS) SLC2A10 Syndromic ACTA2 FTAAD ACTA2	
Types of non-syndromic FTAAD all caused by mutations in exons	Caused by mutations in exons of the indicated genes that affect the: VSMC extracellular matrix FBN1,ELN,FBLN4,MFAP5 VSMC cytoskeleton MYH11, ACTA2 (e.g., Arg39Cys, Arg258 Hi/Cys mutation), MYLK,PRKG1, TGF beta pathway, cell proliferation TGFBR1,TGFBR2,SMAD2, TGFB2, TGFB3, Other pathways LOX, FOXE3, 11q23, 5q13–14	
Types of familial AAA all caused by specific mutations outside of exons but close to the indicated specific genes		Caused by predisposing mutations indirectly affecting expression of the indicated genes involved in: Lipid metabolism LDLR, SORT1 Immune response IL6R VSMC proliferation 9p21/ANRIL, DAB2IP, ERG, SMYD2, LINC00540 Extracellular matrix remodeling MMP9

patients has become routine clinical practice. To date in the USA, most routine genetic tests performed for all individuals with aortic aneurisms assess the presence of mutations in at least 20 separate loci, such as the commercially available Ambry Genetics TAADNext test which assesses 22 loci. This has allowed early treatment of at-risk family members with beta-blockers or blood pressure drugs, regular screening for aneurysms, and/or corrective surgery. A striking example of progress is Marfan syndrome where the average life expectancy has dramatically increased with modern diagnosis, monitoring, and treatment (for details see also section describing MFS below).

Syndromic Familial Thoracic Aneurysms and Dissections (Syndromic FTAADs)

The most well-understood syndromic FTAADs include the dominantly inherited Marfan syndrome (MFS), Loeys–Dietz syndrome (LDS), vascular Ehlers–Danlos syndrome (vEDS), and arterial tortuosity syndrome (ATS) [24]. They are all characterized by acute thoracic aortic dissections and ruptures, which cause sudden cardiac death. In addition they exhibit characteristic features unrelated to the aorta, such as long limbs in the case of MFS and widely spaced eyes for LDS. The first of these syndromic FTAADs was MFS. In 1991, MFS was found to be caused by mutations in the fibrillin 1 gene (FBN1) [22, 25, 26]. Since then, LDS, vEDS, and ATS as well as BAV have been defined by causative mutations in specific genes, and all FTAADs together are now either known or being suspected to be caused by inherited mutations in an increasing number of different genetic loci (currently more than 20), with a few loci being affected most frequently.

Early theories of the causes of human aneurysm mostly focused on inherited or acquired defects in components of the extracellular matrix in the aorta. Although several mutations in the genes encoding extracellular matrix proteins have been recognized, more recent discoveries have also shown important perturbations in cytokine signaling cascades and intracellular components of the smooth muscle contractile apparatus.

There is great utility of genetic diagnostics in the management of syndromic FTAAD, and often essential conclusions for optimal downstream treatment can be drawn since the optimal clinical management of individual FTAADs can be quite distinct. More fundamentally, genetic diagnostics is necessary to diagnose syndromic FTAAD, to exclude syndromic FTAAD and to specify disease types. Combining phenotype with genotype information maximizes the predictability of the course of disease and contributes to a better timing of elective surgery and to a better choice of procedures. Perhaps most importantly, with genetic diagnostics it is possible to predict the birth of children with causative mutations for syndromic FTAAD and to initiate timely drug therapy to prevent the onset of aortic dilatation or to slow down its progression to aortic aneurysm. For all these reasons, it is now standard procedure to apply genetic diagnostics to all new patients with aortic disease.

Marfan Syndrome (MFS)

MFS is a Mendelian disorder of the connective tissue clearly shown to be familial and autosomal dominant in 1931 [27]. MFS is associated with many striking features including long limbs and the presence of dislocated ocular lenses (ectopia lentis). In 1943, it also was shown to be the first Mendelian disorder to cause TAA [28]. In 1991, MFS was shown to be caused by mutations in a specific gene, the fibrillin 1 (FBN1) gene [22]. It is by far the most prevalent Mendelian disease of the aorta and occurs in about 1 of 3000–5000 individuals [29]. The name is a misnomer because the disease originally discovered in 1896 by the French physician Antoine Marfan was almost certainly not MFS but a related disease with similar skeletal malformations, named congenital contractural arachnodactyly (Beals–Hecht syndrome) [30]. MFS is a heritable disorder of fibrous connective tissue and shows highly variable but strongly systemic pathology in the skeletal, ocular, and cardiovascular systems [31, 32]. The current international standard for diagnosis of MFS is documented in the revised Ghent criteria [33]: two of the following four major criteria are needed to diagnose MFS – (1) the presence of dislocated ocular lenses (ectopia lentis), (2) the dilatation or dissection of the aortic root, (3) the presence of a mutation in the FBN1 gene, and (4) the sum of several other features, such as increased height, disproportionately long limbs and digits, anterior chest deformity, mild to moderate joint laxity, and vertebral column deformity (scoliosis and thoracic lordosis) as well as highly arched palate with crowding of the teeth and overbite [34].

In addition to ectopia lentis, myopia, increased axial globe length and corneal flatness are frequent ocular findings [31]. Besides aortic root dilatation and dissection, mitral valve prolapse, mitral regurgitation, and aortic regurgitation are cardiovascular features and together represent the major life-threatening conditions of Marfan patients [31, 35].

Other common manifestations are striae distensae, pulmonary blebs (which predispose to spontaneous pneumothorax), and spinal arachnoid cysts or diverticula [36–38]. By CT scanning, widening of the lumbosacral spinal canal (dural ectasia) was found in 36 of 57 patients with the Marfan syndrome and in none of 57 age- and sex-matched non-Marfan control patients [39]. Severe changes were present in 13 patients, 2 of whom had associated neurologic signs, and included meningoceles or near-total erosion of a pedicle [39].

Due to improved diagnosis, monitoring and treatment, life expectancy for MFS patients has increased from about 44 (men) and 47 years (women) in 1972 to an almost normal life-span as of today as mentioned on website of the Marfan Foundation (https://www.marfan.org/). As a consequence, new MFS features have emerged. These include aortic dilatation beyond the root, type B aortic dissection, aneurysms in arterial branches of the aorta, and cardiomyopathy, as well as cataracts, glaucoma, obstructive sleep apnea, hepatic and renal cysts, degenerative arthritis, osteoporosis, myopathy, and truncal obesity. These new features are an important field of future clinical research.

The fibrillin gene (FBN1) Mutations in the FBN1 gene are the cause of Marfan syndrome, and most FBN1-mutant alleles lead to Marfan syndrome through a dominant negative effect [40, 41]. While the majority of FBN1 mutations cause MFS, a few of the mutations in the FBN1 gene do not cause syndromic disease but asymptomatic FTAAD (OMIM#132900), ectopia lentis (OMIM #129600), or several other very rare conditions. Interestingly, about one-quarter of FBN1 mutations arise spontaneously, but most are inherited from one parent in an autosomal dominant fashion with high penetrance.

The FBN1 gene codes for the extracellular protein fibrillin 1 which is the main and essential component for generation and maintenance of extracellular microfibrils. It is found in connective tissue throughout the body and is a major component of the extracellular matrix (ECM) of the aortic media.

The large size of the FBN1 gene (~200kB) and corresponding fibrillin 1 protein (~350kD) helps explain why more than 1800 different pathogenic mutations, about 1200 of these single nucleotide polymorphisms (SNPs), have been found in the gene, affecting all areas of the corresponding long and repetitive fibrillin 1 protein, according to the current status of the FBN1 Universal Mutation Database that was founded in 2003 [42].

Clearly different functional classes of mutations are seen, although the exact cause and effect relationship is not well understood: 54% of the Marfan patients listed had ectopia lentis, and a higher probability of ectopia lentis was found for patients with a missense mutation substituting or producing a cysteine, when compared with other missense mutations. In addition, patients with a premature termination codon had a more severe skeletal and skin phenotype than did patients with an in-frame mutation. Mutations in exons 24 through 32 were associated with a more severe and complete phenotype, including younger age at diagnosis of type I fibrillinopathy and higher probability of developing ectopia lentis, ascending aortic dilatation, aortic surgery, mitral valve abnormalities, scoliosis, and shorter survival; most of these results were replicated even when cases of neonatal MFS were excluded (FBN1 Universal Mutation Database) [42].

Animal models and molecular pathology of Marfan disease Human MFS affects primarily the aortic root, usually starting with aortic root dilatation.

Indeed, mouse models with mutated FBN1 show overlapping but distinct pathological mechanisms compared to mouse models of AAA. Historically one of the originally most important MFS animal models is the fibrillin 1 mutant mouse used by the Dietz lab to show that either anti TGF-beta neutralizing antibody or angiotensin type 1 receptor blocker losartan reduces aneurysm formation [43]. The potential explanation for this effect was that fibrillin 1 binds to inactive forms of TGF-beta and acts normally as a sump to keep TGF-beta inactive, while mutations in fibrillin 1 protein would set free a pathologically high amount of TGF causing many of the syndromic effects of MFS not only in the aorta but the rest of the body. This finding resulted in the use of losartan (a presumed anti TGF-beta agent) in human subjects of MFS with existing aneurysm (see below). Paradoxically, more recently, using the Angiotensin II-induced mouse model of AAA, TGF-beta inhibition by neutralizing antibodies did not prevent but instead significantly augmented AAA formation mice [44], indicating that perhaps generation and progression of TAA requires different TGF-beta-related stimulation.

Even more intriguing, a more recent mouse model of MFS, the FBN1 C1039G mouse model, altogether questions the original idea that increased TGF-beta levels and thus increased TGF-beta-signaling cause MFS. This is based on the discovery that smooth muscle cell-specific deletion of the TGF-beta receptor TGFBR2 in neonatal FBN1 C1039G mice, which should decrease TGF-beta signaling, actually accelerates, rather than diminishes, aortopathy [45]. In addition, deletion of TGFBR1 (which forms a complex with TGFBR2) in smooth muscle cells of normal adult mice which should diminish TGF-beta signaling causes TAA with 100% penetrance [46]. Losartan, a blocker of the angiotensin receptor, fully rescues this mutation and prevents TAA formation in this mouse model [46], and this is consistent with the fact that the renin angiotensin signaling pathway is upregulated in these mice.

Another twist in the search for novel drug candidates for treatment of TAA was presented by a study in 2016 showing that resveratrol can inhibit specifically TAA progression in the FBN1 C1039G mouse model [47]: resveratrol was administered for 2 months to FBN1 C1039G mice with already existing small TAA, and intriguingly, this completely reversed the TAA to no aneurysm and thus achieved complete cure. Resveratrol promoted extracellular matrix integrity and smooth muscle cell survival and also downregulated the aneurysm-related micro RNA 29b in the aorta [47].

In summary, despite intense efforts using sophisticated genetically engineered mouse models to find new drug targets for MFS, further research will still be necessary. Both

animal models and human genetics of MFS clearly implicate crucial genes of the TGF-beta pathway in TAA formation. While it is clear that in normal mice TGF-beta signaling is necessary for normal early development of arteries including the aorta, it is at present still unclear how the causative FBN1 mutation causes TGF-beta pathway-dependent or -independent development and long-term progression of TAA.

Clinical trials of MFS Multiple clinical trials have been finished, and several are ongoing that are testing angiotensin receptor blockers and/or beta-blockers. Unfortunately, even if a reduction of enlargement of TAA was present due to such treatment, neither beta-blockers nor losartan has so far convincingly changed rates of aortic root surgery, dissection, or death [48]. Several recent trials failed to show a clear positive effect of losartan, the most widely used angiotensin receptor inhibitor, or of any beta-blockers, and careful meta-analysis of all major trials, as well as additional recruitment of patients, may be needed to get a better idea of benefit. Interestingly, the beneficial effect of losartan may depend on the exact nature and location of the causative mutation within the large fibrillin 1 gene as reported for a clinical trial in 2015 [49, 50]. Specifically, losartan alone could very significantly slow down TAA diameter growth in patients with fibrillin 1 mutations that cause haploinsufficiency but no effect at all in patients with dominant negative fibrillin 1 mutations [49, 50]. Indeed, these two types of mutations are functionally distinct as only the dominant negative mutation generates a novel type of fibrillin 1 protein, whose function is not yet well understood, while haplo-insufficient mutations do not change the fibrillin 1 protein but instead cause the fibrillin 1 protein level to fall by about 50% in all tissues.

Additional trials are underway testing more potent versions of angiotensin-receptor blockers [51] such as telmisartan [52]. Truly effective and curative medication for TAA has yet to be discovered, and the multiple recent genetically engineered mouse animal models of TAA will no doubt result in novel candidate drugs to be tested in clinical trials. The recent discovery that resveratrol inhibits TAA expansion in mice is an example of such a new candidate drug [47]. Of note, resveratrol has already shown some positive effects on lipid profiles, body fat, blood pressure, inflammation, and glucose metabolism in some clinical trials [47].

Loeys–Dietz Syndrome (LDS)

LDS was first described in 2005 [53], is exceedingly rare (less than 3 in 100,000 individuals), and is characterized by an aortic pathology similar to Marfan syndrome but lacking a mutation in the FBN1 gene. LDS is an autosomal dominant connective tissue disorder characterized by rapidly progressive thoracic aortic aneurysmal disease, generalized arterial elongation with abnormal twists and turns (vascular tortuosity), increased distance between the eyes (hypertelorism) and bifid/broad uvula or cleft palate [53, 54]. While LDS is similar to MFS in some respects, there are important differences: hypertelorism, cleft palate/bifid uvula, and arterial tortuosity are associated with most forms of LDS but are absent in MFS. Reversely, ectopia lentis (misplacement of the lens) is exclusive to MFS and perhaps directly caused by misfolding or haploinsufficiency of fibrillin 1 which is likely part of the ECM components that hold the lens of the eye in its correct location. Thus ectopia lentis is routinely used to distinguish MFS from LDS and other FTAADs [54].

Based on the most recent genetic studies, mutations in five genes, TGFBR1, TGFBR2, SMAD3, TGFB2 and TGFB3, have been recognized to cause LDS [54, 55] (OMIM database https://www.omim.org/). A single mutation in any one of these genes can be sufficient for full expression of the syndrome. However, not all types of mutations found in these genes cause an "LDS-type" syndrome with the above strong phenotype, and indeed several mutations in these genes have been found that cause only mild symptoms including mild aortic phenotype [56]. This finding highlights the fact that specific mutations in the same gene can have different phenotypes and can cause indeed different diseases. Interestingly, all of the above five genes are involved in the TGF beta pathway that regulates multiple developmental processes including the development of the cardiovascular system and potentially the progression of aneurysmal disease, even perhaps in MFS, as described above. While some of the reported LDS mutations are expected to downregulate TGF beta signaling, as they reduce activity of the corresponding genes, they are not autosomal recessive but autosomal dominant. Whether simple haploinsufficiency or a novel pathological pathway is generated by the different dominant mutations causing LDS must still be determined by further research.

While mutations in the five genes mentioned above can all cause severe TAA, they often do exhibit somewhat different syndromic pathologies. For example, TGFB3 mutations may not cause arterial tortuosity in contrast to mutations in the other four genes [55, 57]. For the characteristic phenotypes of LDS patients with TGFBR1 and TGFBR2 mutations, the Montalcino Aortic Consortium published updated criteria in 2016 [57].

Animal models A recent animal model of LDS confirmed that mutations of Tgfbr1 or Tgfbr2 that are analogous to human mutations (TGFBR1 M318R and TGFBR2 G357W) act in a dominant negative way to contribute to LDS-like symptoms in mice – mostly TAA but not any of the other syndrome-like features [58]. Another study confirmed the importance of Tgfbr2 in TAA development by deleting TGFBR2 specifically in postnatal VSMC, which caused aortic damage, including moderate TAA [59]. On the other

hand, TGFBR1 deletion in VSMC caused severe aortic damage including severe TAA with 100% penetrance, strongly confirming its importance in LDS [46]. Loss of TGFBR1 seems to act through multiple pathways including the TGFBR2, the ERK (extracellular signal-regulated kinases), and the angiotensin receptor AT1R pathways [46]. These data suggest that multiple pathological pathways can be expected for generation of TAA in humans as well. SMAD3 is a downstream target of TGFBR1 and TGFBR2, and some SMAD3 mutations cause LDS in humans and indeed cause severe TAA in SMAD3−/− mice, leading to sudden rupture at death after 6–30 weeks of age [60]. In addition, TGFB2 (transforming growth factor beta 2) haploinsufficiency in mice leads to TAA confirming the finding in LDS patients [61].

At this time it is still too early to make conclusions about the proposed exact molecular mechanisms in LDS and MFS pathogenesis, especially as there are apparently contradictory findings about the importance of TGF beta signaling, as discussed above. It is also possible that some of the discrepancies are simply a consequence of incomplete analysis of the molecular pathology often relying on only one or two maker proteins supposedly proving one type of signaling over another. Thus, it may be beneficial if researchers revisit the various existing animal models to study additional components of possible signaling pathways (TGF beta, SMAD3, ERK, and angiotensin signaling). This could be facilitated by the use of functional genomic techniques such as RNAseq of the aortic tissues to generate unbiased whole genome mRNA expression profiles.

Clinical trials Because LDS is exceedingly rare and it is very difficult to recruit enough LDS patients to reach statistical power, clinical trials of LDS have been performed mostly in form of sub-studies of Marfan clinical trials (see above) and using MFS-specific medications, such as losartan and beta-blockers.

Vascular Ehlers–Danlos Syndrome (vEDS)

Vascular Ehlers–Danlos syndrome (vEDS), also called EDS type IV, is an autosomal dominant disorder caused by mutations in the COL3A1 gene [62, 63]. All EDS types together occur at a frequency of about 1 in 5000, and they are distinguished by the exact type of collagen gene that is mutated (COL1A1, COL1A2, COL3A1, COL5A1, or COL5A2). In the 1920s, individuals with EDS, such as the Indian rubber man and other circus performers, showed off the extreme elasticity of their skin and ligaments common for many types of EDS. vEDS is rare and occurs only in about 1 out of 100,000 people [63]. It is distinct from other EDS types by its arterial complications and therefore included in the syndromic FTAAD conditions. Unlike other FTAAD syndromes, large aneurysms in the aorta are rare but nevertheless ruptures occur with high frequency even in small diameter aneurysms indicating a much more fragile vessel wall than that in MFS and LDS patients [63]. Importantly, not only the aorta but many other types of arterial vessels are often affected already at a young age and therefore vEDS features a significantly more severe pathology than MFS or LDS. In addition dangerous perforations of other hollow organs, especially the sigmoid colon, are common [64].

The median life expectancy for patients with vascular EDS is 40–50 years [63]. Death is most frequently due to complications associated with vascular and hollow organ rupture. Surgical intervention, especially stent grafting, has only limited value in vEDS patients. Indeed, vascular stents and endografts are not often used, as the long-term durability of these repairs often is poor due to suboptimal graft–aortic wall interaction caused by the underlying connective tissue disease and vessel fragility. Unfortunately, even minimally invasive, relatively simple therapeutic interventions can have adverse events – even with standard tools, such as catheters and guide wires – and the need for careful intravascular manipulation cannot be stressed strongly enough.

The COL3A1 gene Mutations in this gene are inherited in an autosomal dominant manner. Interestingly, approximately 50% of cases represent new mutations that occur sporadically and lack a family history of disease but are then inherited to the next generation. The other half inherits the COL3A1 mutation from at least one parent. Women and men seem to be affected with comparable frequency. As with MFS and LDS, pregnancy can be associated with severe complication in vEDS women.

To date, more than 1000 COL3A1 mutations have been identified, and more are being added continuously, and they all can be accessed free of charge from the COL3A1 section of the online Ehlers–Danlos Syndrome Variant Database (https://eds.gene.le.ac.uk/home.php?select_db=COL3A1). This is an invaluable and very detailed resource that allows stratification for types of mutations, frequencies of occurrence, and known pathogenic effect. Most pathogenic mutations are missense mutations leading to substitutions for one of the glycine residues that are part of the many repeating G X Y (G = Glycine, X and Y representing any amino acid) triple repeats in the long triple helical region of the collagen molecule [65–67].

Animal models One of the more informative of the few animal models of vEDS shows that mice haploinsufficient for COL3A1 develop thoracic aortic aneurysms at high rate if infused with AngII by implanted pump, the same procedure used to model TAA and AAA in hyperlipidemic mice [68, 69]. This certainly confirms the crucial role of COL3A1 in aneurysm and rupture seen in vEDS patients, although the

mutation is different from those usually found in patients, which are single amino acid changes that confer a dominant negative phenotype. At this early stage, it is premature to make any definitive statements about candidate drug targets deduced from the known molecular pathology of vEDS.

Clinical trials Because of the paucity of patients, it is not surprising that few clinical trial data describing beneficial effects of candidate drugs were done, despite the great need due to the major perioperative problems associated with surgical procedures. So far only one trial has shown positive pharmacological effect for vEDS patients. It was performed in France involving 53 vEDS patients and shows beneficial effect of a beta-blocker, celiprolol, which interestingly may not act primarily through lowering of blood pressure but through stabilization of the arterial wall [70]. The originally planned 5-year study was stopped already after 5 months due to an unexpected positive effect so that all patients, including control group patients, could benefit from celiprolol [70].

Bicuspid Aortic Valve Syndrome (BAV)

Bicuspid aortic valve syndrome [24] describes an aortic valve with two rather than three leaflets [24, 71]. It is the most frequent congenital heart defect and is present in 1–2% of the population. It is also highly heritable, but so far elucidation of the mutated genes responsible has explained only a few percent of total heritability, and additional mutated loci are expected to be identified in the future.

BAV is frequently followed by aortic valve stenosis or insufficiency. Valve calcification is also observed frequently. About 20% of BAV patients develop TAAD, a significant number, given the high overall frequency of BAV.

Mutations in the signaling and transcription regulator NOTCH1 may cause an early developmental defect leading to BAV according to some genetic linkage analysis studies and functional mouse studies [72]. It is well documented that the few BAV individuals that also carry a NOTCH 1 mutation are either asymptomatic or feature valve calcification, aortic valve stenosis, coarctation, and/or hypoplastic left heart but do not usually develop TAA [24]. Further studies are needed to determine if NOTCH1 signaling plays any role in development of TAA in BAV patients.

A recent extensive large genetic linkage study testing more than 20 genes previously associated with BAV with or without TAA through numerous, mostly small, genetic linkage studies and/or animal models yielded intriguing results: based on 441 BAV patients that also had TAA, and 183 controls, only one clear genetic association with BAV/TAA syndrome could be confirmed, namely, mutations in the functionally important MH1 and MH2 domains of the transcription factor SMAD6, which could explain the molecular pathology in 2.5% of the BAV/TAA patients in the study population [24]. The NOTCH 1 gene could not be confirmed in this study to be associated with BAV/TAA [24]. Remarkably, mice lacking the mouse SMAD6 homologue also present with misplaced septation, thickening of the cardiac valves, and ossification of the outflow tract, although TAA was not yet documented [73].

Because of the considerable uncertainty about the involvement of additional proposed candidate genes that may contribute to inheritance of BAV with and without TAA, we will not discuss these here further and instead refer to recent detailed reviews of the subject [74–76].

Arterial Tortuosity Syndrome (ATS)

This very rare syndrome with autosomal recessive inheritance is caused by mutations in the SLC2A10 gene which codes for glucose transporter 10 (GLUT10) protein [77]. It is usually diagnosed in infants and consists primarily of tortuosity of the aorta and of other arteries throughout the body, such as in the pulmonary, subclavian, and renal arteries, and all of these are subject to sudden rupture. Additional clinical features in some of these patients are hyperlax skin and joints and/or dilation of the ascending aorta, thoracic aortic aneurysms, and stenosis of the ascending aorta and/or the pulmonary arteries.

ACTA2 (Alpha 2 Actin, VSMC-Specific) Syndromic FTAAD (OMIM#611788)

Mutations in the ACTA2 gene, which codes for the VSMC-specific actin, inherit in dominant fashion and cause severe early-onset FTAAD, often in children [78]. Interestingly, mutations in this gene also often cause coronary artery disease and stroke. Indeed, like other genes causing TAA, ACTA2 can cause both syndromic and non-syndromic FTAADs (see also below), depending on the exact mutation in the gene. *ACTA2* missense mutations that disrupt arginine 179 lead to a syndromic FTAAD with distinctive smooth muscle dysfunction syndrome characterized by aortic and cerebrovascular disease, fixed dilated pupils, hypotonic bladder, intestinal hypoperistalsis, and pulmonary hypertension [79]. This particular mutation causes severe and early-onset vascular disease, including TAA, and has so far only been identified as a de novo mutation in affected individuals. In addition, preliminary studies have shown correlations between specific *ACTA2* mutations and increased risk for early-onset stroke or coronary artery disease [80].

Non-syndromic Familial Thoracic Aortic Aneurysms and Dissections (Non-syndromic FTAADs)

In contrast to syndromic FTAADs, non-syndromic FTAADs lack additional unrelated symptoms, especially in children and young adults [6, 8, 81] but may acquire later in life some

weak symptoms. When such weak, often nonspecific, symptoms do occur, potentially life-threatening aneurysms have often already developed. Therefore any person with a blood relative that has experienced non-syndromic FTAAD should be genetically tested. Often this is the only way to predict and prevent potentially dangerous aneurysms. The inherited mutations that cause non-syndromic FTAAD are strikingly different from those that cause syndromic FTAAD and have been discovered more recently with the advent of modern genetic screening tools. According to the Online Mendelian Inheritance in Man (OMIM) database, there are at least 11 non-syndromic FTAAD subtypes caused by inherited mutations in at least 11 different genes. Each mutated gene by itself is sufficient to cause non-syndromic FTAAD, and by definition genetic analysis is always required to ascertain a specific genetic subtype because of the lack of an easily discernible phenotype [6, 8, 81]. Like syndromic FTAADs, most if not all non-syndromic FTAAD subtypes are inherited in an autosomal dominant manner with high penetrance.

Non-syndromic FTAAD Caused by Mutations in Genes Coding for Proteins That Are Part of the Elastin Microfibril Units in the Extracellular Matrix

FBNI *(fibrillin 1)* (OMIM#134797)

As mentioned above, in rare cases, mutations in the FBN1 gene do not cause MFS-like symptoms and instead only cause isolated FTAAD.

ELN (Elastin) (OMIM #130160)

Specific types of mutations in the ELN gene cause FTAAD. For example, individuals with triplicate copies of the ELN gene have been found to have FTAAD [82]. This is one of the most rare forms of FTAAD but with highly penetrant and autosomal dominant inheritance. Patients have also often cutis laxa (OMIM#614437). However, the most frequently observed mutations in the ELN gene do not cause FTAAD but instead cause supravalvular aortic stenosis (OMIM #185500), which is often present at birth. In some cases aortic stenosis can cause aneurysms at high age of the patient. Some types of mutations in the ELN gene cause inherited intracranial aneurysm in the absence of FTAAD [83].

FBLN4 (fibulin4) (OMIM #604633)

Patients with recessive FBLN4 mutations are predominantly characterized by aortic aneurysms, arterial tortuosity, and stenosis [84, 85]. Certain mutations in FBLN4 cause autosomal recessive cutis laxa, often in the absence of aneurysms (OMIM #614437). Mouse models with VSMC-specific KO of FBLN4 reproduce human FTAAD very well [86, 87]. The exact relationship between cutis laxa and FTAAD is subject of ongoing research.

MFAP5 (Myofibrillar-Associated Protein 5), **FTAAD Type 9** (OMIM #616166)

Very recently discovered mutations in the MFAP5 gene, coding for an extracellular matrix protein, cause FTAAD and at times mild skeletal features similar to MFS. The aneurysms and aortic dissections are caused by alterations to the structure or function of the VSMC elastin-contractile unit, of which MFAP5 is an integral part of, together with other structural components such as FBN1, ELN, and FBLN4 [90].

Non-syndromic FTAAD Caused by Mutations in Genes Coding for Proteins That Are Part of the Actin-Myosin Contractile Units in the VSMC

MYH11 (Myosin Heavy Chain 11, VSMC-Specific), FTAAD Type 4 (OMIM #132900)

MYH11 functions as key cytoskeletal protein in VSMC as do ACTA2, MYLK, and PRKG1. Heterozygous mutations in MYH11 cause severe FTAAD causing life-threatening aortic dissections [90]. These rare MYH11 mutations are often associated with ductus arteriosus [88]. Force generation by VSMC requires interactions between filaments composed of VSMC-specific isoforms of myosin heavy chain (encoded by MYH11) and of α-actin (encoded by ACTA2). MYH11 and ACTA2 mutations that disrupt this cyclic interaction cause FTAAD (see also below).

ACTA2 (Alpha 2 Actin, VSMC-Specific), FTAAD Type 6 (OMIM#611788)

ACTA2 can cause both syndromic (see above) and non-syndromic FTAAD, depending on the exact nature of the mutation in the gene. ACTA2 is the most frequently mutated gene causing non-syndromic FTAAD and is responsible for about 12–21% of such cases [89, 90]. Unlike many other genes associated with TAA, Acta2 mutations tend to cause thoracic dissections, rather than true aneurysms. The lifetime risk for an aortic event (dissection or repair) is about 76%, suggesting that additional environmental or genetic factors play a role in expression of aortic disease in individuals with ACTA2 mutations. Mutations within the ACTA2 gene at amino acid position R179 or R258 were associated with significantly increased risk for such aortic events, whereas R185Q and p.R118Q mutations showed significantly lower risk of aortic events compared with other mutations [90].

VSMC from the aorta of ACTA2 −/− mice present with a reduced number of elastic lamellae and progressive aortic root dilatation, confirming the causative role of ACTA2 in TAA [91].

MYLK (Myosin Light Chain Kinase), FTAAD Type 7, 2010 (OMIM#613780)

Mutations of the MYLK gene cause non-syndromic FTAAD [92, 93]. Individuals carrying a mutation in the MYLK gene that inactivates its function have a high risk of acute aortic dissection or rupture at an early age. Importantly, aortic events are often not preceded by obvious aortic dilatation. This means that elective surgery should be done at a lower aortic diameter than for other inherited forms of TAA. Surprisingly, in mice, the same MYLK mutations found in humans (R247C/R247C) do not present a severe phenotype; however they do cause TAA if challenged with hypertensive drugs such as angiotensin – a note of caution when trying to compare the same mutations in humans and mice [94].

PRKG1 (cGMP-Activated Protein Kinase), FTAAD Type 8 (OMIM#615436)

PRKG1 is a protein kinase, and a constitutively hyperactive mutant form of this kinase indirectly leads to dysregulation of the regulatory light chain of MYH11, thus leading to inability of the contractile cytoskeletal apparatus to generate force. Heterozygous mutations in PRKG1 genes cause severe FTAAD causing life-threatening aortic dissections [87, 93].

Non-syndromic FTAAD Caused by Mutations in Genes Coding for Proteins That Are Part of the TGF-Beta Signaling Pathway Active in VSMC and Immune Cells

TGFBR1 (Transforming Growth Factor Beta Receptor 1: Also Presents as LDS) FTAAD Type 5 (OMIM#609192)

Mutations in the TGFBR1 gene can cause LDS type 1 but can also present without the related non-aortic syndrome, thus causing isolated TAAD. It is possible that the exact nature of the mutation within the TGFBR1 gene determines whether it presents as syndromic or non-syndromic FTAAD. TGFBR1 is a complex molecule with multiple functional domains that could, if mutated separately, cause different downstream metabolic effects. No doubt the future will tell if that is the case. It is also possible that lifestyle choices and/or additional genetic factors play a role.

TGFBR2 (Transforming Growth Factor Receptor 2: Also Presents as LDS) FTAAD Type 3 (OMIM#610168)

Mutations in the TGFBR2 gene can cause LDS but, as for TGFBR1 mutations, can also cause aneurysms and dissections without the related non-aortic syndrome. The same considerations as for TGFBR1 above apply.

SMAD2 (SMAD Family Member 2) Isolated FTAAD

Recently discovered mutations in the SMAD2 gene cause early-onset thoracic aortic aneurysms [95, 96], a gene structurally and functionally similar to SMAD3 which, when mutated, can cause LDS (see above).

TGFB2 (Transforming Growth Factor Beta 2) Isolated FTAAD

Some mutations in the TGFB2 gene cause non-syndromic FTAAD rather than syndromic FTAAD (LDS; see above).

TGFB3 (Transforming Growth Factor Beta 3) Isolated FTAAD

Some mutations in the TGFB3 gene cause non-syndromic FTAAD rather than syndromic FTAAD (LDS, see above).

FTAAD Caused by Mutations in Genes Coding for Proteins That Are Part of Other Signaling Pathways

LOX (Lysyl Oxidase) (OMIM#617168), **FTAAD Type 10**

Mutations in LOX cause autosomal-dominant FTAAD in humans [97, 98]. LOX belongs to a group of copper-dependent oxidodeaminases that cross-link lysyl residues on the ECM proteins elastin and collagen, the structural proteins necessary for formation of elastic lamellae and collagen fibers in the media of the aorta [99]. Mice homozygous for a missense mutation in the LOX gene that causes FTAAD in humans die shortly after birth because of ruptured aortic aneurysms [98].

FOXE3 (Forkhead Transcription Factor 3) (OMIM#617349), **FTAAD Type 11**

Mutations in the FOXE3 gene cause autosomal dominant FTAAD [100]. In mice, FOXE3 deficiency reduced smooth muscle cell (SMC) density and impaired SMC differentiation in the ascending aorta. FOXE3 expression was induced in aortic SMCs after transverse aortic constriction, and FOXE3 deficiency increased SMC apoptosis and ascending aortic rupture with increased aortic pressure [100].

FTAAD type 1, Genetic Locus 11q23 (OMIM #607086): Mutated Gene(s) Unknown

The locus q on chromosome 11q23.3–11q24 has been shown already in 2001 to cause FTAAD at young age with extensive medial necrosis, VSMC loss, loss of elastic fibers, fibrotic remodeling, and accumulation of polysaccharides [101, 102]. However, a causative gene within this chromosomal location has yet to be identified. The difficulty may be due to incomplete penetrance of the mutation, or perhaps the mutation is not located in a gene but in a regulatory region outside of genes that regulate gene expression at a long distance. Indeed recent whole genome sequencing studies have revealed that at least eight times more DNA is reserved for active regulatory regions, such as promoters, enhancers, and non-coding RNAs, than for the protein-coding genes itself. Modern RNAseq analysis combined with whole genome sequencing and whole genome epigenetics will no doubt eventually reveal the exact mutation in locus 11q that causes non-syndromic FTAAD.

FTAAD Type 2, Genetic Locus 5q13–14, (OMIM#607087): Affected Gene(s) Unknown

Like FTAAD1, FTAAD2 presents at young age with extensive medial necrosis and is caused by a mutation whose general location, 5q13–14, is known since 2001. The exact location and nature of the causal mutation(s) have not yet been identified [103]. The same considerations as described above for locus 11q23 apply.

Cellular Pathology of the Thoracic Aorta

As detailed in Table 5.1, the cellular pathology of the dilated thoracic aortic wall is clearly distinct from the pathology of the abdominal aortic wall and is characterized by a paucity of atherosclerotic plaques and calcification, absence of intraluminal thrombus, and presence of Th1 immune cells. On the other hand, thoracic and abdominal aortic pathologies of both the non-inherited and inherited forms have important similarities: for both thoracic and abdominal aortic aneurysms, the aortic media is the predominant location of the causative injury which consists of infiltration of multiple types of inflammatory cells mostly through the adventitia; abnormal activation of metalloproteinases, such as MMP9; destruction of the elastic fibers; and loss of VSMC which together lead to structural weakness.

FTAAD, but not AAA, is caused by dominant mutations in specific genes, including those coding for proteins of the cytoskeleton as described above. Strikingly, mutations that directly affect the VSMC cytoskeleton have mostly been found in non-syndromic FTAAD, pointing toward a highly specific pathogenic mechanism. Some of these mutations seem to cause a unique pathological appearance of the aortic wall [23], and this is subject to current research. For a more detailed description of the cellular pathology of aneurysmal thoracic aortic walls including images of stained diseased aortic wall sections, visualizing the intima, media, and adventitia, the reader may be referred to several specialized reviews [23, 81, 104].

Note that the sections below on animal models for FTAAD and AAA, respectively, frequently refer to specific pathological features of the aortic media caused by either mutations in genes that affect ECM remodeling, VSMC proliferation/metabolism and/or inflammatory cells of the media, or by drugs that act on these cells via specific genetic pathways.

How Comparison of Animal Models of FTAAD with Those of Sporadic TAAD Helps Elucidate the Molecular Pathology of Sporadic TAAD

Animal models have been instrumental in the study of syndromic and non-syndromic FTAAD ever since genetically engineered mutations in the FBN1 gene were found to cause Marfan syndrome in mice (see above). Since then, almost everyone of the more than 20 genes subsequently shown to be mutated in FTAAD patients was also shown to cause TAAD in animal models, almost always mice, because of the relative ease of genetic engineering of these animals, the lower cost of animal handling, and the speed of breeding. The information from these genetic models has been useful for establishment of candidate drug targets that repress progression of TAA. Both beta-blockers and angiotensin receptor blockers (losartan) have first been proven to prevent establishment and/or progression of TAA in mice with FTAAD, and this has directly led to the – albeit modest – success of these drugs in clinical trials and in clinical practice. Several of the relevant animal models for MFS, LDS, vEDS, and ATS have already been discussed in the sections for these conditions above and will not be discussed here further, except if there are potential functional overlaps.

However two new animal models of sporadic TAAD, caused by non-genetic factors such as age, diet, smoking and others, are described below. These models would not have been possible without prior knowledge obtained from animal models of FTAAD as they recapitulate some of the molecular pathology found in FTAAD. These animal models of sporadic FTAAD are of potentially high significance as they connect findings from the study of FTAAD with the much more prevalent sporadic TAAD.

High-Fat Diet-Induced Sporadic TAAD Animal Model Involving Inflammation and the VSMC Contractile Apparatus

Interestingly, aortas from patients with sporadic TAAD (not caused by the above familial mutations but either by envi-

ronmental factors or by other yet uncharacterized mutations) show significant degeneration of contractile proteins, including MYH11, in the ascending aorta and to lesser degree also in the descending aorta [105].This suggests that degeneration of contractile proteins such as MYH11 in VSMC of the aorta of sporadic TAA patients may be an important disease causing characteristic even in the absence of a clear-cut genetic cause. Importantly, this observation links FTAAD caused by mutations in MYH11 (see above) with non-genetic TAA.

The authors further show that palmitic acid, used to simulate high-fat diet, induces caspase 1- and NLRP3-mediated inflammatory action in VSMC from TAA patient aorta removed for prophylactic surgery [105]. This was confirmed in mice with inactivating mutations in either caspase 1 or NLRP3, which resulted in reduced MYH11 degradation and attenuation of TAA generation after angiotensin challenge [105]. Finally, this study shows that TAA generation is reduced in AngII challenged in mice if anti-inflammatory glyburide is administered.

This study connects high-fat diet that causes a specific form of inflammation in the aortic media with degradation of the cytoskeleton in VSMC of the media and, thus, with the occurrence of sporadic TAA. Since VSMCs cannot generate force without connections to the extracellular matrix through focal adhesions [87], and mutations in the extracellular matrix component fibrillin 1, which links VSMC to the elastin/collagen fibrils in the matrix, also cause thoracic aortic disease (MFS) as discussed above, it is possible that disruption of the ability of the aortic VSMC to generate force through the elastin-contractile units in response to pulsatile blood flow may be a primary cause for both inherited and non-inherited thoracic aortic aneurysms and dissections.

In summary, this work is a prime example of how the study of genetic disease (FTAAD) can help elucidate the molecular mechanism of its non-genetic counterpart (sporadic TAAD) and how it can potentially assist in the development of drugs for the treatment of the non-genetic disease, by far the most frequent form of TAA.

Sporadic TAA Animal Model of NAD+ Signaling

A recent animal model of spontaneous TAA suggests that the healthy aortic media depends on an intrinsic NAMPT-NAD^+ fueling system which is necessary for ATP production, to protect against DNA damage and premature SMC senescence. In mice with NAMPT-deficient VSMC, NAD+ levels are reduced, and aortas dilate and become prone to aneurysm and rupture when challenged with angiotensin II [106]. This corresponds with the reduced levels of NAMPT found in dilated, aneurysmal aortic tissue of sporadic TAA patients [106]. VSMC in diseased aortas were not apoptotic but showed signs of senescence, including DNA double strand breaks.

The Present Pharmacological and Surgical Management of Syndromic and Non-syndromic FTAAD

While it is tempting to draw from gene defects conclusions on pathogenic pathways that are amenable to pharmacological therapy, more preclinical studies and clinical trials that test novel drug candidates will be necessary before true progress is made on drug-mediated prevention of aneurysm progression and rupture. Beta-blocker therapy is at this time still the initial medication for management of aortic aneurysm in all syndromic and non-syndromic FTAADs. Losartan may be added if beta-blocker monotherapy is not effective. Losartan may also be administered on its own if patients do not tolerate beta-blockers [107].

In contrast to prophylactic medication, the precise timing of prophylactic aortic surgery strongly depends on the diagnosis of a specific aortic disease and the availability of patient-specific genetic information, because specific FTAADs influence the choice of the surgical procedure. Major recommendations based on the genetic subtype of FTAADs are that elective surgery and invasive angiography should be avoided in vEDS and that stent graft prostheses should not be placed in native aortic tissue in MFS or LDS [107]. Further, the recommendations for the extent, type, and timing of diagnostic imaging reflect the different patterns of aortic, vascular, and systemic manifestations related to the different syndromic and non-syndromic FTAADs [107]. Additionally, there are different recommendations on the timing of elective surgery at smaller or larger aortic diameters based on the knowledge that different FTAADs, based on different genetic defects, vary in their risk for dissection or rupture [107].

The Increasing Role of Medical Genetics in the Clinical Practice of FTAADs

The explosion of knowledge regarding the genetic basis of FTAADs starting in the 1990s – and especially in the past ~5 years – has profoundly changed diagnosis and clinical treatment of these conditions, and the rapid current progress will continuously lead to further refinement of diagnosis and treatment.

Initially genetic associations with FTAADs were discovered exclusively by painstaking traditional genetic linkage analysis consisting of slowly narrowing down the approximate chromosomal location of the inherited mutation conferring a specific FTAAD in more and more affected families, requiring long-term approach and refined cytogenetic capabilities. This was followed by DNA sequencing of all the genes located in the identified broad chromosomal location that usually resulted in the discovery of a single gene whose

mutations follow Mendelian inheritance in a given affected FTAAD family, as expected from single gene disorders.

Whole exome sequencing (WES) has recently replaced much of traditional genetic linkage analysis, and a dramatic example of its success is the multiple recently identified genes that cause FTAAD, including ACTA2, MYLK, LOX, and FOXE3. Indeed, WES is on a path toward implementation in clinical practice, due to dramatically lower costs of high throughput sequencing caused by key technological advances [108–110]. The unique advantages of WES in the clinical setting are similar compared to the more comprehensive (and much more expensive) GWAS studies used in some clinical trials (see section below for GWAS studies of AAA). WES employs high throughput (HTP) DNA sequencing to simultaneously identify any type of mutation in the DNA of all ~20,000 known genes (defined as all DNA sequences that code for all-known proteins) without any preconceived ideas about which genes would be most important, and currently costs about $1000–2000 per sample.

While the human DNA coding for all the known proteins, which are by definition located in the exons, only represents about 2–3% of total human DNA, mutations in exons are especially important for FTAADs because, so far at least, FTAADs have been shown to be caused by mutations located inside these exons, directly affecting protein expression of the genes. In contrast, for AAA almost all relevant mutations are SNPs that are located in intergenic regions (introns) and not in exons, a common property of mutations that are inherited not in strictly Mendelian fashion but are predisposing for a disease in concert with non-genetic factors, such as lifestyle, age, diet, smoking, and others (see below).

One recent example for routine clinical application of WES in FTAADs was established by the Elefteriades group in 2015 at the Yale Aortic Institute who subjected 102 new and returning TAAD patients to WES [108]. The findings confirmed the presence of already known FTAAD causing mutations in many of the more than 20 genes known to be functionally associated with FTAAD (see also above). Such mutations were found in more than 20% of the patients – the rest of the patients did not seem to carry known medically important genetic alterations based on current knowledge. Importantly, in addition to known FTAAD mutations, 22 not previously reported types of mutations, mostly novel types of amino acid changes, were found in the FTAAD-associated genes described in the previous paragraphs, although their potential role in the FTAAD should await further study, especially in animal models.

The most immediate clinical benefit of WES is that FTAAD patients can be given personalized treatment depending on the gene and type of mutation causing FTAAD. Specifically, for patients with mutations prone to dissect without prior severe aneurysmal dilatation, which are mutations in the ACTA2 (FTAAD type 6), MYLK (FTAAD type 7), TGFBR1 (LDS), TGFBR2 (LDS), SMAD3(LDS), TGFB2 (LDS), and TGFB3 (LDS) genes, a policy of more frequent imaging and earlier prophylactic surgery (at lower aortic diameter) should be applied [108].

Toward Diagnosis of FTAAD via Blood Test

Since more than 90% of TAAs are asymptomatic before dissection or rupture occurs, a biomarker that could detect and monitor the progress of an early, small aneurysm would be extremely useful, especially for individuals with family members that were affected by FTAAD and that therefore are at very high risk of developing TAA.

Many potential biomarkers have been investigated for their utility in diagnosing and/or monitoring TAAs, and some are promising. None, however, are ready to be reliably used in the clinical setting [111]. D-dimer has perhaps been the most studied potential biomarker of TAAs. It is a by-product of fibrin degradation that has been shown to be up to 99% sensitive in detecting acute aortic dissections. However, the specificity of D-dimer as a test for acute aortic dissection is relatively low, as D-dimer is elevated in a number of other conditions, including pulmonary embolism and coronary thrombosis [111]. In addition, the abundance of ECM proteins such as MMPs or elastin fragments in blood may prove predictive of dangerous aortic dilatation and TAA. Immune components such as C-reactive protein and some interleukins are also being tested as biomarkers for TAA [112].

In the future, ribonucleic acid signature sequences may be reliable biomarkers of TAAs. A study looking at 33,000 mRNA expression patterns in the blood of TAA patients and comparing them with those of control patients without TAAs showed that measuring the mRNA expression of a panel of 41 genes could distinguish, with about 80% accuracy, between patients with and without TAAs based on a blood test alone [51]. In addition, microRNAs (miRNAs), short RNA molecules that function in the regulation of hundreds of genes, have recently been shown to be involved in aortic dissections.

Toward a Pharmacological Cure of FTAAD

Further delineation of the pathological pathways in inherited TAA will be needed to narrow down potential therapeutic targets. This will require more refined preclinical mouse studies testing promising drug candidates, especially those types of models that test specifically aneurysm or dissection progression rather than generation. Interesting data from a powerful recent meta-analysis of inherited AAA studies [11] (see section below for details) may also be important for

FTAAD: MMP9 was very strongly implicated in pathogenesis of AAA and appears to be an attractive drug target, and such drugs (doxycycline) already have been tested in clinical trials. Remarkably, MMP9 has been shown to be overexpressed in the media of aortas of FTAAD patients as well, although MMP9 has not been directly genetically linked to FTAAD. Further, some authors believe that MMP9 and other metalloproteinases could be promising drug targets not only for inherited FTAAD and inherited AAA but also for the much more prevalent non-inherited forms [113]. Clearly the coming years will be exciting as we may be on the cusp of defining the best drug targets for both TAA and AAA, a potentially unprecedented and life-changing advance.

Gene and Cell Therapy of TAA

Due to recent dramatic advances in gene therapy methods, direct correction of the inherited genetic defect specifically in the aortic wall may be possible within the next 10–20 years. Importantly, TAA caused by mutations in certain affected genes that are not believed to be good drug targets can possibly best be ameliorated by gene therapy. These mutations include amino acid changes in transcription factors, such as SMAD6 and FOXE3, as well as in the cytoskeletal proteins ACTA2, MYH11, and MYLK. Over the past 15 years, several different approaches of cell therapy have been used in cardiovascular disease, especially to target ischemia of the heart, but the clinical trials showed limited success so far. However improved existing and novel methods are emerging, and these should also be applicable for aortic diseases. In addition to choosing the correct cell type to repair a genetic defect, such as autologous genetically repaired vascular endothelial cells, vascular smooth muscle cells, mesenchymal stem cells, and/or macrophages, the delivery method is crucial and is often the most challenging aspect: a recent review of the use of stem cells in cardiac regeneration describes several advanced examples of using magnetic cell targeting, as well as ultrasound-mediated delivery of stem cells [114]. Such procedures could also be done for the aorta at an early stage of the disease, and if engraftment is successful and long term, they could obviate the need of life-long drug treatment or invasive and risky surgery of the patient.

Given the rapid pace of investigation in the nonsurgical treatment of TAA, it is probably only a question of time until gene/cell therapies, perhaps combined with novel drugs, are discovered that truly prevent progression of existing TAA in patients in the long term.

Once this happens, the need for surgery to treat TAA, which is associated with significant perioperative complications, will be significantly reduced.

Genetics of Abdominal Aortic Aneurysms (AAAs)

The field of AAA genetics has been rapidly progressing in the past several years, due to increasing use of modern high throughput DNA sequencing technology that enabled genome-wide association studies (GWAS). Indeed in the past several years, more than 100 independent AAA-centered genetic studies, including GWAS studies, have revealed more than 100 specific novel genetic loci that may be associated with AAA. However, only 5 of these original >100 AAA-associated genetic loci have been clearly confirmed by 2 recent large-scale meta-analyses [11, 115] that attempted to combine the data from these earlier studies. Intriguingly, one of these two meta-studies, which is the largest one published so far [11] (4972 AAA cases and 99,858 controls), also discovered four novel AAA-associated loci with very high certainty, due to its unprecedented statistical power. Indeed, this study is an impressive testament to successful international collaboration in AAA genetics. In the interest of brevity and focus, only the genetic loci supported this study [11] will be described in detail below – with the caveat that some of the previously described loci that were not confirmed may still have functional significance in genetic subpopulations.

Since the 1970s, it has been known that an important risk factor for AAA formation is a positive family history for the disease with an increased individual risk between two- and 11-fold [116–120]. It is estimated that at least 15% of all cases of AAA are in part caused by one or more inherited mutations [117]. Since AAA in general (non-inherited plus inherited) affects ~8% of males and ~1% of women over the age of 65 [2, 120, 121], genetically conditioned AAA represents about 1–2% of all people over the age of 65, a significant part of the population.

Currently, the diagnosis and management of AAA is challenging: aneurysm development and progression are mostly asymptomatic, and diagnosis is often accidental, during imaging of other medical indications. Once diagnosed there are no blood tests for monitoring aneurysm growth. Instead monitoring is done by repeated imaging until the aortic diameter approaches about 55 mm, when usually surgical intervention via open repair or endovascular stenting is performed [122]. Unfortunately open surgery is associated with significant perioperative mortality, and endovascular stenting fails in up to 20% of patients, thus requiring re-stenting for this group [122]. One typical complication is bacterial infection that can lead to septic shock if untreated [123]. Early detection is very important for success exactly as for stenting in coronary artery bypass surgery [124]. Imaging, surgical repair, and perioperative care cost at least US$20,000 per patient [122]. If no alternative treatments are found, requirement for AAA-related surgery will rise in parallel

with the global ageing population. Given these high costs, there is an urgent need for improved diagnosis, monitoring, and treatment of AAA. Optimally AAA expansion should be stopped at an early stage in order to prevent rupture completely, thus converting it from a condition treated exclusively by surgery to one mostly managed by drugs and/or cell/gene therapy.

Indeed, several novel animal models of AAA have begun validation of new therapeutic targets in vivo, and this has led to multiple recent clinical trials involving a few novel drugs. These animal models are based on the novel genetic AAA-associated loci found through genetic linkage and GWAS studies.

Most inherited AAAs are of multifactorial origin with genetic mutations as well as lifestyle choices such as diet and exercise contributing to the disease, in striking contrast to familial thoracic abdominal aneurysms (FTAADs) which show autosomal dominant inheritance with high penetrance regardless of other factors (see also below). The most important non-genetic risk factors for AAA are advanced age, male gender, cigarette smoking, hypertension, and cardiovascular risk factors, such as dyslipidemia [1].

Interestingly, some of the known AAA-predisposing mutations functionally contribute to dyslipidemia and cardiovascular disease: the best examples may be mutations affecting the expression of the SORT1 gene and to a lesser degree, the LDLR gene. However, it should be emphasized that many other AAA-predisposing mutations are not directly associated with dyslipidemia or cardiovascular disease. Indeed, several AAA-associated mutations act via remarkably diverse mechanisms that affect the immune system, the angiotensin system, extracellular matrix remodeling, and VSMC proliferation. Both the structure and function of the well-known AAA-predisposing loci have been under intense investigation. Enough functional data seem now available for several of these loci to allow the design and testing of novel drugs that potentially could prevent enlargement and rupture of existing AAA [1, 2].

Subtypes of Genetically Conditioned and/or Inherited AAA

Genetic subtypes of AAA are listed in Tables 5.1 and 5.2, as well as in more detail below, according to their associated genetic locus and mode of function, as published in [11]. This meta-study includes most previous original studies and analyzed an unprecedented 4972 AAA cases and 99,858 controls, about twice as many as the previously biggest meta-analysis [10]. Its only major bias is that many more men than women were analyzed, for the simple reason that men suffer from AAA at ~5 times higher rate than women and are thus much more easily available for screening. Surprisingly, this study confirmed only 5 of >100 previously published specific SNPs. These five SNPs have been confirmed with very high certainty, and they are located in five different genetic loci: 9p21 (CDKN2BAS/ANRIL), IL6R, SORT1, DAB2IP, and LDLR. In addition, four previously unknown SNPs in four new loci were also found by [11] to strongly associate with AAA, and these are the SMYD2, LINC00540, MMP9, and ERG loci. As shown in Tables 5.1 and 5.2, all nine strongly AAA-associated loci fall into two main subtypes, first those known to contribute to dyslipidemia and cardiovascular disease (LDLR, SORT1) and second, those not associated with dyslipidemia (IL6R, 9p21, SMYD2, LINC00540, MMP9, ERG). Confirming absence of genetic association with dyslipidemia for the second group, neither the patients nor the corresponding animal models, if available, exhibit obligate dyslipidemia or cardiovascular disease. Intense efforts are currently underway to develop novel anti-AAA drugs based on the suspected molecular mechanisms of AAA pathology of all of the proven AAA-associated loci.

Table 5.2 The nine loci strongly associated with AAA according to Jones et al. 2017 [11]

Gene locus	SNP	Major/ minor allele	Proposed primary pathogenic mechanism
LDLR	rs6511720	G* > T	Lipid metabolism
PSRC1- CELSR2-SORT1	rs602633	G* > T	Lipid metabolism
IL6R	rs4129267	C* > T	Immune response
CDKN2BAS1/ ANRIL	rs10757274	G* > A	VSMC proliferation
DAB2IP	rs10985349	C > T*	VSMC signaling and immune response
ERG	rs2836411	C > T*	Endothelial cell homeostasis
SMYD2	rs1795061	C > T*	Unknown
LINC00540	rs9316871	G* > A	Unknown
MMP9-PCIF1-ZNF335	rs3827066	C > T*	Extracellular matrix remodeling

Of note, the most recent individual AAA GWAS study, which was conducted in Japan (456 patients with aortic aneurysm, 8326 control individuals), found that the *EGFLAM* and *SPATC1L* loci are significantly associated with true aortic aneurysm, and *RNASE13* was significantly associated with dissecting aortic aneurysm [125]. What is unusual about these loci is that all three are mutated within protein-coding regions, which will strongly facilitate future functional analysis [125], in contrast to most other known AAA-associated loci which are mutated outside of protein-coding regions, making functional analysis more challenging. However, since these new protein mutations are not yet confirmed independently, they will not be further discussed here.

Since the above nine confirmed loci from the largest meta-analysis to date are contributing factors and usually not the sole factor causing AAA, it seems prudent to list the associated-AAA phenotypes not as separate diseases but as genetic subtypes of AAA. Remarkably, most of these loci

Table 5.3 The most important mouse models of AAA

AAA mouse model	Mode of AAA induction	Pathological pathway causing AAA	Human-like aspects of AAA	Disadvantages	Ref
Hph1 −/− mice deficient in the protein GTPCHI which produces tetrahydrobiopterin (BH4) a cofactor of NADPH oxidase	AngII infusion by pump for 4–6 weeks	Perturbation of endothelial nitric oxide synthase (eNOS) signaling in the aorta as well as angiotensin renin system	Leads to aneurysm formation, progression, and rupture	Unclear if the eNOS pathway acts the same way in humans. No clear genetic association of Hph1 or eNOS signaling in humans	[161]
ApoE1−/−	AngII infusion by pump for 4–6 weeks induces supra renal AAA which progresses continuously as long as AngII is infused	Perturbation of angiotensin renin system. Increased blood pressure. High-fat diet induces atherosclerosis and increases speed and frequency of AAA formation	Pathological pathways may be similar to humans. Shows also male gender bias found in humans. Focal aneurysm. Features inflammation and extracellular matrix remodeling found also in humans.	Affects suprarenal aorta not infrarenal as found in humans. No strong genetic association of human AAA with Angiotensin/renin system	[162–166]
LDLR−/−	AngII infusion by pump for 4–6 weeks	Perturbation of angiotensin renin system. Similar to ApoE1−/− mice above	See ApoE1−/− mice above	See ApoE−/− mice above	[134, 164, 166]
Apo E $^{-/-}$ TIMP-1 $^{-/-}$	30 days of cholesterol-rich diet	MMP are activated due to absence of TIMP-1 (tissue inhibitor of metalloproteinase-1	Slow induction of AAA, partly diet mediated	TIMP1 mutations not known to cause AAA in humans	[167] 2002, not repeated since
Normal mice (129/sv)	Elastase perfusion of abdominal aorta over 5 min, following surgical preparation leads to immediate AAA formation	Degrades aortic media by degradation of elastin, the major protein component of the extracellular matrix of the media. This induces chronic inflammation and infiltration of MMP9 producing macrophages over the next months	Elastin treatment in the aortic media induces MMP9 action, which is similar in human AAA: MMP9 misregulation found in AAA patients. MMP9 mutations strongly associated with human AAA	Procedure requires surgery on the aorta. Mostly an acute AAA model as most AAA resolve gradually over several weeks, while human AAA is chronic	[165, 168]
C57BL6 mice	BAPN (a lysyl oxidase inhibitor) was provided in drinking water 2 d before periaortic elastase application	Persistent irreversible AAA formation, thrombus formation, and spontaneous rupture more than 100d after elastase induction	Only model for chronic late stage aneurysm development, which is the most relevant human AAA condition. Most ruptures happen during late stage as for humans	BAPN amount needs to be balanced very carefully to prevent early AAA dissection	[169]

have been experimentally confirmed in animal models to strongly contribute to formation and progression of AAA (see Table 5.3 and detailed descriptions of individual loci in the text below). Some, but not all, of these loci are already assigned in the OMIM (Online Mendelian Inheritance in Man) database as familial abdominal aortic aneurysms type 1, 2, 3, or 4. However at this time, we find it more prudent to simply list them with their associated gene names according to their known metabolic function. See also Tables 5.1 and 5.2.

AAA-Associated Loci Affecting Lipid Metabolism

We list here only the lipid metabolism-associated loci clearly confirmed by [11] although other such loci may still be relevant for certain subpopulations not captured by this large meta-study.

LDLR *(LDL Receptor)* G* > T (rs6511720)

This single SNP G* > T (rs6511720) is one of many known mutations in the LDLR locus but was so far the only one found in GWAS studies to be strongly associated with AAA. In contrast to most other known LDLR mutations, it is not located in the protein-coding region but in the first intron of the LDLR gene and overlaps with a potential enhancer site that could regulate LDLR gene expression [126]. In contrast to many other mutations found in the LDLR locus, rs 6511720 may increase expression of the LDLR protein: it does so at least in human liver-derived cell lines and would thus potentially lead to lower, potentially cardioprotective LDL-C levels [126]. However, based on these data, it is also tempting to speculate that rs6511720 causes higher levels of the LDLR protein in macrophages that infiltrate aneurysmal

aortic walls. This would potentially lead to higher fat accumulation in the macrophages (generation of "foam cells") which in turn would lead to inflammation in the arterial wall and ultimately to AAA formation.

Consistent with the above, SNP rs6511720 has not been clearly associated with coronary artery disease and atherosclerotic plaque formation [127, 128] in striking contrast to many other SNPs in the LDLR locus which cause a reduction of LDLR activity, especially those causing familial hypercholesterolemia [127–129] (see also below).

The above strongly suggests that rs6511720 acts through a fundamentally different pathological mechanism than the many other SNPs found in the LDLR gene and that causes cardiovascular disease but no increased prevalence of AAA. The exact pathological mechanism that causes this SNP to promote AAA formation is not yet fully understood and requires significant additional research.

To begin to understand the role of rs6511720 in AAA, it is useful to consider the known LDLR function: LDLR mediates endocytosis of cholesterol-rich LDL particles from plasma and is necessary for maintenance of the optimal plasma level of LDL. LDL endocytosis occurs in many cell types including hepatocytes in the liver and macrophages that can infiltrate the aortic wall. The hepatocytes of the liver remove ~70% of LDL from the circulation. Likewise, the macrophages in the aortic wall which infiltrate during AAA formation can take up LDL from the blood using the LDLR and other receptors. LDL particles are internalized via LDLR-rich clathrin-coated pits. After internalization, the receptors dissociate from their ligands when they are exposed to lower pH in endosomes. After dissociation, the receptor recycles to the cell surface [130]. The rapid recycling of LDL receptors provides an efficient mechanism for delivery of cholesterol to cells [131, 132]. The crucial role of LDL and LDLR in maintenance of plasma lipid levels was originally discovered by Goldstein and Brown using cell culture of fibroblasts derived from patients of familial homozygous hypercholesterolemia (hoFH, see below) which lack functional LDLR [131]. It was confirmed in genetically engineered LDLR−/− mouse models (see below) and therefore immediately suggested a causal role of high plasma LDL particle levels for development of atherosclerosis.

Inactivating mutations in the LDLR locus cause familial hypercholesterolemia (FH), one of the most prevalent Mendelian disorders [129]. Interestingly, these mutations are not associated with a higher occurrence of AAA [127, 128]. These mutations usually, but not always, occur in the coding region of the very large LDLR gene, and more than 1000 different mutations have been found to cause FH. Heterozygotic FH can usually be managed by lipid lowering drugs, but the homozygotic form (hoFH) is severe and causes premature death through cardiovascular disease, and there is no satisfactory treatment available [129].

FH-like symptoms can be reproduced well in LDLR KO mice, which is very helpful for development of therapies. They include severe atherosclerosis, steatohepatitis, and high frequency of cardiovascular disease. However, in contrast to human FH patients, LDLR−/− mice also show significantly increased frequency of AAA. However, this increased AAA formation in LDLR−/− mice is conditional on angiotensin II treatment [133]. This potential difference of AAA formation frequency in LDLR−/− mice and FH patients is subject to research.

The LDLR−/− angiotensin II mouse is one of the most used and best characterized AAA animal models [134]. This model also shares many features with human AAA and has become one of the most commonly used AAA animal models. However, this mouse model does not fully recapitulate human AAA pathogenesis, in particular as homocygotic FH (hoFH) patients, all of whom are LDLR−/−, do not seem to show increased AAA occurrence.

LDLR gene therapy for hoFH patients is being performed currently only in clinical trials and attempts to deliver the normal LDLR gene to the liver – this is thought to be the only way towards a true cure of hoFH. However, this approach likely will not cure rs6511720-induced AAA as this LDLR mutation may act primarily through the macrophages in the aortic wall, as discussed above. Therefore, LDLR gene therapy for AAA may have to repair the genetic defect in macrophages instead of in the hepatocytes of the liver, an approach that seems feasible in principle (see also below).

PSRC1- CELSR2 - SORT1 G* > T(rs 602633)

The non-coding SNP G* > T (rs 602633) is one of the most strongly AAA-associated mutations [10, 11, 135] and at the same time is associated with hyperglyceridemia and coronary artery disease [136–138]. It is located close to the three genes PSRC1, CELSR2, and SORT1. Of these three, the SORT1 gene is by far the most well understood, and it has been known since 2010 that its biological expression level strongly influences risk of cardiovascular disease [139]. Interestingly, another SORT1 genetic variation at SNP rs12740374 seems to significantly mediate the variation in SORT1 gene expression and also SORT1-mediated risk of cardiovascular disease found in humans but so far has not been associated with risk of AAA [139].

In mice, macrophage SORT1 deficiency protects against atherosclerosis by reducing macrophage uptake of LDL, through lowering LDLR expression [140]. Since LDLR in the outer cell wall of macrophages is necessary for efficient uptake of LDL into macrophages, it is tempting to speculate that rs602633-mediated normal or high macrophage LDLR levels may not only lead to atherosclerosis but also to AAA, through conversion of macrophages into foam cells in the aortic wall [140].

Although much has been learned about the role of SORT1 in lipid metabolism and atherosclerosis, there is no associated SORT1 animal model of AAA. Since SNP rs 602633 is very strongly associated with AAA and also with coronary artery disease [25] and SNP rs 12740374 is strongly associated with CAD but not at all with AAA, these two SNPs, which are both located close to the SORT1 PSRC1 and CELSR2genes, clearly work through distinct mechanisms.

The clearly different disease associations of the two SNPs provide strong evidence that AAA and CAD pathologies act at least in part through separate mechanisms in humans and strongly caution against simplified assumptions that anti atherosclerotic drugs like statins would automatically be the best therapeutics for AAA although statins do seem to be somewhat beneficial. Given the extremely strong correlation of rs 602633 with human AAA, careful functional studies of the role of all three genes PSRC-CELSR2-SORT1 in AAA are well justified and likely to significantly improve our understanding of human AAA pathology.

AAA-Associated Loci Affecting the Inflammatory Response

Interleukin 6 Receptor (IL6R) C* > T(rs 4129267)

IL6R is a receptor for the pro-inflammatory cytokine interleukin 6. SNP rs 4129267 is found to be strongly associated with AAA [11] and is located close to the exon regions of the IL6R gene – but not within exons – and thus its precise function cannot be easily studied in animals. Instead current animal models study IL6R over expression or deficiency, and especially interleukin 6 (IL6, the ligand of IL6R) over expression or deficiency (see below). SNP rs 4129267 is not only strongly associated with AAA but is closely associated with occurrence of SNP rs 7529229 which causes an Asp358Ala change in IL6R [141]. In human lymphoblastoid cell lines, this Asp358Ala mutation in IL6R caused a reduction in the expression of downstream targets (STAT3, MYC, and ICAM1) in response to IL-6 stimulation [141]. Indeed, two remarkable animal models have already shown that IL6R signaling strongly influences the risk of rupture of AAA, but not de novo generation of AAA, and therefore these models may be highly relevant for risk of rupture of preexisting human AAA.

The first animal model of the role of IL6 signaling in AAA [142] is not an IL6R −/− mouse, but it is a mouse with inactive IL6, the ligand of IL6R, with expected similar phenotypes. Importantly, IL6 signaling in this model does not affect AAA generation per se: AAA generation is triggered by angiotensin II administration given to lysyl oxidase inhibitor-preconditioned mice, which stimulates CXCL1/granulocyte colony-stimulating factor expression and very rapid abdominal aortic dissection of AAA and AAD generation within 24 hours, in both IL6+/+ and IL6−/− mice at the same frequency and intensity: the dissections were initiated at the proximal site of the descending thoracic aorta and propagated distally into an abdominal site. Remarkably, only in IL6+/+ mice dissection of the aorta caused dilatation, and ~70% of the IL6+/+ mice died of aortic rupture. Importantly, in IL6+/+ mice, the adventitia of the expanded dissected aorta demonstrated high levels of interleukin-6 (IL-6) expression. Neutrophils were the major sources of IL-6. Remarkably, in IL6 −/− mice, dilatation and rupture of the dissected aorta as well as death was strongly suppressed even though the presence or absence of IL6 did not influence the timing of emergence or the initial number of AAAs or neutrophil mobilization [142].

In summary, adventitial CXCL1/granulocyte colony-stimulating factor expression in response to AAD triggers local neutrophil recruitment and activation. This leads to adventitial inflammation via IL-6 and results in aortic expansion and rupture. This model could be highly relevant for human AAA, because it is prevention of progression of existing small AAA that requires treatment in humans and not prevention of AAA generation, mostly because there is no efficient method available allowing anticipation of AAA generation in humans before it occurs [142].

Another recent noteworthy animal model of AAA confirms that inflammatory cytokine production plays a major role in AAA in expansion and progression of existing small AAA [143]. In this case downregulation of the mTOR pathway by rapamycin dramatically limits the expansion of the abdominal aorta following intraluminal elastase perfusion. Furthermore, actual reduction of aortic diameter is achieved by inhibition of the mTOR pathway, which preserves and/or restores the contractile phenotype of VSMCs and downregulates macrophage infiltration, matrix metalloproteinase expression, and inflammatory cytokine production [143]. These data highlight the importance of preservation and/or restoration of the smooth muscle cell contractile phenotype and reduction of inflammation by mTOR inhibition in AAA.

In summary, the two above mouse models of AAA implicate pro-inflammatory cells as a major driver of rupture of preexisting AAA, and thus targeted anti-inflammatory drug treatment may be a promising strategy for clinical trials.

AAA-Associated Loci Possibly Affecting Cell Proliferation and/or the Inflammatory Response or of Unknown Function

***CDKN2BAS/ANRIL* (*Part of 9p21 Locus*)** G* > A(rs 10757274)

The **CDKN2BAS and ANRIL** genes are located within the 9p21 locus that has been implicated in cardiovascular disease by a seminal GWAS study in 2007 [144] whose main conclusions have indeed been largely confirmed by multiple

follow-up GWAS studies. This 9p21 region contains multiple SNPs that span an area of about 58kB that are among the strongest risk factors ever discovered for MI, cardiovascular disease, and AAA. Homozygotes for the risk allele were estimated to make up 20–25% of Caucasians and have an approximately 30–40% increased risk of CAD.

Importantly the SNPs in the 9p21 locus are highly disease specific. For example, according to the most powerful and most recent meta-analysis of GWAS studies on AAA in 2017 [11], only the 9p21 SNP rs10757274 but not any of the other several major 9p21 SNPs is very strongly associated with AAA, while the other SNPs are only associated with MI and cardiovascular disease. The underlying pathological molecular mechanism that is triggered by SNP rs10757274 and contributes to AAA and cardiovascular disease is under intense investigation and not yet well understood, in part because it is located in an intergenic region characterized only by multiple transcription factor binding sites as well as a large non-coding RNA called ANRIL [11]. Interestingly, directly adjacent to the 58kB span, a tumor suppressor gene named CDKN2B is present.

A seminal mouse study by Leeper et al. in 2013 [145] showed that absence of CDKN2B in all tissues causes an increased aortic diameter in the elastase-driven mouse model of AAA. They also elegantly showed by bone marrow transplantation experiments that absence of CDKN2B directly caused apoptosis in VSMC of the aorta via the p53 pathway and not primarily through a defect in infiltrating immune cells [145].

This study contrasted with a later study showing that in culture, aortic smooth muscle cells obtained from mice with a 70 kb deletion encompassing the location of the human SNP rs10757274 showed excessive proliferation (instead of apoptosis) and altered regulation of the neighboring CDKN2B gene [146].

A European case control study involving 4251 patients with coronary artery disease and 4443 controls [147] replicated association for 7 separate MI-associated SNPs in the above 58 kB region of chromosome 9p21, including rs 10757274 and 10757278. In addition this study found that the large non-coding RNA (ANRIL) co-locates with the high-risk haplotype of 9p21 and is expressed in tissues and cell types that are affected by atherosclerosis, including VSMC and HVECs.

Harismendy et al. [148] identified 33 potential enhancers (short DNA sequences that promote activation of specific genes from a distance of up to several kB) in the 9p21 locus; this enhancer-rich region features a six times denser occurrence of potential enhancer sequences than the whole genome (P less than 6.55 × 10(−33)). Using a new, open-ended approach to detect long-distance interactions, they found that in human vascular endothelial cells the enhancers located close to the CAD-associated sequences of the 9p21 locus physically interact with the neighboring CDKN2B gene, consistent with the above mouse studies implicating downregulation of CDKN2B in AAA [145].

Based on the above, it will be interesting to study if drugs that upregulate ANRIL and CDKN2B can help restore the vessel wall in animal models of AAA.

DAB2IP C > T*(rs 10985349)

In humans, DAB2IP (also named AIP1) mRNA is detected in most tissues and organs but is very low or absent in blood cells. In the aortic media, it is expressed in the VSMC and is an important regulator of signal transduction of cancer-related pathways. DAB2IP acts as an adaptor, or scaffold, in protein complexes relevant for signal transduction, and it can function as a competitor, or scavenger, by binding signaling proteins and preventing their interaction with upstream activators or downstream effectors. Through these actions, DAB2IP has the potential to modulate a remarkable array of cancer-related pathways.

DAB2IP (AIP1)-deficient mice showed no obvious developmental defects including vascular development. However, they exhibited dramatically enhanced angiogenesis in two models of inflammatory angiogenesis. In one of these models, the enhanced angiogenesis observed in the DABIP-deficient mice was associated with increased VEGF-VEGFR2 signaling [149]. Consistent with this, VEGF-induced ear, cornea, and retina neovascularization were greatly augmented in DABIP KO mice, and the enhanced retinal angiogenesis was markedly diminished by overexpression of DAB2IP [149].

In a syngeneic aortic transplantation mouse model [150] in which wild-type or DAB2IP-knockout mouse aortas were transplanted into IFNγ receptor-deficient recipients and in which neointima formation was induced by intravenous administration of an adenovirus that encoded a mouse IFN-γ transgene, donor grafts from DAB2IP-knockout mice enhanced IFN-γ-induced VSMC proliferation and neointima formation. Mechanistically, knockout or knockdown of DAB2IP in VSMCs significantly enhanced IFNγ-dependent VSMC migration and proliferation, a critical step in neointima formation. Thus, DAB2IP functions as a negative regulator in IFN-γ-induced intimal formation, in part by downregulating IFN-γ-JAK2-STAT1/3-dependent migratory and proliferative signaling in VSMCs.

Taken together, these two animal models suggest that removing DAB2IP in VSMC may increase VSMC signaling and inflammation and may induce aneurysm. Drugs that prevent AAA-specific inflammation in the aortic wall should be considered in additional preclinical studies.

ERG C > T* (rs 2836411)

The recent large AAA meta-studies [4, 11] and also an earlier study [150] strongly implicate mutations in the ERG

gene: the protein product of the ERG gene physically interacts with the proteins made by the IL6R, LDLR, and MMP9 genes, which are all genes strongly associated with AAA [11] (see also above). It is particularly intriguing that each of these genes was discovered by multiple independent GWAS studies.

ERG is a transcription factor and has emerged as a major regulator of endothelial function. Multiple studies have shown that ERG plays a crucial role in promoting angiogenesis and vascular stability during development and after birth [151]. In the mature vasculature, ERG also functions to maintain endothelial homeostasis, by transactivating genes involved in key endothelial functions, while repressing expression of pro-inflammatory genes. Its homeostatic role is lineage-specific, since ectopic expression of ERG in non-endothelial tissues such as prostate is detrimental and contributes to oncogenesis.

ERG is highly expressed in differentiated quiescent endothelial cells of the aorta and of all other arteries, as well of as veins and microvasculature, and has been shown to maintain the endothelium in an anti-inflammatory state by repressing expression of pro-inflammatory molecules such as vascular cell adhesion molecule (VCAM), plasminogen activator inhibitor (PAI)-1, and interleukin (IL)-8 [152, 153]. Consistent with this, endothelial ERG expression is down-regulated by inflammatory stimuli, including tumor necrosis factor (TNF)-α and lipopolysaccharide (LPS) [154, 155]. In addition, ERG expression was lost from the endothelium near the shoulder regions of human coronary plaques which contain inflammatory infiltrate [153].

In summary, ERG is anti-inflammatory and necessary for normal homeostasis of endothelial cells, and the AAA associated ERG mutation rs 2836411 may decrease ERG activity and increase inflammation of the aortic intima and media which in turn may contribute to AAA formation. Additional research, including animal models, is required to confirm this hypothesis.

SMYD2 *C > T**(rs 1795061)

The AAA meta-study by Jones et al. [11] for the first time provided evidence that SMYD2 may be associated with AAA pathology. SMYD2 is a histone methyltransferase and may be involved in regulation of gene expression of specific genes. However in the absence of functional data regarding the role of SMYD2 in AAA, it is also possible that two other genes located nearby the AAA-associated SNP (rs 1795061) are involved. However, among the three candidate genes located near rs 1795061, SMYD2 is the best characterized gene and could be connected to AAA via its ability to regulate methylation of the heat shock protein HSP90 [156] and the fact that inhibition of HSP90 reduces AAA formation in mice [157]. Interestingly, computational network analysis based on existing database data compiled and analyzed by the consensus PathDB program revealed that SMYD2 interacts functionally with LDLR via TNF. Clearly it is premature to reach conclusions on the functional effect of (rs 1795061) on AAA and much further research is needed to clarify.

LINC00540 *G* > A*(rs 9316871)

This non-coding RNA has no known function, but a SNP located close to it was discovered in the recent meta-study with very high probability to be associated with AAA in humans [11]. rs 9316871 is not only close to LINC00540 but also to the FGF9 gene which shows increased expression in human AAA tissue. GWAS3D and eQTL analysis indicates a function of LINC00540 with FGF9 [11]. GWAS3D and eQTL (expression quantitative trait loci) analysis is similar to the consensus path DB program (see above) and combines existing GWAS DNA data with existing gene expression profile data. They are a powerful unbiased computational tool allowing determination of probable functional associations of mutations with phenotype. However, animal models lacking or overexpressing LINC00540 are still needed to study the role of this non-coding RNA in AAA in vivo.

AAA-Associated Loci Affecting ECM Remodeling

MMP9 C > T* (rs 3827066)

In addition to being very strongly implicated in AAA by multiple GWAS studies [11], computational gene network analysis reveals a central role for MMP9 in AAA [11]. MMP9 is a matrix metalloproteinase that is highly expressed in VSMC of the aortic wall. Mechanical insults, inflammatory cytokines, and other factors that act on VSMC strongly upregulate MMP9 expression and excretion, leading to immediate remodeling of the media by locally dissolving elastic laminae and other structural components of the extracellular matrix. Abnormal MMP9 overexpression can strongly contribute to eventual rupture of preexisting AAA. Computational network analysis utilizing large existing databases containing experimental gene expression data of most known genes in most known tissues has recently been devised and is commercially available as online services. Two of the most powerful network analysis tools are IPA and Consensus PathDB. IPA network analysis (IPA = Ingenuity Pathway Analysis, Qiagen) compiles and evaluates data from omics experiments, such as RNA-seq, small RNA-seq, microarrays including miRNA and SNP, metabolomics, proteomics, and small-scale experiments. Similarly, Consensus PathDB consists of a comprehensive collection of human (as well as mouse and yeast) molecular interaction data integrated from 32 different public repositories and applies computational analysis to report interaction network modules, biochemical pathways, and

functional information that are significantly enriched by the user's input.

Both IPA and Consensus PathDB programs report direct physical contact between ERG, IL6R, LDLR, and MMP9 proteins, and Consensus PathDB reveals signaling without contact (e.g., phosphorylation) between SMYD2 and LDLR as well as SMYD and MMP9 [11]. These computed outputs which are based on a huge amount of independently collected publicly available experimental data are highly significant because they establish a novel gene network that, based on GWAS studies, is directly involved in AAA formation and progression with high certainty.

In addition, two powerful animal studies of AAA have been published, showing strong involvement of MMP9 in the long-term expansion of experimentally induced AAA. In particular, these models show that pharmacological inhibition of MMP9 in the aortic wall, using imidapril administration, prevents further dilatation of experimentally induced aneurysms in mice [158]. A second related study suggests that pharmacological inhibition of necroptosis, a process intrinsic to the vasculature, using administration of small molecule drug Necrostatin1-s, stabilizes preexisting aneurysms by diminishing inflammation and promoting connective tissue repair via reduction of MMP9 activity in the aorta [159].

MMP9 is multifunctional and plays also a role in brain inflammation through modulation of the blood-brain barrier. Its overexpression is also connected to progression of certain types of cancers. Because of its role in multiple important disease processes, several small molecule drugs have already been developed that are currently in ongoing clinical trials, including AAA trials.

Animal Models of AAA

In humans, true and dissecting aneurysms of the aorta develop as a result of progressive weakening of the vessel wall. This weakening of the vessel wall is associated with and likely caused by medial degeneration, which usually starts with degeneration and fragmentation of elastic fibers, infiltration of inflammatory immune cells, as well as weakening and/or loss of smooth muscle cells through apoptosis, and also increased production of reactive oxygen intermediates causing high lipid peroxidation and general oxidative stress. Persistent growth of AAA over the years, combined with accumulation of intraluminal thrombus and an increasing number of atherosclerotic plaques, frequently occurs in humans. However AAA progression can occur in the absence of atherosclerosis in patients and animal models, and most patients with extensive atherosclerosis in the aorta indeed never develop AAA. Both the inherited and non-inherited forms of aneurysm formation are therefore clearly functionally distinct from atherosclerosis.

Ideally, a model for AAA would mirror the pathology of human disease, permitting specific investigation into the mechanisms underpinning human aneurysms. To date, no model that features all important human AAA properties exists; however specific aspects of human AAA can be studied well in animal models. For example, the arterial morphology of pigs is similar to that of humans, the coagulation pathway of sheep is analogous to humans, and primate species have similar clotting and fibrinolytic systems [122]. However, despite these advantages of large animal species, the cost of purchasing and maintaining stocks and ethical considerations have limited their use in vascular research.

In contrast, mouse models are currently by far the most important models of AAA, primarily because of their ease of genetic manipulation combined with low cost of animal husbandry and short time span till fertility. In mice AAAs typically manifest within the suprarenal aorta, while in humans they primarily affect the infrarenal aorta [160], but this difference has not been enough to justify use of large animal models.

Many AAA mouse models have been studied in detail and have indeed revolutionized our understanding of AAA (see Table 5.3). No doubt, additional mouse models with refined, more human-like AAA features will further improve our understanding of AAA pathogenesis.

Cellular Pathology of the Abdominal Aorta

In surgical pathology studies of aortas resected primarily for aneurysms, ruptured aneurysms, and dissections, the frequency and severity of atherosclerosis is much greater in abdominal aortic segments than in thoracic aortic segments [23, 170]. In adults, prominent atherosclerosis typically involves less than 10% of resected thoracic aortic segments but usually more than 80% of resected abdominal aortic segments [23, 170]. This is consistent with the finding that mutations in the LDLR and SORT1 genes are strongly associated with both atherosclerosis and AAA formation/progression, but not with TAA formation, as mentioned above. Complications of abdominal aortic atherosclerosis that may prompt surgical correction include aneurysm formation, aneurysm rupture, occlusive aortic thrombosis, fistula formation, infection, penetrating ulcer with dissection, and distal embolization of thrombus and/or plaque material.

Innate and adaptive immune cells, including neutrophils, macrophages, mast cells, natural killer cells, dendritic cells, B cells, and several types of T cells, have been identified in abdominal aortic aneurysms and have been shown to contribute to AAA development, as described in detail in [171]. Preceding aortic wall dilatation, these immune cells often enter the aorta from the microvessels of the adventitia, causing strong adventitial inflammation, and from there migrate

into the media and cause inflammation as well as breakdown of the elastin fibers through MMP9 expression by macrophages, which is strongly genetically implicated in AAA, as described above. Distinct monocyte and macrophage subsets have critical and differential roles in initiation, progression, and healing of the abdominal aortic aneurysmal process as reviewed in detail by Raffort et al. [172].

Monocytes and macrophages can also enter through lesions in the vascular endothelial cells layer of the intima and together with the vascular endothelial cells are responsible for uptake of LDL particles from the blood via the LDL receptor [173], and the gene coding for LDLR is indeed genetically strongly implicated in AAA (see above). LDL uptake often leads to foam cell formation, an important first step toward atherosclerosis that starts in the intima. It has been hypothesized that intimal plaque formation causes a compensatory inflammation of the adventitia, thus eventually causing AAA. The presence of inflammatory cells and their associated cytokines and proteases in the adventitia and media may protect from arterial narrowing by promoting outward remodeling [171, 174].

However, there is strong epidemiologic evidence against a strict causal relationship between atherosclerosis and AAA formation: it is firmly established by multiple meta-studies that diabetic patients have a significantly lower rate of AAA than the normal population, although diabetic patients show a tenfold higher propensity to atherosclerosis than the normal population [66].

Toward a Pharmacological Cure of AAA

Pharmacological treatment for AAA has been standard for many years although it only has served to slow down enlargement and rupture, not prevent it. Consistent with the strong genetic correlation of AAA with mutations affecting genes that regulate lipid metabolism (LDLR, SORT1), statins, and other blood lipid-lowering drugs as well as blood pressure-lowering drugs such as beta-blockers are often being used on diagnosed AAA patients in the hope to reduce AAA growth.

Initial clinical trials using statins to reduce dyslipidemia and atherosclerosis or beta-blockers to reduce mechanical stress did not find slowing of abdominal aortic diameter growth below ~1.5 mm per year (assuming a typical growth rate of 1.5–2.5 mm per year) [175]. More recently several clinical studies [176–180] show a significant benefit of statins, but unfortunately others do not, even though the number of enrolled subjects has been increasing to several thousand in several of these studies [181]. Of note, statins and beta-blockers are often used together, even in clinical trials, and therefore the isolated effect of these drugs is often difficult to determine.

In many countries beta-blockers and statins have become a routine treatment for AAA patients awaiting surgery. In addition, losartan, an angiotensin receptor-blocking drug that binds the AT1 receptor and also reduces blood pressure, is being used, especially when beta-blockers show insufficient results. Other completed clinical trials used antihypertensive or anti-inflammatory drugs such as perindopril, pemirolast, propranolol, and amlodipine but showed no slowing of AAA diameter growth [182].

Fortunately, population-wide AAA frequencies have been reduced somewhat in several western countries, most likely due to better diagnosis and monitoring of AAA, due to improved intraluminal stenting procedures, and perhaps also due to drug treatment [179, 180].

As mentioned above, extensive meta-analyses of recent clinical AAA trials reveal mixed results for existing AAA drugs – some seem to work better for one genetic type of AAA than for another, or there are other unexplained factors that cause often contradictory data. Therefore there is an urgent need for novel types of drugs that prevent AAA enlargement and rupture reliably and completely. At this time, the only effective treatment to prevent sudden rupture of AAA remains open surgery or catheter-based insertion of stents, as reviewed in Chaps. 19 and 22 of this book.

Fortunately, due to the recent large-scale genome-wide association studies (GWAS) of AAA that we have discussed above, many fundamentally novel animal models of AAA were published in the past ~4 years, and this has led the pharmaceutical industry to develop several new AAA drug candidates that have either already entered clinical trials or will shortly do so. These novel drugs target not only AAA-associated genetic loci involved in dyslipidemia and atherosclerosis (LDLR, SORT1) but also AAA loci that are known or suspected to affect the immune system (IL6R, ERG), extracellular matrix remodeling (MMP9), and VSMC proliferation or homeostasis (9p21/CDKN2BAS, ERG, DAB2IP). Indeed, the animal studies described above and in Tables 5.3 and 5.4 in detail show that IL6R, MMP9, and 9p21 (CDKN2BAS) can contribute to AAA generation via a different mechanism than LDLR and SORT1.

Very well-documented clinical data showing that the AAA expansion rate is significantly lower in patients with diabetes than in those without diabetes, despite the high susceptibility to advanced atherosclerosis as well as calcification in diabetic patients [65, 66], indicate that inflammation-mediated AAA formation and atherosclerosis-mediated AAA formation may not act in concert in all patients. In diabetic patients AAA calcification which is associated with stiffening of the wall may even be inversely correlated with AAA expansion [65, 183]. In addition, it has been suggested that drugs taken by almost all diabetic patients may be in part responsible for the lower AAA incidence, and this is subject to intense research [65, 66]. Multiple preclinical animal models of AAA, mostly

Table 5.4 Anti-AAA drugs tested in mouse animal models of AAA

Pre-clinical animal model and mechanism of action	Administered anti-AAA drug or agent or causative genetic change	Prevention of AAA generation	Prevention of AAA growth and rupture	Affected metabolic pathway	Human AAA-associated genes or loci	Ref
Inhibition of matrix metalloproteinases by small drug delivery using elastin antibody-coated nanoparticles	Small molecule batimastat-loaded nanoparticles	Yes	Yes	MMP remodeling of EM	MMP9	[185]
Overexpression of Sirtuin1 suppresses AAA formation in old ApoE−/− mice	Sirtuin1 transgene	Yes	Yes	VSMC proliferation and immune system	9p21, IL6R	[186]
Elevation of adiponectin levels prevent AngII-induced AAA formation	AAV adiponectin	Yes	Yes		?	[134]
Hypoxia inducible factor 1 alpha inhibition reduces AngII-induced AAA formation	Small molecule digoxin	Yes, acts in the first 10 days	No	MMP regulation	MMP9	[187]
Inhibition of receptor interacting kinase 1 ameliorates elastase-induced AAA progression	Necrostatin-1s small molecule drug	?	Yes	VSMC necroptosis	MMP9?	[159]
Factor Xa/IIa inhibitors reduce size of AAA and atherosclerosis	Small molecule dabigatran	Yes	Yes	Intraluminal thrombus formation	?	[188]
Inhibition of micro RNA-29b reduces AAA development in AngII-induced AAA in mice and also in Elastin-induced AAA in mice	Anti mi29b siRNA expression	Yes	?	Perivascular fibrosis and collagen and elastin expression. Col1a1, Col3a1, Col5a1, and Eln are miRNA29B targets	MMP9 important for extracellular matrix protein homeostasis	[189, 190]
SMAD3 deficiency promotes AAA in CaCl2-induced AAA	Mouse SMAD3−/− model	Yes	?	MMP expression and infiltration of macrophages and T cells	?	[191]
Long non-coding RNA ANRIL may be involved in development of AAA	Mouse 9p21 −/− model	Yes	?	Proliferation of VSMC?	9p21 ANRIL locus	[190]
Angiotensin receptor type 1 blockade attenuates 10 week high-fat diet-induced AAA	Telmisartan (improved over losartan) small molecule drug	?	yes	Renin-angiotensin system	?	[192]
Doxycycline may inhibit MMPs and reduce AngII-induced AAA development	Doxycycline	Yes	Yes	MMP expression in aortic media	MMP9	[163]
Cigarette smoke induces MMP9 secretion from mouse aortic VSMC	N/A	N/A	N/A	Inhibition of the JAK/STAT pathway may alleviate AAA in smokers	MMP9	[193]
Elastase-induced AAA in C57Bl6 male mice	Dietary phytoestrogens	Yes	?	Anti-inflammatory action	?	[194]
CaCl2-induced AAA in mice	Resveratrol	Yes		Anti- inflammatory	?	[195]
AngII-induced AAA in non-cholesterolemic mice. Such mice usually show little AAA	Systemic TGF beta antibodies cause strong AAA induction	Yes	?	Protects against AAA through inhibition of MMP12	?	[44]
Elastase-induced AAA in CDNK2B−/− mice	Deletion of CDKN2B causes induction of VSMC proliferation in the aorta and AAA	Yes	?	CDKN2B protects against aortic inflammation and AAA	9p21	[145]

knock-in mice with genetically engineered mutations in these loci, have shown that AAA expansion can indeed occur in the complete absence of atherosclerosis and calcification (see also Tables 5.3 and 5.4).

Perhaps the best known of the currently completed clinical trials targeting one of the new strongly AAA-associated loci (MMP9) is the long-term administration of doxycycline, an anti-inflammatory drug that is also an MMP9 inhibitor. As described above, individuals with mutations in MMP9 are predisposed to AAA, likely because they cause abnormal MMP9 expression within the extracellular matrix of the media of the aortic wall, which in turn leads to structural

weakness and, thus, aneurysms. A recent animal model has shown clearly that doxycycline can strongly reduce generation of AAA in mice and importantly also can help prevent further expansion and rupture [184]. Doxycycline was tested in an AAA clinical trial starting in 2013, but this was stopped after 1 year of administration, as doxycycline turned out to slightly *increase* the diameter of the AAA instead of stabilize or decrease it [182]. It was assumed that this increase was statistically and biologically insignificant, and an additional study is currently underway using higher doxycycline concentrations [182].

Other current clinical AAA trials test the angiotensin II blockers telmisartan and valsartan [182] which are improved versions of losartan, which has been previously used for both AAA and FTAAD to reduce hypertension and other effects of angiotensin II but with variable success so far (see above). Cyclosporine A and ticagrelor are also in clinical trial in hopes to reduce AAA growth by reducing inflammation and matrix remodeling [182].

To improve the relevance of animal models for human disease, new animal studies aim at testing the effect of novel drugs specifically on existing aneurysms rather than initiation of new aneurysms (see Table 5.4 for details). Indeed, a novel C57BL6 mouse AAA model was published in 2017 that uses oral BAPN administration combined with periaortic elastase application and that for the first time demonstrates all major stages of human AAA formation, including aneurysm formation, slow enlargement over ~ 100 days, thrombus formation, and spontaneous rupture [169]. No doubt such models will be invaluable for the development of drugs that can stop growth and rupture of diagnosed aneurysms in humans.

The most recent mouse models increasingly test drug effects on AAA progression: Table 5.4 lists more than 15 novel pharmacological approaches toward slowing AAA development as well as reducing AAA progression (many of them published within the past 3 years), including long-term treatment of existing AAA, that makes AAA pharmacology a very exciting field. Table 5.4 also lists the suspected mode of action of the treatment and the relevant characteristics of the models for human drug development.

Gene and Cell Therapy of AAA

As described above in the FTAAD section, direct correction of the genetic defect, and/or modulation of the lipid metabolism, of the extracellular matrix remodeling, and of the immune response by gene therapy could be possible within the next 10 years. Importantly, AAA caused by mutations in certain affected genes can potentially be cured by a one-time treatment with gene therapy. These include mutations in transcription factors, such as CDKN2B, DAB2IP, SMYD2, and ERG, which may not directly be targetable by drugs. One interesting way to perform cell/gene therapy in the aorta and other blood vessels was recently published for mice: the use of magnetic field-aided seeding of magnetic genetically engineered vascular endothelial cells into the aortic wall [196]. Such a procedure could be done at an early stage of the disease and could obviate the need of life-long drug treatment or invasive and risky surgery of the patient. Such procedures could also be done for the abdominal aorta at an early stage of the disease, and if engraftment is successful and long term, they could obviate the need of life-long drug treatment or invasive and risky surgery of the patient.

Given the rapid pace of investigation in the nonsurgical treatment of AAA, it is probably only a question of time until novel drugs or even gene/cell therapies are discovered that truly prevent progression of existing AAA in patients in the long term. Once this happens, the need for surgery to treat AAA, which is associated with significant perioperative complications, will be significantly reduced.

Summary and Conclusions on Abdominal Aortic Aneurysm

Familial/genetically conditioned AAA is a complex disorder that is associated with both lifestyle-associated risk factors and predisposing genes, similar to cardiovascular disease. The most recent and largest meta-analysis [11] finds strong support for statistically significant association of nine loci with AAA, four of which have never been recognized before. The nine loci are named 9p21 (CDKN2BAS/ANRIL), SORT1, IL6R, DAB2IP, LDLR, SMYD2, LINC00540, MMP9, and ERG. Since the original AAA studies that were subjected to meta-analysis proposed more than 90 candidate loci, it is clear that majority of these loci could not be confirmed after increasing statistical power [10, 11] and may either not be relevant or will have to await further studies for independent confirmation in specific patient subpopulations. This high failure rate among proposed AAA loci is not atypical of studies in medical genetics in general. Unfortunately, it is often caused by lack of statistical power, lack of sufficient normal controls, systematic error, or bias [197]. Crucially, several of the above nine genetic loci have also been clearly confirmed in multiple independent animal models, usually in the mouse. The presence of mutations in these nine loci should be determined in all AAA patients as well as their non-symptomatic blood relatives. This would allow identification of relatives with high risk of AAA. These individuals would benefit from frequent monitoring of the abdominal aorta and would be motivated to initiate preventative measures such as diet and lifestyle changes – long before an aneurysm develops. However, further research into the

function of the nine AAA-associated genes and especially the establishment and/or refinement of the corresponding animal models to allow monitoring of the effect of drugs on long-term AAA growth (and not AAA generation) is necessary to find novel candidate curative AAA drugs. Indeed, multiple current and planned clinical AAA trials promise possible future pharmacological treatment of AAA, with the potential to greatly reduce the need for surgery.

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