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Review Article

Experimental *in vivo* and *ex vivo* models for the study of human aortic dissection: promises and challenges

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Abstract: Aortic dissection (AD) is a life-threatening aortopathy with high mortality. To mimic spontaneous AD, investigate the pathogenesis of AD and develop novel therapeutic targets and measures, multiple AD experimental models have been generated, including drugs or chemicals induced experimental models, genetically modified experimental models, surgically or invasively induced experimental models, and *ex vivo* models. However, the perfect model of AD that replicates every aspect of the natural disease has not be generated yet. This review provides an overview of the experimental models used in AD preclinical research. The value and challenges of each *in vivo* and *ex vivo* model are discussed.

Keywords: Aortic dissection, experimental model, β-aminopropionitrile monofumarate, angiotensin II, marfan syndrome

Introduction

Aortic dissection (AD), with high mortality, is one of the extremely dangerous aortic diseases around. Patients with AD regardless of its classification are at high risk for an adverse outcomes and in the patients with an acute Type A AD there are a mortality of 50% chance of death within the first 48 hours if not operated [1]. Although a variety of operations developed and the application of intravascular stent greatly improved survival rate and period of AD patients, the therapeutic results of some patients are disappointing. In addition to all of this, there is almost no specific medicine to treat AD. The major reason for this may be due to the fact that the pathogenesis of AD is not fully elucidated. Therefore, unravelling the pathogenesis of AD is urgently needed, and in generating a good experimental model we have the potential to go along way.

An AD is initiated by an intimal tear which further penetrates through the medial layer, resulting in separation of the aortic wall layers and subsequent formation of a true lumen and a false lumen [1]. The dissection can extend either an antegrade or retrograde manner from the location of the entry tear. In some patients, a second intimal tear may form to communicate a true lumen and false lumen, which will reduce impulse and shear force of blood flow on adventitial layer and thrombosis. However, the most serious consequence is an aortic rupture in the case of adventitial disruption before surgery, which will directly lead to hypotension shock, or even the death of patients. AD can be classified according to the segments involved. In this light, the Debakey system and the Stanford system were developed and are the two most commonly used classifications in practice today [2]. AD is subdivided into Debakey Type I, II, and III by Debakey system, and are depicted as Stanford Type A or B by Stanford system (**Figure 1**) [3-5]. In addition to this, AD can also be divided into acute (<14 days), sub-acute (15-90 days), and chronic (>90 days) AD according to duration [1]. As stated in 2014 European Society of Cardiology (ESC) guidelines on aortic

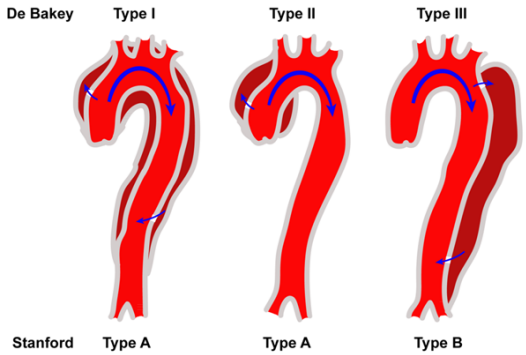

Figure 1. Classification of aortic dissection. Schematic diagram of aortic dissection subdivided into DeBakey Type I, II, and III, or Stanford Type A and B.

diseases, AD is rarity in epidemiology [1]. Specifically, the results of Oxford Vascular study show that the incidence of AD is estimated 6 cases per 100,000 persons per year and seems to be increasing [2, 6], as evidenced by the study of Clouse and his colleagues performed in Olmsted county, MN, USA between 1980 and 1994, in which they reported the incidence of acute AD was 3.5 cases per hundred thousand people in the county [7]. The improvements made in the field of diagnostic imaging might have contributed a lot to the increased incidence of AD cases. Particularly, males are found to be more vulnerable to AD than females and the incidence increases with age [8]. In the International Registry of Aortic Dissection (IRAD) study, 65% of patients with AD were male, with the mean age of 63 years [9]. Data from IRAD also told the truth that up to 16% of patients with AD have a known aortic aneurysm [9, 10]. Poorly controlled hypertension, as observed in 65-75% of individuals, is the most common risk factor of AD [9, 11-14]. Other risk factors include age, sex, atherosclerosis, aortic aneurysm, aortic valve disease, family history of aortic diseases, inherited disorders (e.g. Marfan syndrome (MFS), Ehlers-Danlos syndrome, Loeys-Dietz syndrome), history of cardiac surgery, iatrogenic related to coronary catheterization, cigarette smoking, pregnancy, direct blunt chest trauma and abuse of intravenous drugs (e.g. cocaine and amphetamines) [3, 15-18]. In addition, previous publications demonstrated that up to 20% of road accident fatalities had a ruptured aorta [19]. The major pathological change of AD is medial degeneration, as evidenced by loss of smooth muscle cells, deposition of mucoid material or glycosaminoglycans in cystic-like spaces, fragmentation of elastic fibers [20]. Inflammatory cells infiltration and inflammatory cytokines release also contribute a lot in initiating the intimal entry tear, destroying the medial layers of the aortic wall, and activating matrix metalloproteinase, which finally promote the formation of human AD [21, 22].

The purpose of this review is to discuss the most up-to-date experimental models of AD and to provide comprehensive information for researchers. Although the research on the pathogenesis of AD has made great progress, many problems still remain unresolved. Therefore, choosing or generating a good animal model is in urgent need for the study of AD.

Experimental models

In the following text we will discuss the most up to date experimental models of AD including drugs or chemicals induced experimental models, genetically modified experimental models, open surgery or minimally invasive surgery induced experimental models, and *ex vivo* models.

Drugs or chemicals induced experimental models

Normally, the aortic wall is composed histologically of three layers: the tunica intima, media, and adventitia [23]. The structural and functional changes in the aortic wall provide a physical basis for the occurrence of AD. The tensile strength and elasticity of the aorta reside in the medial layer, which is composed of concentric sheets of elastic and collagen fibres, as well as smooth muscle cells (SMCs) [1, 23]. The pathological hallmark of AD is medial degeneration, as evidenced by fragmentation and loss of the elastic lamellae, focal areas lacking smooth muscle cells, and accumulation of proteoglycans in the aortic media [23, 24]. It's reported that medial degeneration was detected at multiple sites in 96.4% of patients via histologic examination [20]. However, an earlier study showed that only 18% of patients with AD exhibited medial degeneration in the ascending aorta [25]. The disparity between these two researches may due to the earlier study just counting medial degeneration in the ascending aorta but not the entire aorta. In most patients, the intimal tear is the exactly primary event that

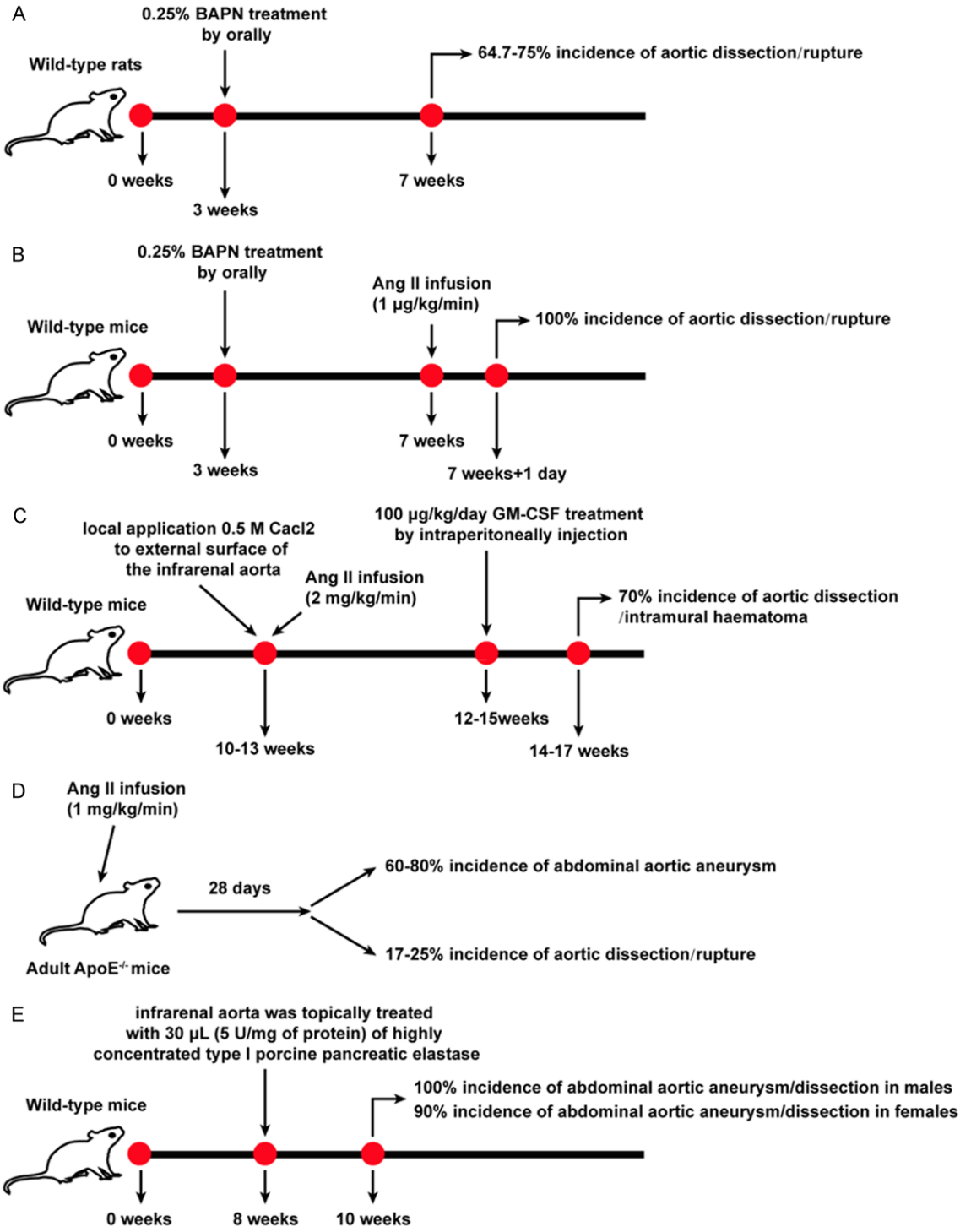

Figure 2. Drugs or chemicals induced aortic dissection animal models. A. Wild-type rats were stimulated with 0.25% β-Aminopropionitrile (BAPN) by orally at 3 week-old age for 4 weeks with 64.7-75% incidence of aortic dissection/rupture. B. Wild-type mice were treated with 0.25% BAPN by orally at 3 week-old age for 4 weeks, and then challenged with 1 μg/kg/min of Ang II by Alzet osmotic minipumps for indicated times, and 20%, 80%, 100% aortic dissection incidence was observed after Ang II treated for 6, 12, 24 hours, respectively. C. The wild-type mice were first locally disposed with 0.5 M $CaCl_2$ at external surface of the infrarenal aorta, and then stimulated with 2 mg/kg/min of Ang II for 2 weeks via Alzet osmotic minipumps. After that the mice were injected with 100 μg/kg/day recombinant murine granulocyte macrophage colony-stimulating factor (GM-CSF) by intraperitoneally for 2 or 4 weeks. D. The adult $ApoE^{-/-}$ mice were treated by subcutaneous implanting an Alzet osmotic minipumps loaded with 1 mg/kg/min of angiotensin II (Ang II) for 28 days. E. The adult wild-type mice were topically challenged with 30 μL (5 U/mg of protein) of highly concentrated type I porcine pancreatic elastase at infrarenal aorta to induce aortic aneurysm/dissection.

allowed the blood to spread through the aortic media, and medial degeneration reduces the resistance of the aortic wall to hemodynamic stress, leading to subsequent dissection [23].

The β-aminopropionitrile (BAPN) monofumarate, a lysyl oxidase inhibitor, is known to induce AD in young rats by inhibiting the cross-linking of collagen fibers [26, 27]. A recent study performed by *Li* and his colleagues showed that 0.25% BAPN treatment orally, instead of 0.4% or injecting 667 mg/kg/day, might be an appropriate dosage to induce rat aortic dissecting aneurysm or AD model [28]. 75% of the rats had an aortic dissecting aneurysm formation or death in the 0.25% BAPN treatment group, which was much higher than in the other two BAPN treatment groups (**Figure 2A**). The diameter of thoracic aorta, media thickness and area of thoracic aorta were significantly increased after 0.25% BAPN treatment which was consistent with the pathologic changes of the patients with aortic dissection. These results indicated that aortic dissecting aneurysm formation was not BAPN dose-dependent [28]. However, they did not compare whether the incidence of aortic dissecting aneurysm and mortality was different between orally or injection the same concentration of BAPN in rats. Another study conducted by the same research team demonstrated that compared with normal control or 0.25% BAPN treatment without AD, aortic longitudinal elastic strength was dramatically decreased in rats with 0.25% BAPN treatment with AD, as evidenced by decreased maximum stretching length, maximum strength, maximum extensibility, draw ratio, maximum load, and elasticity modulus [29]. Similarly, the mouse AD model was generated by administrating freshly prepared BAPN solution dissolved in the drinking water (1 g/kg/day) to three week-old male mice for 4 weeks [30]. Their results showed that in 16 out of 18 developed thoracic aortic aneurysm/dissection (TAAD) and of these 10 died from rupture, and mechanical stress-induced endoplasmic reticulum stress promoting smooth muscle cells apoptosis, inflammation and degeneration may be responsible for TAAD formation and progression [30]. Three-week-old Sprague-Dawley rats were also treated with 0.25% BAPN, after 1 week, and temocapril (an angiotensin-converting enzyme inhibitor, ACEI) or CS-866 (angiotensin II receptor type-1 blocker) was administered orally for 2 weeks. A spontaneous dissection was observed in 64.7% rats treated with 0.25% BAPN only, while only 26.7% rats have AD after temocapril treatment, which indicated that temocapril significantly prevented BAPN induced AD, cystic medial degeneration, and vascular smooth muscle cells apoptosis. In contrast, CS-866 had no statistically significant effect on BAPN induced AD [31]. Therefore, these results indicated that ACEIs might be of clinical value for the prevention and treatment of aortic diseases. Since the pathological process of AD could be prevented by ACEIs, angiotensin II (Ang II) infusion may have accelerated AD development. Given this, a novel AD model was established by infusing Ang II (1 μg/kg/min) to immature mice (three-week-old) that had been receiving BAPN for 4 weeks [32]. In this mouse model, 20%, 80%, 100% AD incidence were observed after Ang II treated for 6, 12, 24 hours, respectively (**Figure 2B**). Their further study demonstrated that AD was initiated by infiltrated neutrophils in aortic intima and released MMP9 in response to Ang II infusion. However, blood pressure elevated equivalently in the BAPN-treated wild-type mice infused with norepinephrine (NE, 1.3 μg/kg/min) or Ang II (1 μg/kg/min), but only 10% of the mice treated with NE infusion or BAPN treatment alone had AD, which indicated in the induction of AD, Ang II has other function other than vasopressor [32]. In comparison with older C57BL/6J mice, reduced incidence of AD was observed after Ang II infusion into mice that lacked either IL-6 or CCR2, and adoptive transfer of $Ccr2^{+/+}$ monocytes into $Ccr2^{-/-}$ mice could restore of IL-6 secretion and increase incidence of AD [33]. Administration of granulocyte macrophage colony-stimulating factor (GM-CSF) in wild-type mice subjected to aortic inflammation (local application $CaCl_2$ and Ang II infusion for 2 weeks) caused AD/intramural haematoma (**Figure 2C**) [34]. It seems that a combination of aortic inflammation with GM-CSF infusion is necessary for the phenotype, as evidenced by Ang II, $CaCl_2$ or GM-CSF alone was not sufficient to induce AD/intramural haematoma [34]. The results indicated that this method could induce AD/intramural haematoma within 2 weeks with high reproducibility (at least 70%) even in young wild-type mice [34]. Given Nox2 is an important source of reactive oxygen species (ROS) production in response to pathological

stimuli such as Ang II [35], endothelium-specific Nox2 overexpression mice have increased endothelial ROS production, cyclophilin A secretion, endothelial vascular cell adhesion molecule-1 expression, matrix metalloproteinase activity, and $CD45^+$ inflammatory cell infiltration, which remarkably increases susceptibility to AD in Ang II-induced mouse model [36].

Though Ang II or BAPN alone could induce AD in $ApoE^{-/-}$ or immature wild-type mice, respectively, it's more sufficient to generate AD experimental model by combination of Ang II with BAPN or other chemical/drugs. Notably, in this process, Ang II not only just functions as vasopressor, but also a pro-inflammatory factor. Hypertension affects the arterial wall in several ways, including causing intimal thickening, calcification, fibrosis, and extracellular fatty acid deposition, as well as spontaneous rupture of the aortic vasa vasorum, which were the initiating factors leading to intramural hematoma and subsequently to intimal tear [37-39]. In addition, increased intimal thickening and adventitial fibrosis resulted in lack of nutrient and oxygen supply to the arterial wall, leading to degradation of the extracellular matrix, endothelial cells and smooth muscle cells apoptosis, and elastolysis with hyalinization of collagen. All these changes will increase stiffness and vulnerability of aorta to pulsatile forces, which may eventually lead to intimal disruption [25, 37, 38, 40-42]. Hypertension augments mechanical strain on the aortic wall. Mechanical forces affect aortic dissection by following ways: the shear stress of the blood, the radial impact of the pressure pulse, and flexion forces of the vessel at fixed sites [38]. There is ample evidence demonstrating that inflammatory cells often accompany medial degeneration [23, 32-34]. Inflammatory response could destroy the medial layers of the aortic wall via releasing inflammatory factor and activating matrix metalloproteinase which leads to weakening, expansion, and dissection of the aortic wall. On the other hand, autoimmune processes may affect vasa vasorum, resulting in nutrient deficiency of aortic wall layers [21, 22]. Given that the major pathologic changes of human AD, including increased diameter of thoracic aorta, media thickness and area of thoracic aorta, decreased longitudinal elastic strength, accelerated smooth muscle cells apoptosis, inflammation and degeneration, activation of matrix metalloproteinase, and elevated blood pressure, were reproduced in mice or rats treated with combination of BAPN and Ang II, these experimental models could largely mimic the pathological process of AD in human beings.

TAAD is characterized by excessive SMC loss, extracellular matrix (ECM) degradation and inflammation. The conventional model of abdominal aortic aneurysm (AAA) model is treating apolipoprotein E-deficient ($ApoE^{-/-}$) mice with Ang II for 28 days, which could achieve 60-80% incidence of AAA [43, 44]. As aneurysms and atherosclerosis are the risk factor for AD occurrence, in $ApoE^{-/-}$ mice with Ang II stimulation, accumulation of macrophages in medial regions of elastin degradation led to aneurysm and medial dissection [43]. A research demonstrated that among 65 Ang II-infused $ApoE^{-/-}$ mice, 16 mice (25%) suffered from aortic rupture with 44% in the aortic arch and 56% in the suprarenal region and most of these occurred within the first 7 days after Ang II infusion (**Figure 2D**) [44]. In the same model, another research group observed one or several focal dissections in the ascending aorta, and the volume of the dissections moderately correlated to the volume of the aneurysm as measured *in vivo* ($r^2 = 0.46$) [45]. Notably, approximate 58% of animals developed an interlaminar hematoma which could be linked to an intimal tear after 3 days of Ang II infusion [45]. Their data also showed that progressive enlargement of the focal dissections over time may have been due to a significant increase in single laminar ruptures, and fatal transmural dissection was observed in 17% of mice at an early stage of the disease [45].

In recent years, many other methods had emerged to establish AD experimental model [34, 46-49]. For example, intracranial aneurysm (IA) and aortic dissection rat model was generated by salt loading and hemi-lateral ligation of renal and carotid arteries, and their results also demonstrated that prostaglandin F-receptor antagonist AS604872 accelerated degeneration of the media in both cerebral artery and aorta and promoted IA and AD [47]. This model maybe more suitable for investigating the role of hypertension in AD occurrence. After exposing the abdominal aorta via midline laparotomy, the infrarenal aorta was treated with 30 μL (5 U/mg of protein) of highly concentrated type I porcine pancreatic elas-

tase. Elastase, not washed off prior to closing the abdomen, was dropped on the anterior aorta from a 2-cm height for 5 min to achieve topical application. After 14 days, abdominal aortic aneurysm was occurred in both the external elastase-treated males (100%) and females (90%) (**Figure 2E**) [48]. Fragmentation of elastic fibers is an obvious pathological change in the aorta of patients with AD [20], and the model induced by type I porcine pancreatic elastase is the critical way of studying AD that results from the elastic fibers disruption or reduction.

Genetically modified experimental models

As we known, numerous genetic syndromes predispose individuals to AD. Three major inherited connective tissue disorders, MFS, Loeys-Dietz syndrome, and Ehlers-Danlos syndrome (vascular form), affect the integrity of the arterial wall, which provides the substance for AD occurrence [50-54]. Patients with MFS show marked clinical heterogeneity, though its primary characteristics are having development of cardiovascular, skeletal, and ocular organ systems manifestation [55, 56]. However, aortic aneurysm and dissection are common life-threatening complications for patients with MFS [55]. Many mutations of multiple genes (*e.g. ACTA2, COL3A1, EFEMP2, FBN1, FLNA, MYH11, MYLK, NOTCH1, SKI, SLC2A10, SMAD3, TGFB2, TGFBR1,* and *TGFBR2*) were identified and related to AD in patients with inherited connective tissue disorders [57, 58]. Ample evidences demonstrated that mutations in fibrillin-1 (FBN1) gene are prerequisite for MFS, and approximate 1800 different mutations of this gene have been identified, and with most of them related to MFS [59, 60]. FBN1 contains 47 epidermal growth factor (EGF)-like domains in which cysteine substitutions mutation are frequent causes of MFS, and the most typical one is FBN1 (C1663R) [61]. However, the transgene mice overexpressing mutant FBN1 (C1663R) have no apparent clinical or histologic vascular phenotype despite high-level production of mutant protein up to 2 years old [62]. The effect of mutant FBN1 may be nullified by endogenous FBN1. $Fbn1^{C1039G/+}$ (a comparable missense mutation with human C1663R) heterozygous mice were produced via homologous recombination [62]. Though the $Fbn1^{C1039G/+}$ mice showed a normal life span and did not die from AD, after 2 months of age, the aorta of the mice progressively deterioration within the medial layer, as evidenced by elastic fiber fragmentation, disarray of vascular smooth muscle cells, ectopic expression of MMP2 and MMP9 [62]. The aortic wall was also gradually thickening due to the excessive deposition of amorphous matrix, including collagen and proteoglycans and the TGF-β-Smad2 signalling pathway activation may be responsible for extracellular matrix deposition [62]. More importantly, losartan, an angiotensin II type 1 receptor blocker, prevented aortic aneurysm and partially rescued noncardiovascular manifestations of MFS in $Fbn1^{C1039G/+}$ mice [63]. Caspase activity also enhanced in $Fbn1^{C1039G/+}$ mice, which contributes to aortic wall remodelling and early aortic aneurysm development [64]. $Fbn1^{C1039G/+}$ mice did not suffer from AD in its lifetime, but aortic lesions related to AD were observed. Therefore, if the $Fbn1^{C1039G/+}$ mice stimulated with precipitating factor (*e.g.* Ang II) at the indicated times, AD is likely to occur. In addition, scientists may develop AD animal model by generating other FBN1 mutation knockin or overexpression animals.

Except for FBN1, the identified genes predisposing to AD encode proteins participate in the following biology processes: the extracellular matrix of the aortic wall (MFAP5), smooth muscle cell contraction or metabolism (ACTA2, MYH11, MYLK, PRKG1, MAT2A) and canonical TGF-β signaling (TGFBR1, TGFBR2, TGFB2, SMAD3) [24, 65-72]. For example, TGFB2 mutations p.Glu102* (frameshift mutation in exon 6) and p.Cys229* (nonsense mutation in exon 4) are predicted to cause haploinsufficiency for TGFB2, but TGF-β2 expression level was increased. These haploinsufficiency of TGFB2 predispose to thoracic aortic aneurysms and acute Ads [70]. Two knockin mouse strains were generated with mutations in either *Tgfbr1* (M318R) or *Tgfbr2* (G357W) and a transgenic mouse overexpressing mutant *Tgfbr2*. Their results showed that knockin and transgenic mice, with TGF-β signalling activation, recapitulated the Loeys-Dietz syndrome phenotype, but not haploinsufficient mice [50]. After conditional inactivation of Tgfbr2 in smooth muscle cells of $Fbn1^{C1039G/+}$ mice (MFS murine model), thoracic aorta rapidly thickened, dilated, and dissected in these animals and MAPK signaling pathway activated but not canonical Smad signaling decreased [73]. Further studies demonstrated that loss of TGF-β signaling impairs

the contractile apparatus of vascular smooth muscle and elicits vascular cell proliferation, which indicates that Tgfbr2 disruption in postnatal smooth muscle impairs aortic wall homeostasis [73]. Similarly, conditional deletion Tgfbr2 in smooth muscle cells of wild-type mice also caused severe aortopathy, including aorta hemorrhage, ulceration, dissection, dilation in young mice [74]. This report is consistent with the research that deletion of SMC Tgfbr2 causes aortic dissection in younger but not in older mice [73]. Therefore, these results suggested that it is should be careful to blockade TGF-β signaling in humans by drugs, which may cause aortic disease rather than preventing it.

Col3α1, which haploinsufficiency causes a complex phenotype with AD in human beings, encodes the α1 chain of type III collagen [75-77]. Based on these findings, previous studies attempted to generate such a mouse model through gene-targeted ablation of Col3α1. The homozygous Col3α1 deletion animals having both bruising and AD, but only 10% of them survive to adulthood and rupture of the major blood vessels maybe responsible for the death of mutant mice, whereas the Col3α1 heterozygous mice were not subject to death from arterial rupture, which maybe the reasons for limited success in this mouse model [78, 79]. Col3α1$^{+/\Delta}$ mice with 50% Col3α1 reduction, caused by a spontaneous 185 kb deletion, including the promoter region and exons 1-39, of the Col3α1 gene, displayed spontaneous acute AD with rupture of the ascending or descending thoracic aorta in 28% of mice, but with no evidence of aneurysm [79]. Though part of the Col3α1$^{+/\Delta}$ heterozygous mice exhibited AD phenotype after age of 12 weeks, there is no spontaneous early vascular phenotype. In addition, spontaneous acute AD was not associated with elevated blood pressure and aneurysm formation, but might concern aberrant collagen fibrillogenesis within the aortic wall [79]. A recent research demonstrated that Ang II (1 μg/kg/min) treatment significantly increased systolic blood pressure in Col3α1$^{+/+}$ and Col3α1$^{+/-}$ haploinsufficient mice, which led to 73% premature mortality rate in Col3α1$^{+/-}$ mice, but 36% in Col3α1$^{+/+}$ mice caused by AD and rupture and associated with low aortic collagen fibrils content at the end of the 4-week period [80]. However, norepinephrine infusion (3.9 μg/kg/min) could also induce systolic blood pressure increase, but did not result in significant mortality in both groups [80]. These results indicated that Col3α1 haploinsufficient markedly increased sensitivity to Ang II-induced prematurely developed AD and rupture and associated high levels in blood pressure. Similarly, homozygous Col1α1 mutant mice (Col1α1$^{\Delta/\Delta}$), lacked a large fraction of the first intron, are predisposed to dissection and rupture of the aorta during their adult life [81]. In particular, approximate 54% of Col1α1$^{\Delta/\Delta}$ mice were died with a large hematoma in aorta, which was associated with AD, during the 18-month observation period [81]. However, AD was not detected in autopsies of heterozygous animals or their littermate controls [81]. Notably, similar to Col3α1$^{+/\Delta}$ mice, high-resolution magnetic resonance imaging (4.7 T) detection showed that Col1α1$^{\Delta/\Delta}$ mice developed dissection and rupture of aorta in the absence of aneurysms [82].

Many other genes mutations were also associated with AD or other aortic diseases in human beings [46, 57, 58], and more other genes and mutations will be identified in the near future, but we currently did not produce a stable AD experimental model by introducing the same mutations of genes to the mice or other animals. Since mice with single mutation of one gene have limited AD prevalence [46, 80, 82], one gene with multiple mutations or polygenes mutation in the same mice maybe more sufficient to induce AD. In addition, combination of genetic manipulation and drugs or chemicals (*e.g.* Ang II) stimulation might be an alternative way to effectively induce AD in animal.

Open surgery or minimally invasive surgery induced experimental models

Iatrogenic and traumatic AD affect a small number of patients whose aorta wall might be well developed with no lesions. The intimal tear was caused by accident or inappropriate medical procedures; such as traffic accident or invasive retrograde catheter interventions [83, 84]. Creating an AD model by surgery is an alternative way to reproduce the pathology of this kind of human AD. The dog AD model was established via surgical splitting the aortic media layer to develop false lumen [85]. In detail, a left thoracotomy was performed to expose the descending aorta after the dog was anesthetized. Then the intercostal branches of the

Table 1. The advantages and disadvantages of all types of AD models

Models of AD		Incidence of AD (%)	Advantages	Disadvantages	References
Drugs or chemicals induced	0.25% BAPN (4 weeks)	64.7-75%	Reproducible	Not suitable for genetic or traumatic induced AD	28, 30, 31
	0.25% BAPN (4 weeks) + 1 μg/kg/min Ang II (1 day)	100%	High efficiency and reproducible	Not suitable for genetic or traumatic induced AD	32, 33
	(0.5 M $CaCl_2$ + 2 mg/kg/min Ang II) (2 weeks) + 100 μg/kg/d GM-CSF (2 weeks)	70%	Mimic AD results from inflammation	Tedious process	34
	1 mg/kg/min Ang II (4 weeks)	17-25%	Good for study AD results from aneurysm	• Low efficiency • Poor repeatability	44, 45
Genetically modified		Depend on gene mutations	Suitable for genetic induced AD (e.g. Marfan syndrome, Loeys-Dietz syndrome, and Ehlers-Danlos syndrome)	Low efficiency	57-82
Open surgery or minimally invasive surgery induced		73.3-78.6%	• Short cycle • High reproducibility • Suitable for the study of hemodynamics and stent graft placement after AD	• Only for type B AD • Closer to traumatic AD but not than other type of AD • Requires specialized equipment and condition	85-91
Ex vivo models		100%	• Useful in the study of AD haemodynamics and the impact of the primary entry tear locations on the patterns of dissection propagation • Suitable for experiment of stent grafts designed for human aortas • High reproducibility and short period • Animal ethics and welfare not involved	• Only for type B AD • Not suitable for the study of pathogenesis in AD • Requires specialized equipment	92-99

descending aorta were ligated at 4 cm distal to the ligamentum arteriosum. The aorta was opened transversely to 50% of its circumference at 3 cm distal to the ligamentum arteriosum after cross clamped. Subsequently, the aortic media layer was split with a spatula to form a dissected false pocket. After closing the incision, the aortic cross clamps were removed to restore blood flow in the modified aorta. The results of post-operative aortography showed evidence of AD (type-B) in all animals.[85] Most importantly, the dissection was obliterated after introducing a balloon-expandable intraluminal vascular graft in a dissected aorta [85]. The similar model was used to study the effectiveness of aorta fenestration on alleviating organ ischemia, and the results showed that blood pressure and flow to hypoperfused organs were restored after aortic fenestration in acute descending AD [86]. Another minor modified procedure was performed to generate AD model in pigs which then treated with radiofrequency induction heating therapy [87]. The results indicated that radiofrequency induction heating in combination with a self-expanding Gianturco metallic stent has potential to treat acute AD if properly used [87]. A new AD model in beagle dog was reported, in which the initial dissection of aorta was created surgically, but the dissection was enlarged and propagated by injecting 0.05 mg/kg epinephrine via the peripheral vein to increase blood pressure and pressure gradient in beagle dogs [88]. The results of digital subtraction angiography and computed tomography angiography indicated that 12 of 16 dogs successfully get AD [88]. *Terai* and his colleagues generated type B AD via surgically creating an entry for the aortic dissection just distal to the origin of the left innominate artery and the reentry was 5 cm distal to the entry point, then the dissection was achieved by injecting normal saline solution into the aortic wall between these two points [89]. All the dogs that survived had completely patent true and false lumina without any thrombi, but about 20% of the dogs ended up dying during the perioperative period [89]. Another method in creating AD in swine via femoral or carotid invasive endovascular procedure was developed [90]. The initial subintimal tear was made by using a Colapinto needle, and the dissections were extended to a predefined position in the aorta. In their study, only 11 of 15 swine were successfully developed AD, and the hemodynamics of the aorta did not change after single-balloon fenestration in this model [90]. The similar procedure was performed to create AD model via femoral artery by another research group, their results showed comparable AD incidence (78.6%) [91].

As aforementioned, surgically or invasively induced AD experimental models have its advantages, such as short cycle, high reproducibility, and suitable for the study of hemodynamics and stent graft placement after AD (**Table 1**). In addition, these procedures were performed on swine and dog whose physical characteristics are closer to human beings in most previously published researches. However, there are also some disadvantages, only type B AD can be induced by surgical method in published data. Also, features of these models maybe closer to traumatic AD but not than other type of AD, as the aortic wall is integral with no medial degeneration, inflammation and elastic fibers fragmentation before artificialy creating intimal tear and dissection. Most importantly, invasive endovascular procedure requires specialized equipment and condition (**Table 1**).

Ex vivo models

Except for *in vivo* AD models, *ex vivo* AD models contributed to ascertain the pathological characteristics of AD, especially fluid dynamics, and the impact of the location of the primary entry tear. The *ex vivo* AD models mimic the human circulatory system via an *ex vivo* circuit which common consists of an aorta with dissection, a pulsatile pump, pressure regulator, aortic connectors, flow sensor and reservoir (**Figure 3**). Twenty nonaneurysmal fresh human aortas were obtained from 2 cm above the level of the aortic valve to just above the iliac bifurcation level [92]. The dissection was created by hemi-circumferential aortotomy at 2 cm below the left subclavian artery level as aforementioned [85, 86]. The false lumen was developed by blunting separation free aortic wall media for 2 cm distally. After being loosely fixed the intimal-medial layer detached to the opposite aortic wall by a suture, the hemi-circumferential aortotomy was closed. The stitch was removed once the dissection was propagated. Subsequently, the aorta with dissection was connected into the bench-top closed-system pulsatile flow model. The pump was activated and result-

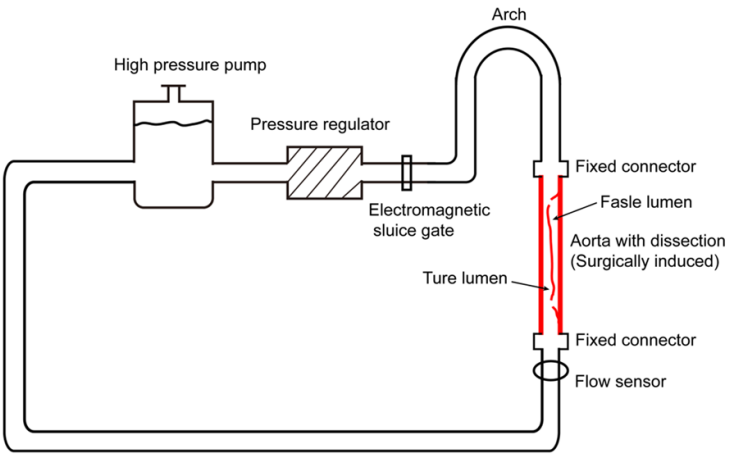

Figure 3. The schematic diagram of *ex vivo* aortic dissection model. The basic components of device for *ex vivo* model, including high pressure pump, pressure regulator, electromagnetic sluice gate, aorta with surgically induced dissection, and flow sensor and reservoir.

ed in a pulsatile flow (60 pulses/min, pressure of 150/80 mmHg) to monitor the propagation of the dissection [92]. Their results showed that extended dissection was achieved in all the aortas which indicated this human *ex vivo* model of type B AD is reproducible [92]. Notably, they found that the locations of the primary entry tear determine the patterns of dissection propagation [92]. The similar AD model was performed by *Dziodzio* and his colleagues in pig aorta, and they got the same conclusion [93]. To assess the feasibility of stent graft treatment of ascending aortic dissections, *Zimpfer at al.* generated the AD model by using the entire thoracic aortic aorta including the supra-aortic branches of adult pigs [94]. The aorta was mounted into the artificial circulatory circuit after an intimal tear was artificially created. The hydraulic motor piston pump worked with a pressure of 200/160 mmHg and constant flow rate (3.5 L/min) to mimic aortic flow and pressure to propagate the dissection. After a dissection developed, a 2 × 2.6 cm covered stent graft was inserted through the brachiocephalic trunk using a specially designed delivery system. Their results demonstrated that stent graft replacement could completely close the false lumen, which indicated that stent graft replacement is feasible for ascending AD [94]. The modified method was used to create dissection in bovine aorta [95]. Firstly, a tiny circumferential separation was surgically made in the aorta to penetrate between the media and the intima, and then the dissection was extended by using a raspatorium. Their results demonstrated that a new inflatable balloon device could increase the adhesive effect of tissue glues and prevent distal embolization of the glue [95].

Another modified model was created by designing and fabricating an aluminum mold to create a compliant dissection model mimicking a chronic type B aortic dissection with hemi-circumference dissected [96]. The artificial aortic arch with dissection was connected into an *ex vivo* circuit to mimic the human circulatory system. Three different dissection models (proximal and distal tear, proximal tear only, distal tear only) with tear sizes of 6.4 mm and 3.2 mm were studied and the results showed that tear size and location impacts false lumen pressure [96]. Recently, a new *ex vivo* AD model was developed by using silicone [97]. Firstly, they obtained clinical computed tomography images of human aorta and then segmented, and reconstructed to form three dimensional models in silico, which were subsequently used as prototype molds for fabrication of silicone models. The silicone models were connected to a single closed loop filled with fluid to mimic human circulatory system [97].

Similar to surgically or invasively induced experimental models, most *ex vivo* AD models are type B AD models. The *ex vivo* AD models may not be suitable for the study of pathogenesis in AD, but it's useful in the study of AD haemodynamics and the impact of the primary entry tear locations on the patterns of dissection propagation [98, 99]. In addition, it maybe also suitable for experiment of stent grafts designed for human aortas. The major advantage of this model is of high reproducibility, short period, and animal ethics and welfare cannot be ignored.

Conclusion and perspective

The *in vivo* and *ex vivo* experimental models of AD provide researchers with experimental models that replicate different features of human AD. No single experimental model is

sufficient for investigation of the pathogenesis and treatment of AD. Drugs and chemicals (especially Ang II and BAPN) induced experimental models maybe the optimum models to mimic the pathological process of AD caused by medial degeneration, hypertension and inflammation in human beings, while surgically or invasively induced experimental models are well suited for investigations of interventional or traumatic AD and developing treatment prevention strategies. Genetically modified models are useful for elucidate the pathological features of AD result from inherited connective tissue disorders. The *ex vivo* models have benefits in studying haemodynamics characteristics of AD. Most importantly, the development and assessment of various novel therapies have relied on these traditional experimental models.

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Disclosure of conflict of interest

None.

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