Mouse models for abdominal aortic aneurysm

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Abstract

Abdominal aortic aneurysm (AAA) rupture is estimated to cause 200,000 deaths each year. Currently the only treatment for AAA is surgical repair, however this is only indicated for large asymptomatic aneurysms, is not always durable and is associated with a risk of serious perioperative complications. As a result, patients with small aneurysms or who are otherwise unfit for surgery are treated conservatively, but up to 70% of small aneurysms continue to grow, increasing the risk of rupture. There is thus an urgent need to develop drug therapies effective at slowing AAA growth. This review describes the commonly used mouse models for AAA. Recent research in these models highlights key roles for pathways involved in inflammation and cell turnover in AAA pathogenesis. There is also evidence for long coding RNAs and thrombosis in aneurysm pathology. Further well-designed research in clinically relevant models is expected to be translated into effective AAA drugs.

Abbreviations

AAA: Abdominal aortic aneurysm;

$CaCl_2$: Calcium chloride;

TGFß: Transforming growth factor-beta;

LOX: Lysyl oxidase;

BAPN: ß3-aminopropionitrile;

MRI: Magnetic resonance imaging;

NLRP3: Nucleotide-binding domain leucine-rich repeat and pyrin domain containing receptor 3;

IL: Interleukin;

COX: Cyclooxygenase;

MMP: Matrix metalloproteinase;

Ig: Immunoglobulin;

LncRNA: Long non-coding RNA;

FOXP3: Forkhead box P3;

CTLA-4: Cytotoxic T-lymphocyte-associated protein 4;

TFEB: Transcription factor EB;

TNF: Tumour necrosis factor;

PI3K: Phosphatidylinositol-3-kinase.

Introduction

Abdominal aortic aneurysm (AAA) is a focal weakening and expansion of the main abdominal artery that affects about 2% of men and 0.5% of women aged over 60 years (Sampson et al., 2014a). The main complication of AAA is aortic rupture, which is responsible for about 200,000 deaths per year worldwide (Sampson et al., 2014b). AAA is usually asymptomatic prior to rupture and diagnosis therefore requires identification through clinical examination or abdominal imaging. In high-income countries, most AAAs are diagnosed through ultrasound, computed tomography and magnetic resonance imaging performed to investigate unrelated abdominal or back problems or via screening programs. To facilitate early identification of asymptomatic AAAs, ultrasound screening programs are offered to people at risk of AAA in a number of high-income countries, such as the USA, UK and Sweden (Force et al., 2019). Important risk factors for AAA include male sex, older age, smoking and family history and these are included as eligibility criteria for screening to varying degrees in different countries (Force et al., 2019; Golledge, Muller, Daugherty & Norman, 2006). Surgical repair is the only current treatment for AAA. Two types of operations are commonly used. Endovascular AAA repair involves placement and fixation of covered stents across the AAA from within the aorta using sheaths placed through a small puncture in the femoral arteries (Cao et al., 2011). Open surgical repair requires a laparotomy or retroperitoneal dissection of the abdominal aorta, clamping of the arteries above and below the aneurysm and the replacement of the AAA with a prosthetic graft (United Kingdom Small Aneurysm Trial et al., 2002). Both types of surgical repair are associated with short and long-term complications. Open AAA repair has a perioperative mortality rate of about 5% and is frequently associated with other perioperative complications, such as myocardial infarction, respiratory infection, atelectasis and wound complications (Landry, Liem, Abraham, Jung & Moneta, 2019; Stather, Sidloff, Dattani, Choke, Bown & Sayers, 2013). Later complications of open repair include incisional hernia, graft infection and false aneurysm (Schermerhorn et al., 2015). In contrast, endovascular AAA repair has a perioperative mortality of around 2% but approximately 20% of patients need re-intervention during long-term follow-up to correct continued perfusion of

the AAA sac (endoleak) and late AAA rupture has been reported in around 3% of patients (Antoniou et al., 2015; Rajendran & May, 2017; Schermerhorn et al., 2015; Stather, Sidloff, Dattani, Choke, Bown & Sayers, 2013).

Due to these complications, surgical repair is only recommended for a patient with an AAA that is considered to be at moderate risk of rupture during the individual's lifetime. The most established predictor of aneurysm rupture is maximum aortic diameter, with yearly rupture rates estimated as 1%, 9%, 19% and 33% for <55, 55-64, 65-69 and ≥70mm AAAs respectively (Lederle et al., 2002; United Kingdom Small Aneurysm Trial et al., 2002). AAA diameter is therefore used in clinical practice to make decisions about when to recommend AAA repair. Randomised controlled trials have found that early elective repair of asymptomatic 40-54mm AAAs does not reduce mortality (Filardo, Powell, Martinez & Ballard, 2015). Current guidelines recommend that surgical repair is considered for asymptomatic AAAs that are ≥50mm in women and ≥55mm in men (Chaikof et al., 2018; Wanhainen et al., 2019). AAAs smaller than this are simply monitored by imaging surveillance, but up to 70% slowly increase in size and ultimately undergo surgical repair (United Kingdom Small Aneurysm Trial et al., 2002). In people for whom surgery is not recommended there are currently no established treatments for AAA (Golledge, 2019).

A key deficiency in the current management of AAA is the absence of drug therapies that are effective in slowing AAA growth and preventing AAA rupture (Golledge, 2019; Golledge & Norman, 2011). Such medications could be used to treat patients with small AAAs or those unfit for surgical AAA repair and could also be an adjunctive therapy for people with endoleak after endovascular AAA repair. The use of animal models is an established way to further the understanding of pathogenesis, identify treatment targets and test potential drugs. This review summarizes available mouse models for studying AAA, and recent findings regarding AAA pathogenesis and drug targets. The key aspects of the most commonly used mouse models and findings from studies published since 2019 using such models are described. Findings from studies published prior to 2019 are described in multiple earlier

reviews (Daugherty A, 2004; Lysgaard Poulsen J, 2016; Patelis N, 2017; Sénémaud J, 2017; Wang Y, 2013a).

Commonly used chemically induced mouse models of AAA

Due to the availability of genetically modified animals, including gene knock-out, knock-in, transgenic and inducible strains, mice are the most commonly used animals in experimental AAA studies although other animals such as rats, rabbits, pigs and dogs have been examined in a smaller number of studies. Readers are referred to previous reviews for a discussion of these less commonly used animal models of AAA (Golledge, 2019; Lysgaard Poulsen J, 2016; Patelis N, 2017; Sénémaud J, 2017; Wang Y, 2013a). In mice, three agents, angiotensin II, elastase and calcium chloride ($CaCl_2$; or phosphate), are commonly used to induce AAA (Golledge, 2019). Characteristics of these three different methods of inducing AAA are summarized in Table 1.

Angiotensin II

It was first reported in 2000 that subcutaneous infusion of angiotensin II at a dose of 0.5 or 1 μg/min/kg for 28 days using a mini-pump implanted in the back of the neck of apolipoprotein E deficient mice induced thoraco-abdominal aortic aneurysm in 20% and 33% of mice, respectively (Daugherty A, 2000). Similar but smaller aneurysms were also reported in low density lipoprotein receptor mice similarly infused with angiotensin II (Cassis LA, 2007). Since that time, angiotensin II infusion has become the most common way of inducing AAA in animal model experiments, with many hundreds of studies reported (Daugherty A, 2004; Golledge, 2019; Patelis N, 2017). This popularity likely reflects both the technical simplicity of the model and the fact that angiotensin II induced aneurysms have some key features of human AAA (Table 1). Male sex is an important risk factor for human AAA (Golledge, Muller, Daugherty & Norman, 2006) and male mice are more susceptible than female mice to aneurysm induction by angiotensin II infusion (Alsiraj Y, 2017). Similar to human AAA, angiotensin II induced aneurysms have marked upregulation of inflammation, extracellular matrix remodeling and thrombosis-associated genes (Biros E, 2015; Rush

C, 2009). Similar to patients, angiotensin II induced aneurysms in mice also commonly rupture (Liu J, 2015). Some of the features of angiotensin II induced aneurysms are not typical of human AAA. Whilst inflammation, particularly macrophage recruitment, has been strongly implicated in the initiation of aneurysm in this model (Daugherty A, 2000), aortic dissection appears to be the key event (Saraff, Babamusta, Cassis & Daugherty, 2003). Histological examination of angiotensin II induced aneurysm walls demonstrates intra-mural haematoma (Daugherty A, 2000; Saraff, Babamusta, Cassis & Daugherty, 2003). Phase-contrast x-ray tomographic microscopy studies suggest that angiotensin II induces an intimal tear and bleed into the aorta wall typical of aortic dissection (Daugherty A, 2000; Trachet B, 2017). This limits the relevance to the usual presentation of human AAA in which dissection is rare and intra-luminal rather than intra-mural thrombus found (Golledge J, 2008). Furthermore, angiotensin II induced aneurysms develop in the supra-renal or thoracic aorta while in patients the most common site affected is the infra-renal aorta (Daugherty A, 2000). Despite these limitations, the angiotensin II induced model remains the most commonly studied AAA model.

Elastase

Elastase is the second most commonly used agent for inducing AAA in mice (Busch A, 2016; Pyo R, 2000). Porcine pancreatic elastase has been used to induce AAA using a number of methods including intra-luminal infusion and painting on the aortic adventitia (Lu G, 2017; Thompson RW, 2006). The original method involved dissecting out the infra-renal aorta, temporarily occluding it, infusing porcine elastase into the lumen of the artery for 5 to 30 minutes via an arteriotomy and then repairing the hole in the aorta and removing the aortic occluding clamps (Thompson RW, 2006). A simpler method of temporarily applying elastase to the adventitia of the infra-renal aorta has been recently used (Lu G, 2017). The intra-luminal elastase perfusion model is far more challenging to learn than the adventitial elastase application or angiotensin II infusion methods with surgery taking about twice as long (Busch A, 2016). Elastase induced aneurysms have true luminal aortic enlargement, in contrast to the dissection formed in the angiotensin II model (Busch A, 2016; Lu G, 2017; Saraff,

Babamusta, Cassis & Daugherty, 2003; Thompson RW, 2006). Both elastase induction methods cause aortic elastin degradation and induce a similar two-fold increase in infra-renal aortic diameter over 4 weeks (Busch A, 2016; Phillips EH, 2015). Inflammation has been reported to be more marked and angiogenesis less marked, after adventitial application than intra-luminal administration of elastase (Busch A, 2016) (Table 1). In contrast to human AAA, elastase induced AAAs do not rupture and there is limited evidence they progressively expand in the longer term. This means the standard elastase model is not useful for the study of advanced AAA pathology or the testing of medications in limiting AAA growth (Table 1).

$CaCl_2$

Application of $CaCl_2$ or calcium phosphate to the adventitia of the infra-renal aorta is another method of inducing AAA although the degree of aortic diameter expansion stimulated is less marked than that caused by angiotensin II or elastase (Phillips EH, 2015; Wang Y, 2013a). Histological examination of $CaCl_2$ induced aneurysm demonstrates inflammation, angiogenesis, elastin breaks and calcification as found in human AAA samples (Chen HZ, 2016; Liu CL, 2016b; Wang Y, 2013a; Xiong W, 2009). $CaCl_2$ induced aneurysms do not have some features of human AAA, such as intra-luminal thrombus and aortic rupture (Table 1) (Wang Y, 2013a).

Recent modifications of the commonly used chemically induced mouse models

A growing number of modifications of the classical mouse AAA models described above have now been reported (Table 1) (Busch A, 2018; Cooper HA, 2020; Kanematsu Y, 2010; Lareyre F, 2017; Lu G, 2017; Yue J, 2020).

Transforming growth factor beta (TGFß) neutralisation

TGFß is strongly implicated in thoracic and AAA pathogenesis, although the pathways involved appear divergent at the two sites (Angelov SN, 2017; Golledge, 2019; Wang Y, 2013b). A number of studies have now shown that systemic TGFß neutralisation, usually by repeated injection of a

blocking antibody, promotes development of more severe AAA within the angiotensin II model (Angelov SN, 2017; Wang Y, 2010). TGFß neutralisation has also been reported to promote more severe AAA within the adventitial elastase model (Lareyre F, 2017). Due to the high incidence of death within 14 days of commencing TGFß neutralization, these models are most appropriate for studying aneurysm rupture (Lareyre F, 2017; Wang Y, 2010).

Inhibition of lysyl oxidase (LOX)

LOX is the enzyme that cross-links collagen and elastin. Mice deficient in LOX develop spontaneous aortic aneurysms (Mäki JM, 2002). ß3-aminopropionitrile (BAPN) fumarate salt is a commonly used oral LOX inhibitor and has been combined with adventitial elastase (Lu G, 2017; Romary DJ, 2019) or subcutaneous angiotensin II infusion (Cooper HA, 2020; Kanematsu Y, 2010) to induce severe AAA. Many mice species, such as C57BL/6, are resistant to the pro-aneurysmal effects of angiotensin II, by comparison to mice with dyslipidemia, such as apolipoprotein E or low density lipoprotein receptor deficient mice (Daugherty A, 2004; Daugherty A, 2000; Kanematsu Y, 2010). This delays the assessment of the role of different genes and pathways in angiotensin II induced AAA, since mice with gene knock-outs or knock-ins need to be backcrossed with dyslipidemic mice prior to experimental testing (Cooper HA, 2020; Hiromi T, 2020). Oral or subcutaneously infused BAPN, like TGFß neutralization (Lareyre F, 2017; Wang Y, 2010), makes C57BL/6 more susceptible to angiotensin II infusion (Cooper HA, 2020; Kanematsu Y, 2010). A combination of angiotensin II infusion for six weeks and BAPN infusion for two weeks has been reported to induce AAA in about half of C57BL/6 mice, of which about one-quarter ruptured (Kanematsu Y, 2010). Like the classical angiotensin II model, aneurysms form in this model as a result of aortic dissection (Cooper HA, 2020; Kanematsu Y, 2010). One of the most promising new models of AAA is one combining adventitial elastase and oral BAPN (Lu G, 2017; Romary DJ, 2019). While so far this model has received limited study the reported findings suggest it has much promise as a clinically relevant AAA model. The model incorporates key features of human AAA including focal fusiform aneurysm formation, development of intra-luminal thrombus, marked inflammation and extracellular matrix remodeling

(Lu G, 2017). Most importantly, aneurysms in this model can be monitored over prolonged follow-up of up to 14 weeks (Lu G, 2017). During this time aneurysms progressively expand to a large size of up to 8-fold the starting aortic diameter and about half of them rupture during this time (Lu G, 2017). This suggests the model is well suited to studying the effects of drugs on AAA growth and rupture.

Modifications of the luminal elastase model

Aneurysms within the luminal elastase model do not typical rupture, however, combining this model with subcutaneous angiotensin II infusion leads to a model with a high frequency of rupture of approximately twice that of angiotensin II infusion alone (Yue J, 2020). The luminal elastase model has also been modified by microsurgical manipulation to incorporate a proximal or distal stenosis that has been reported to promote formation of larger aneurysms (Busch A, 2018). Finally the same researchers have created more extensive aneurysms involving the juxta-renal aorta and iliac arteries through extending the arterial area over which the luminal elastase is infused (Busch A, 2018). These modifications enable the elastase luminal model to simulate some of the unique features of human AAA.

Modification of angiotensin II model

In order to make mice without congenital mutations causing dyslipidaemia susceptible to angiotensin II induced AAA, a number of modification have been proposed to the model (Cooper HA, 2020; Kanematsu Y, 2010; Lu H, 2020). These include feeding mice BAPN or injecting TGFß blocking antibody (as discussed above), as well as high fat feeding, increasing the dose of angiotensin II infused (from 1000 to 1500 or 2000 ng/kg/min) or using viral vectors to induce genetic mutations in lipid related genes, such as proprotein convertase subtilisin/kexin type 9.(Cooper HA, 2020; Kanematsu Y, 2010; Lareyre F, 2017; Lu H, 2020).

Assessing outcomes in mouse models of AAA

A range of methods with various advantages and disadvantages are used to assess aneurysm severity in these mouse models (see Table 2). Commonly used laboratory methods to assess aneurysm severity include analysis of photographs (morphometry), histology, immunohistochemistry and molecular biology techniques such as Western Blotting and real time quantitative polymerase chain reaction (Chen C, 2020; Liu R, 2020; Ren P, 2020; Suh MK, 2020). AAA severity can be graded by maximum diameter of the aneurysm, degree of medial elastin fiber disruption and extracellular matrix degradation, and the degree of aortic inflammation but requires terminal samples (Figure 1). Ultrasound imaging is frequently used at different stages of experiments to monitor AAA size *in vivo* (Cooper HA, 2020; Hiromi T, 2020; Li Z, 2020; Sharma N; Shi X, 2020). High-resolution ultrasound can provide detailed morphological assessment capable of accurately defining AAA size, presence of intra-mural haematoma, dissection, intra-luminal thrombus and also assess wall biomechanical properties, such as circumference strain or pulse wave velocity (Cooper HA, 2020; Hiromi T, 2020; Li Z, 2020; Nandlall SD, 2016; Romary DJ, 2019; Sharma N; Shi X, 2020). A range of molecular imaging methods are increasingly being used to study AAA models (Brangsch J, 2019; Trachet B, 2015). Imaging probes targeting mechanisms implicated in AAA, such as inflammation, extracellular matrix remodeling and thrombosis, (Adams LC, 2020; Botnar RM, 2018; Brangsch J, 2019; English SJ, 2020; Yao Y, 2020) are being used in combination with high resolution imaging, such as micro-computed tomography or magnetic resonance imaging (MRI; Table 2). A study of the angiotensin II model for example, quantified aortic macrophage infiltration and elastin using gadolinium-diethylenetriaminepentaacetic acid complex and ultrasmall superparamagnetic iron oxide enhanced MRI (Brangsch J, 2019). Angiotensin II-induced AAAs that subsequently ruptured had significantly higher iron oxide uptake and significantly lower elastin specific probe signal at one week after starting the angiotensin II infusion (Brangsch J, 2019). Another study reported that uptake of an albumin-binding probe designed to assess vascular permeability was also predictive of subsequent rupture of angiotensin II induced aneurysms (Adams LC, 2020). Use of these methods within the different

mouse models is providing increasing understanding of the stages of AAA development *in vivo*, which is of value for furthering understanding of the pathogenesis of AAA.

Recent findings in mouse models of AAA

It has been proposed that the mechanisms involved in AAA initiation and progression may be distinct (Sénémaud J, 2017). This has implications for the development of therapies that may prevent as opposed to treat AAA (Golledge, 2019; Sénémaud J, 2017). In order to summarise recent discoveries relevant to the development of drug therapies for AAA, the PubMed database was searched for studies in mouse models that have examined the impact of administered interventions on AAA development or progression. All included studies administered a drug or inhibited the expression of a gene or protein and examined how this impacted on the AAA development or progression. In order to focus findings on recent discoveries and in view of the timing of past reviews (Golledge, 2019; Sénémaud J, 2017), the search covered the period 1 January 2019 to 5 May 2020. The following two sections summarise the findings of these recent studies. The first section focused on studies that have examined the effect of interventions in which administration commenced prior to or at the same time as AAA induction commenced, i.e. effect on AAA initiation or development. The second section focused on interventions that were commenced after an AAA was established or at least some time after the AAA induction process began, i.e. effect on AAA growth or progression.

The effect of interventions on AAA initiation in mouse models

Forty-two published studies reporting that different interventions inhibited AAA initiation in mouse models were identified. The Supplementary Table summarizes these studies in terms of the AAA model studied, number of mice investigated in the intervention and control groups, length of time the intervention started before AAA induction commenced, intervention type, how outcome was assessed, the effect on AAA incidence, size and rupture, and effects attributed to the interventions. The interventions studied varied but overall four strategies were most commonly studied, namely modifying inflammation, cell turnover, thrombosis and haemostasis and epigenetic mechanisms.

Studies investigating the effect of modifying inflammation in AAA mice models

Inflammation has long been strongly implicated in AAA (Golledge, 2019; Golledge, Muller, Daugherty & Norman, 2006). Examination of human AAA samples demonstrates an array of different inflammatory cells, including both those from the innate and adaptive immune systems, such as T, B and dendritic cells, natural killer cells, monocyte/ macrophages, neutrophils and mast cells (Dale MA, 2015; Forester ND, 2005; Lindeman JH, 2009; Spear R, 2015; Tsuruda T, 2008). Analysis of relative gene expression at the main site of the AAA in comparison to samples from relatively non-diseased aortas or samples from people that do not have aneurysm, confirms the marked upregulation of an array of inflammation associated genes and pathways (Biros E, 2015; Biros E, 2014). All the mouse models discussed above show evidence of aortic inflammation. A micro-array study of the angiotensin II model, for example, found within aneurysms the transcriptome was enriched with genes involved in cytokine-cytokine receptor interaction, leukocyte transendothelial migration, natural killer cell mediated cytotoxicity and hematopoietic cell lineage (Rush C, 2009).

An array of different inflammation modifying regimens have recently been reported to inhibit or promote the incidence and/ or rupture of AAAs formed in the different mouse models (Figure 2 and Supplementary Table 1) (Amin et al., 2019; Hiromi T, 2020; Krishna, Moran, Jose, Lazzaroni, Huynh & Golledge, 2019; Li et al., 2019a; Li et al., 2019c; Liu, Kong, An, Zhang, Qin & Meng, 2019; Liu et al., 2019a; Nie et al., 2019; Paige et al., 2019; Peshkova et al., 2019; Ren P, 2020; Sharma et al., 2019a; Suehiro et al., 2019; Suh MK, 2020). The nucleotide-binding domain leucine-rich repeat (NLR) and pyrin domain containing receptor 3 (NLRP3)-inflammasome plays an important role in innate immunity through controlling the secretion of pro-inflammatory cytokines. Interleukin (IL)-1β-induced neutrophil extracellular trap formation (NETosis) and circulating IL-1β have been detected in human AAA patients (Meher AK, 2018) (Ahmad M, 2018). MCC950, an NLRP3 inhibitor, was reported to inhibit the incidence and size of AAAs induced by angiotensin II infusion associated with reduced elastin fragmentation and vascular smooth muscle cell apoptosis (Ren P, 2020). Prostaglandin E_2 is a key pro-inflammatory mediator generated from arachidonic acid by the

actions of cyclooxygenase (COX) 2. Upregulation of prostaglandin E_2 has been reported in human AAA samples by comparison to non-diseased (Holmes DR, 1997) but not atherosclerotic aortas (Reilly JM, 1999). Samples of human AAA maintained in culture from people with ruptured AAA secreted greater amounts of prostaglandin E_2 than samples from intact AAAs (Cheuk BL, 2007). Inhibition of prostaglandin E_2 has been reported to limit angiotensin II induced AAA development (King VL, 2006; Wang M, 2008). Deficiency of prostaglandin E receptor 4 in bone marrow cells has also been reported to inhibit angiotensin II induced AAA (Tang EH, 2011). Recently it was reported that upregulation of prostaglandin E receptor 4 in vascular smooth muscle cells promoted AAA development in both the angiotensin II and $CaCl_2$ models, associated with increased aortic inflammatory monocytes, IL-6, matrix metalloproteinase (MMP)-9 and elastin fragmentation, and decreased LOX (Hiromi T, 2020). Deficiency of prostaglandin E receptor 4 in vascular smooth muscle cells inhibited angiotensin II induced AAA development (Hiromi T, 2020). Administration of a prostaglandin E receptor 4 inhibitor has been reported to reduce AAA development in the angiotensin II model suggesting that this could be a legitimate target to prevent AAA (Yokoyama U, 2012). A number of chemokines and cytokines have been reported to be upregulated in human AAA (Golledge AL, 2009). Inhibiting the chemokine CXC receptor 2 was recently reported to limit AAA development in the angiotensin II model through reducing aortic macrophage infiltration and elastin degradation (Nie et al., 2019).

Airways disease is very common in people with AAA and this has been presumed to be due to smoking being an important risk factor for both conditions (Liu CL, 2016a). It was recently proposed that this association relates to direct involvement of immunoglobulin (Ig) E- mediated innate immunity in AAA (Liu CL, 2016b). Ovalbumin sensitization and challenge led to the development of allergic lung inflammation in mice and promoted angiotensin II induced AAA, which was inhibited by anti-IgE antibody administration (Liu CL, 2016b). Furthermore, IgE receptor deficiency was reported to inhibit the incidence and size of angiotensin II induced AAAs (Guo et al., 2019). IgE was

reported to promote expression of a long non-coding RNA (LncRNA-p21) which promoted vascular smooth muscle cell senescence (Guo et al., 2019).

A large body of published research suggests that Foxp3+ regulatory T cells inhibit AAA development within both the angiotensin II and $CaCl_2$ models (Golledge, 2019; Liu, Kong, An, Zhang, Qin & Meng, 2019; Suh MK, 2020; Yodoi K, 2015). Of potential clinical application, it was recently reported that *ex vivo* expansion and administration of Foxp3+ regulatory enriched T cells reduced the size of $CaCl_2$ induced AAAs (Suh MK, 2020). One mechanism implicated in the benefit of regulatory cells is through inhibiting prostaglandin production by decreasing COX-2 expression (Suh MK, 2020). Further supporting the role of adaptive immunity in AAA, a recent report showed that deficiency of antigen presenting CD11c+ dendritic cells limited angiotensin II induced AAA development associated with reduced circulating concentrations of effector CD4+ and CD8+ T cells and B cells (Krishna, Moran, Jose, Lazzaroni, Huynh & Golledge, 2019). Low–molecular mass protein 7 is a proteolytic subunit of the immunoproteasome that regulates the major histocompatibility antigen presenting pathway. In keeping with the importance of antigen presentation in AAA pathogenesis, it was recently reported that low–molecular mass protein 7 deficiency or inhibition reduced AAA development within the angiotensin II model (Li et al., 2019a).

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) is an immune check point important in downregulating the adaptive immune response. It was recently reported that transgenic over expression of CTLA-4 reduced the development and rupture of angiotensin II induced AAA suggesting it could be a strategy to prevent AAA (Amin et al., 2019). This finding also raises some potential concerns with how promotion of adaptive immunity, for example by immune check point inhibitors, as a treatment for cancer might affect people with concurrent AAA.

A growing number of ILs are implicated in promoting or inhibiting AAA in mice models (Li et al., 2019c; Paige et al., 2019; Peshkova et al., 2019; Sharma et al., 2019a; Suehiro et al., 2019). Blocking IL-6 has been reported to inhibit AAA development and rupture promoted by inhibiting TGFß in both

the angiotensin II and adventitial elastase models (Paige et al., 2019). Similarly, IL12p40, IL-18 and IL-27 have been reported to promote AAA development in mouse models (Peshkova et al., 2019; Sharma et al., 2019a; Suehiro et al., 2019). In contrast, IL-33, has been reported to inhibit calcium phosphate induced AAA development (Li et al., 2019c) by expanding Foxp3+ regulatory T cells.

Overall these studies strongly implicate innate and adaptive immunity in AAA development based on studies in mouse models, although how these directly translate to patients remains controversial (Golledge, 2019).

Studies investigating the effect of modifying cell phenotype and turnover in AAA mice models

Turnover of resident aortic cells, such as vascular smooth muscle cells and endothelial cells, is a normal physiological process but in a diseased aorta it is believed phenotypic changes in resident cells contribute to AAA pathogenesis (Petsophonsakul P, 2019). Usually vascular smooth muscle cells have a contractile phenotype but when stimulated by growth factors, inflammatory cytokines and reactive oxygen species, the cells may switch to a synthetic form in which contractile genes are downregulated and proteolytic enzymes, proliferative and migratory genes are upregulated (Petsophonsakul P, 2019). Histological examination of human AAA biopsies typically demonstrates markedly medial thinning with a paucity of vascular smooth muscle cells (Figure 2). Normal cell turnover is controlled by programmed cell death processes called apoptosis and necroptosis, and clearance mechanisms such as autophagy (Gupta K, 2018; Petsophonsakul P, 2019). In AAA these processes are believed to be pathologically promoted by a range of changes in the cell microenvironment, such as the release of cytokines and reactive oxygen species (Gupta K, 2018; Petsophonsakul P, 2019). Studies in dyslipidemic mice that were rendered pro-apoptotic suggest that in areas of atherosclerotic plaque, apoptosis triggered medial thinning and wall degeneration contributes to AAA development (Clarke MC, 2008)

Recent studies highlight that targeting these abnormal cellular changes has potential to prevent AAA (Supplementary Table 1). Transcription factor EB (TFEB) is a master regulator of autophagy. A

recent study found that vascular smooth muscle cell-specific deficiency of TFEB increased apoptosis and inhibited autophagy within experimental AAAs and promoted a greater incidence and size of AAA developed within both the angiotensin II and composite angiotensin II- BAPN models (Lu H, 2020). Administration of hydroxypropyl-β-cyclodextrin (an agent used to dissolve drugs) activated TFEB, reduced apoptosis and reduced aneurysm incidence and size within the angiotensin II model (Lu H, 2020).

Cell proliferation and function is dependent on mitochondrial metabolism (Lopez-Crisosto C, 2017). Mitochondrial turnover is a coordinated process involving binary fission and mitochondrial DNA replication under control of the guanosine triphosphate hydrolysing dynamin-related protein 1 (Peng W, 2019). Relative deficiency in dynamin-related protein 1 has been reported to limit cell senescence and reduce the size of AAAs induced by angiotensin II and BAPN (Cooper HA, 2020). Administration of a mitochondrial fission inhibitor was also reported to lead to significantly smaller AAAs in response to angiotensin II and BAPN, associated with reduced aortic macrophage infiltration, MMP-2 and -9 activity and lower oxidative stress (Cooper HA, 2020).

The cellular function of invading inflammatory cells are also believed to play an important role in AAA pathogenesis. The CD95 receptor and its ligand, CD95L, are members of the tumour necrosis factor (TNF) receptor and TNF family. CD95 is classified as a death receptor involved in the control of apoptosis and is highly expressed on T cells (Seyrek K, 2019). The expression of CD95L has been reported to be significantly higher within human AAA samples by comparison to aortic tissue from age and sex-matched organ donors (Liu et al., 2019b). Mice totally deficient in CD95L have been reported to be relatively resistant to $CaCl_2$ induced AAA, associated with reduced aortic macrophage and T cell infiltration and lower aortic MMP-2 and -9 activity (Liu et al., 2019b). Chimeric mice with bone marrow deficient in CD95L, not mice with systemic but not bone marrow CD95L deficiency, were resistant to $CaCl_2$ induced AAA development (Liu et al., 2019b). CD95L deficiency was associated with caspase 8 deficiency, which is believed to play an important role in activation of the NLRP3 inflammasome through cleaving pro-IL-1β (Liu et al., 2019b).

Phosphatidylinositol-3-kinase (PI3K) plays a key role in cell growth, proliferation and migration. The PI3Kγ isoform is mainly found in myeloid cells (Fruman DA, 2017). PI3Kγ expression has been reported to be significantly greater in human AAA samples than aortic tissue from organ donors (Liu R, 2020). Administration of a PI3Kγ inhibitor lowers the incidence and size of aneurysms induced by intra-luminal elastase perfusion in mice (Liu R, 2020). This was reported to be secondary to reduced aortic infiltration by macrophages and T cells and reduced neo-angiogenesis (Liu R, 2020).

Spermidine, is a histone acetyl transferase inhibitor that induces autophagy and stabilises DNA (Ren J, 2018). Oral administration of spermidine has been reported to reduce the size of AAAs that developed in mice following intra-luminal elastase infusion (Ren J, 2018). This was associated with reduced aortic accumulation of macrophages, T cells and markers of autophagy (Ren J, 2018).

Overall, these studies suggest an important pathological role of vascular smooth muscle cell apoptosis and a protective role of autophagy. Furthermore, inhibiting mitochondrial fission, blocking the CD95 death receptor, inhibiting PI3Kγ and promoting autophagy through spermidine are novel ways to limit aortic inflammation and potentially prevent AAA.

Studies investigating the effect of modifying thrombosis and haemostasis in AAA mice models

Most human AAAs have intra-luminal thrombus and the volume of this is strongly positively correlated with the maximum diameter of the aneurysm (Golledge J, 2008). Aortic thrombus contains a collection of products from leukocytes and platelets with the potential to promote extra-cellular matrix remodeling (Golledge, 2019). Circulating thrombus turnover markers, such as D-dimer, are increased in patients with AAA and people with higher levels have faster AAA growth (Golledge J, 2011). Larger volume of intra-luminal thrombus has also been associated with faster aneurysm growth (Parr A, 2011). As a result of these findings, it has been proposed that anti-platelet agents and/ or anti-thrombotic drugs might inhibit AAA development and progression. A number of recent mice studies have reported that different methods of inhibiting thrombosis, such as factor XII deficiency or inhibition (Moran CS, 2020) or factor Xa inhibition (using rivaroxaban),(Allen-Redpath et al.,

2019) reduce the incidence and size of AAAs induced by angiotensin II infusion (Supplementary Table 1).

Studies investigating the effect of modifying epigenetic mechanisms in AAA mice models

Twin studies suggest that over 70% of the variance in AAA penetrance is determined by genetic components as opposed to environmental determinates (Joergensen TM, 2016). A number of genetic risk alleles have been identified through genome wide association studies but it is believed epigenetic changes also likely contribute to the inherited risk (Golledge J, 2016). There has been particular interest in non-coding RNAs, including microRNAs and long coding RNAs. A wide range of different microRNAs have been reported to be differentially expressed in the aorta and blood of people with AAA (Golledge J, 2016; Iyer V, 2017). In mouse models, upregulating or antagonising a number of different microRNAs has also been reported to limit AAA development (Golledge J, 2016). Most recently it has been reported that microRNA-144-5p agomirs,(Shi X, 2020) silencing of long non-coding RNA plasmacytoma variant translocation 1 (Zhang et al., 2019) and downregulation of long noncoding RNAs GAS5 (He et al., 2019), limited AAA development induced by angiotensin II infusion. In addition, interfering RNA targeting Runt-Related Transcription Factor 2, which is strongly implicated in calcification, was reported to inhibit angiotensin II induced AAA development (Li Z, 2020). In the later study positron emission tomography-computed tomography was used to show the micro-calcification proceeded aneurysm formation in apolipoprotein e deficient mice infused with angiotensin II (Li Z, 2020). Peri-adventitial hydroxyapatite nanoparticles applied to the supra-renal aorta promoted aneurysm formation in response to angiotensin II infusion (Li Z, 2020). The findings are in keeping with those from a patient study reporting that uptake of fluorine-18-sodium fluoride on positron emission tomography-computed tomography, representative of micro-calcification, is predictive of subsequent AAA growth (Forsythe RO, 2018).

Miscellaneous other interventions recently investigated

Supplementary Table 1 lists a number of other interventions that have recently been reported to promote or inhibit AAA development in mouse models. These include ulinastatin, a serine protease inhibitor,(Li G, 2020) probucol, a lipid lowering agents,(Chen et al., 2020) an apelin analogue,(Wang et al., 2019) a sodium-glucose cotransporter-2 inhibitor,(Ortega et al., 2019), niacin,(Horimatsu et al., 2019) licochalcone A, a traditional Chinese medicine,(Hou, Yang & Zheng, 2019) and gambogic acid, a derivative of resin (Liu, Shan & Li, 2019) which inhibited AAA development. In contrast, vascular smooth muscle-specific deficiency of the alpha subunit of the heterotrimeric G stimulatory protein, responsible for receptor-stimulated cAMP generation and activation of protein kinase A pathway,(Qin et al., 2019) has been reported to promote AAA formation in mouse models . Similarly, liver kinase B1P, implicated in the activation of adenosine monophosphate kinase,(Li et al., 2019b) dihydrofolate reductase deficiency, which leads to hyper-homocysteinaemia,(Li, Youn, Siu, Murugesan, Zhang & Cai, 2019; Shao et al., 2019) caloric restriction,(Gao et al., 2019) and endothelial-specific deficiency of retinoblastoma protein, a tumour suppressor that controls cell proliferation,(Cao et al., 2019) have been reported to promote AAA formation in mouse models (see Supplementary Table 1 for details).

The effect of interventions on AAA growth in mouse models

The main therapeutic deficiency in the management of people with AAA is the absence of medication effective at limiting the growth of established AAAs and reducing the requirement for surgery or AAA rupture (Golledge, 2019; Golledge J, 2020b; Golledge, Muller, Daugherty & Norman, 2006; Golledge & Norman, 2011; Golledge J, 2017). In contrast to the large number of interventions reported to prevent AAA initiation in mouse models, relatively few investigators have studied the effects of pathways or drugs on progression of established AAAs (See Supplementary Table 1 and compare to Table 3). One problem is that most of the available mouse models cause acute aortic dilatation without progressive expansion making it difficult to study the effect of drugs on aneurysm

growth (Table 1). Table 3 lists recently published studies that investigated the effect of drugs, diets or cell depletion, which commenced after the aneurysm induction process started. The period given for aneurysms to establish prior to the intervention varied between 3 and 28 days (Dhital S, 2020; He Y, 2020; Krishna, Moran, Jose, Lazzaroni, Huynh & Golledge, 2019; Li G, 2020; Liu J, 2020; Liu S, 2020; Lu H, 2020; Park, Hong, Kim, Jung, Kim & Choi, 2019; Sharma et al., 2019b; Tomimori, Manno, Tanaka, Futamura-Takahashi, Muto & Nagahira, 2019). The models used were either the angiotensin II or luminal elastase. Interventions reported to successfully limit AAA progression included a number also reported to prevent aneurysm initiation (discussed above), namely hydroxypropyl-β-cyclodextrin,(Lu H, 2020) spermidine,(Liu S, 2020) ulinastatin,(Li G, 2020) and depletion of CD11c+ dendritic cells,(Krishna, Moran, Jose, Lazzaroni, Huynh & Golledge, 2019). Other interventions successfully reported to limit AAA growth include kallistatin (via inhibition of the wingless pathway)(He Y, 2020), pentagalloyl glucose-loaded nanoparticle, a polyphenol that increases elastin deposition by vascular smooth muscle cells (Dhital S, 2020), an inhibitor of notch signalling (Sharma et al., 2019b), the beta-blocker carvedilol (Park, Hong, Kim, Jung, Kim & Choi, 2019), the angiotensin converting enzyme inhibitor ramipril (Park, Hong, Kim, Jung, Kim & Choi, 2019), and a chymase inhibitor (Tomimori, Manno, Tanaka, Futamura-Takahashi, Muto & Nagahira, 2019) (Table 3). Mechanisms implicated in the ability of these interventions to limit AAA growth included reduced inflammation and extracellular matrix remodelling (Table 3).

Clinical relevance and implications of mouse AAA research for the discovery of AAA drugs

The clinical relevance and feasibility of translating the findings of AAA mouse model research to patients has been questioned (Golledge J, 2017). This concern has mainly been fueled by the finding that targeting a number of pathways, shown to be key in AAA pathogenesis in mouse models, has not slowed AAA growth in human randomized controlled trials (Golledge, 2019; Golledge J, 2017). Doxycycline, pemirolast, perindopril, telmisartan, ticagrelor and fenofibrate are examples of drugs that have been shown to be ineffective in human randomized controlled trials, after promising results in mouse models (Golledge, 2019; Golledge J, 2017). Doxycycline, a tetracycline antibiotic, mainly

became of interest as an AAA drug due to widespread reports that it was able to inhibit MMP activity (Thompson RW, 1999). MMP have been strongly implicated in AAA in studies using many of the mouse models mentioned in this review. Deficiency of tissue inhibitor of metalloproteinase-1, an MMP inhibitor, has been reported to promote spontaneous AAA formation in apolipoprotein e deficient mice (Silence J, 2002). Similarly, MMP-9 deficiency has been reported to inhibit luminal elastase induced AAA (Pyo R, 2000). Doxycycline has been reported to limit AAA development in the luminal elastase (Pyo R, 2000), $CaCl_2$ (Prall AK, 2002) and angiotensin II (Manning MW, 2003) models. None of the three randomised controlled trials have found that doxycycline significantly reduced growth of small AAAs (Baxter BT, 2020; Meijer CA, 2013; Mosorin M, 2001). One trial actually reported that doxycycline significantly increased AAA growth (Meijer CA, 2013). Similar to this, promising results from mice studies related to pemirolast (Tsuruda T, 2008), perindopril (Inoue N, 2009), telmisartan (Xuan H, 2018), ticagrelor (Owens AP 3rd, 2015) and fenofibrate (Krishna SM, 2012) have not translated to positive results in human randomised controlled trials (Bicknell, Kiru, Falaschetti, Powell, Poulter & Collaborators, 2016; Golledge J, 2020a; Golledge J, 2020b; Pinchbeck JL, 2018; Sillesen H, 2015; Wanhainen A, 2020). How much this reflects lack of clinical relevance of the mouse models used, poor design of prior mice studies or poor design of the clinical trials, remains uncertain (Golledge J, 2017). There is a need to use more clinically relevant designs of mouse model studies, for example by studying the effect of drugs on growth of established aneurysms rather than prevention of AAA development (Golledge, 2019). Pre-clinical research studies also need to be designed to reduce biases, through incorporation of blinding of outcome assessors, sample size estimates, randomisation of mice to different groups and intention to treat analyses, typical of human clinical trials. In addition human clinical trials need to be much larger to enable them to be sufficiently powered to test plausible moderate treatment effects (Golledge, 2019). It is hoped through using newer mouse models, which are more clinically relevant, and employing improved study design, it will be possible to identify drugs that can be translated to successful therapies in large clinical trials.

Conclusion

A large range of different mouse models are now available to study AAA. Some models are better suited to the study of AAA initiation, others optimal for the investigation of aneurysm rupture and relatively few ideal for the study of AAA growth. So far, findings from mice models have not been translated into evidence from human randomized trials that a drug can successfully limit AAA growth. With the increasing understanding of the need to model the clinical situation more accurately and design pre-clinical studies with the same rigor as human clinical trials, it is expected that the discovery of translatable AAA drugs will be achieved in the coming decade.

Conflicts of interests

The authors have no relevant conflicts of interest.

Figure legends

Figure 1: **A schematic diagram illustrating outcome assessments that are commonly used in mouse models of AAA.** Examples of outcome assessment in the different mouse models. Morphometry assessment showing the grading scale (Type 1-4) according to Daugherty A, *et al*, 2001. Histology assessment using haematoxylin and eosin (H&E) stain is used to assess the severity of extracellular matrix degradation. Elastin degradation is assessed and graded (Grade 1-4) according to the degree of elastin fibre breaks evident by elastin Van Gieson (EVG) staining. Severity of inflammation is commonly measured using Immunohistochemistry (IHC) using antibodies staining macrophages or T lymphocytes. Protein assays commonly used include Western Blotting and ELISA assays. Molecular biology assessments employed include real time quantitative polymerase chain reactions using mRNA for differential expression. *In vivo* assessments include ultrasound measurements to measure diameter, intra-mural haematoma, intra-luminal thrombus and wall biomechanical properties. Micro-computed tomography and high sensitive magnetic resolution imaging coupled with imaging probes can be used to assess inflammation, extracellular matrix remodelling and thrombosis. AAA: abdominal aortic aneurysm; CT: computed tomography; ECM: extracellular matrix; IHC: immunohistochemistry; MRI: magnetic resonance imaging; RT-PCR: real time polymerase chain reaction; US: ultrasound; SRA: supra-renal aorta; IRA: infra-renal aorta.

Figure 2: Cartoon illustrating the mechanisms implicated in AAA pathogenesis and recently discovered interventions effective at limiting AAA development or growth in mouse models. Both innate and adaptive immunity are strongly implicated in AAA and recent studies suggest that upregulating T regulatory (reg) cells or interleukin (IL) 33, blocking IL-6, upregulating cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and inhibitors of prostaglandin E receptor 4 (PgER4), C-X-C Motif Chemokine Receptor 2 (CXCR2) or Phosphatidylinositol-3-kinase (PI3K) γ inhibit AAA development in mouse models. Phenotypic changes in vascular smooth muscle cells (VSMCs) favour apoptosis, senescence and necroptosis. The damaged cells can be effectively cleared by autophagy.

Cyclodextrin, spermidine and mitochondrial fission inhibitors have been reported to aid autophagy and limit apoptosis thereby limiting AAA development in mouse models. Proteases, such as matrix metalloproteinases and chymase, are strongly implicated in remodeling of the extracellular matrix and fragmentation of the aortic media. The actions of these proteinases can be blocked by a range of novel agents that successfully limit AAA development in mouse models. Micro-calcification involving hydroxyapatite formation has been demonstrated in both experimental and human AAA and correlated with AAA progression. Small interfering RNA targeting Runt-Related Transcription Factor 2 (RTF2) is effective at limiting AAA development in mouse models. Epigenetic mechanisms implicated in AAA include long non-coding RNAs (Lnc-RNA) p21, GAS5 and plasmacytoma variant translocation 1 (PVT1). MicroRNA (miR)-144-5p has been reported to inhibit AAA development in mice. Intra-luminal thrombus is a consistent feature of human AAA and implicated in release of inflammatory cells and proteases that promote AAA. Blocking factors Xa or XII have been reported to limit AAA development in mice. Targeting these aspects has been shown to decrease AAA development in mouse models. NLRP3:nucleotide-binding oligomerization domain–like receptor pyrin domain containing 3; SGLT-2: sodium-glucose cotransporter 2. Adapted from a previous published figure with permission {Golledge, 2019 #16}.

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Table 1: Human relevant characteristics of commonly used and new mouse models of AAA

Model	Severity of aortic dilatation	Aortic rupture	Progressive dilatation	Abdominal aorta dilation only	Risk factor for AAA				Histological, genomic and imaging features	Longest period studied (weeks)
					Old age	Male sex	Smoking or cigarette products	Dyslipidaemia		
AngII (subcutaneous) (Daugherty A, 2000)	Moderate	Common	Yes	No	No	Yes	Yes	Yes	Aortic wall dissection and haematoma; inflammation, angiogenesis and proteolysis	12
Elastase (luminal)(Thompson RW, 2006; Yue J, 2020)	Moderate	Rare	Limited	Yes	NR	Yes	Yes	No	Transmural inflammation, elastic fibre destruction and angiogenesis	8
Calcium chloride or phosphate (adventitial)(Wang Y, 2013)	Mild	No	No	Yes	No	NR	NR	Yes	Aortic calcification, inflammation, angiogenesis and proteolysis	6

Elastase (adventitial)(Busch A, 2016)	Moderate	No	No	Yes	No	No	NR	NR	Adventitial inflammation and mild elastic fibre thinning and inflammation	14
Elastase (adventitial) and BAPN oral(Lu G, 2017; Romary DJ, 2019)	Very severe	Common	Yes	Yes	No	NR	NR	NR	Intraluminal thrombus formation, medial elastin fragmentation, medial thinning, influx of T cells to the aorta and MMPs	14
Elastase (adventitial) or angiotensin (subcutaneous) and TGFβ-blocking antibody(Larey re F, 2017)	Severe	Very common	Yes	Yes	NR	NR	NR	No	Intraluminal thrombus formation, medial elastin fragmentation, angiogenesis, leukocyt	2

									es and MMPs	
Angiotensin (subcutaneous) and TGFβ-blocking antibody(Wang Y, 2010)	Severe	Very common	Yes	No	NR	NR	NR	NR	Intramural haematoma, aortic dissection, inflammation and extracellular matrix degradation	4
Elastase (luminal) and Angiotensin II (subcutaneous) (Yue J, 2020)	Severe	Very common	NR	No	NR	NR	NR	NR	Upregulation of cytokines	2
AngII (subcutaneous) and BAPN oral or subcutaneous(Cooper HA, 2020; Kanematsu Y, 2010)	Moderate	Common	NR	No	NR	NR	NR	NR	Medial elastin fragmentation, influx of macrophages, upregulation of markers of oxidative stress, inflammation and MMPs	6
Elastase (luminal) and flow restriction (Busch A, 2018)	Severe	NR	Limited	Yes	NR	NR	NR	NR	Presumed to be same as standard model	4

Extended elastase (luminal)(Busch A, 2018)	Moderate	Rare	Limited	Extended to Juxta-renal aorta or iliac arteries	NR	NR	NR	NR	Presumed to be same as standard model	4

BAPN: ß3-aminopropionitrile fumarate salt; MMP: Matrix metalloproteinase; NR: not reported; TGF: transforming growth factor-β. Adapted from a previously published table.(Golledge, 2019)

Table 2: Methods of assessing outcomes in mouse models of AAA

Outcome assessment method	Adaptations	Outcome measures	Advantages	Disadvantages	Examples references
Histology and molecular biology techniques	Electron microscopy RNA sequencing Proteomics	Morphometry Aortic inflammation Aortic matrix degradation Expression of genes and proteins	Widely available Can assess wide range of molecular pathways	Require terminal samples	(Lareyre F, 2017; Xiong W, 2009; Yao Y, 2020)
Ultrasound	High resolution Four dimensional Pulse wave imaging	Maximum AAA diameter Circumferential strain	Assessment *in vivo* Easily repeated Rapid Suitable machines commonly available Used in clinical practice	Measurement error	(Cooper HA, 2020; Hiromi T, 2020; Li Z, 2020; Nandlall SD, 2016; Romary DJ, 2019; Sharma N; Shi X, 2020)
Magnetic resonance imaging	Elastin-specific probe Iron oxide particles Fibrin-specific probe MMP-specific probe Albumin-binding probe	Maximum AAA diameter Intra-luminal or mural hematoma Aortic inflammation Aortic matrix degradation Vascular permeability	Assessment *in vivo* High resolution Wide ranging assessments	Suitable machines not widely available Expensive Time consuming Not widely available	(Adams LC, 2020; Botnar RM, 2018; Brangsch J, 2019; Yao Y, 2020)
Computed tomography	Positron emission tomography CCR2-targeted Gold nanoparticles conjugated with an elastin antibody (EL-GNP)	Maximum AAA diameter Intra-luminal or mural hematoma Aortic inflammation Aortic matrix degradation	Assessment *in vivo* Rapid to complete Wide ranging assessments	Expensive Not widely available Require radiation protection	(English SJ, 2020; Gandhi R, 2020; Shannon AH, 2020)

MMP: matrix metalloproteinase; CCR-2: chemokine receptor type 2.

Table 3: Studies examining mechanisms involved in AAA progression in mouse models

AAA induction	Strain	N	AAA establishment period (days)*	Monitoring period (days)	Intervention	Outcome assessment	Effect on AAA growth	Mechanism	Reference
AngII	Cre-Lox system plus AAV-PCSK9 gain of function	18	28	28	HPβCD	US	Reduced	Activates TFEB, reduced elastin fragmentation and apoptosis	(Lu H, 2020)
AngII	ApoE$^{-/-}$	20	7	21	Kallistatin	NR	Reduced	Reduced Wnt pathway and ICAM-1 expression	(He Y, 2020)
AngII	LDLR$^{-/-}$	31	28	56	High fat diet	US Morphometry	Promotes	NR	(Liu J, 2020)
Elastase (luminal)	C57BL/6	17	3	11	Spermidine**	Morphometry	NSA	NA	(Liu S, 2020)
Elastase (luminal)	C57BL/6	20	14	14	pentagalloyl glucose (PGG)-loaded nanoparticles‡	US Morphometry	Reduced	Decreased macrophage infiltration & TGFß-1	(Dhital S, 2020)
Elastase (luminal)	C57BL/6	16	4	10	Ulinastatin	US	Reduced	Reduced elastin degradation, macrophages, T & B cells and angiogenesis	(Li G, 2020)
AngII	ApoE$^{-/-}$	33	14	4	Depletion of CD11c+	US, Morphometry	Reduced	Down-regulated circulatin	(Krishna, Moran,

					dendritic Cells			g effector T cells and attenuated matrix degradation	Jose, Lazzaroni, Huynh & Golledge, 2019)
AngII	ApoE$^{-/-}$	36	28	4	Notch inhibitor	US, Morphometry	Reduced	Reduced inflammatory response	(Sharma et al., 2019)
AngII	ApoE$^{-/-}$	36	28	8	Ramipril or Carvedilol	Micro-computed tomography	Reduced	Decreased MCP-1	(Park, Hong, Kim, Jung, Kim & Choi, 2019)
Ang II	ApoE$^{-/-}$	21	3	28	Chymase Inhibitor	Morphometry	Reduced	Decreased pro-MMP9	(Tomimori, Manno, Tanaka, Futamura-Takahashi, Muto & Nagahira, 2019)

* before intervention started; ** A natural polyamine; † definition of AAA varied; ‡ A natural polyphenol; AAA: abdominal aortic aneurysm; AAV: Adeno virus associated; AngII: angiotensin II; Cre-Lox: site-specific recombinase technology; ICAM: intercellular adhesion molecule; LDLR$^{-/-}$: low density lipoprotein receptor deficient; ApoE$^{-/-}$: Apolipoprotein E deficient; Morphometry include analysis of photographs of excised aortas or direct measurement at laparotomy; NA: not applicable; NR: not reported; NSA: No statistically significant effect of intervention, i.e. $p \geq 0.05$; PCSK9: Proprotein convertase subtilisin/kexin type 9; TGF: transforming growth factor; US: ultrasound; Wnt: wingless pathway.

Table 1: Human relevant characteristics of commonly used and new mouse models of AAA

Model	Severity of aortic dilatation	Aortic rupture	Progressive dilatation	Abdominal aorta dilation only	Risk factor for AAA				Histological, genomic and imaging features	Longest period studied (weeks)
					Old age	Male sex	Smoking or cigarette products	Dyslipidaemia		
AngII (subcutaneous) (Daugherty A, 2000)	Moderate	Common	Yes	No	No	Yes	Yes	Yes	Aortic wall dissection and haematoma; inflammation, angiogenesis and proteolysis	12
Elastase (luminal)(Thompson RW, 2006; Yue J, 2020)	Moderate	Rare	Limited	Yes	NR	Yes	Yes	No	Transmural inflammation, elastic fibre destruction and angiogenesis	8
Calcium chloride or phosphate (adventitial)(Wang Y, 2013)	Mild	No	No	Yes	No	NR	NR	Yes	Aortic calcification, inflammation, angiogenesis and	6

									proteolysis	
Elastase (adventitial)(Busch A, 2016)	Moderate	No	No	Yes	No	No	NR	NR	Adventitial inflammation and mild elastic fibre thinning and inflammation	14
Elastase (adventitial) and BAPN oral(Lu G, 2017; Romary DJ, 2019)	Very severe	Common	Yes	Yes	No	NR	NR	NR	Intraluminal thrombus formation, medial elastin fragmentation, medial thinning, influx of T cells to the aorta and MMPs	14
Elastase (adventitial) or angiotensin (subcutaneous) and TGFβ-blocking antibody(Larey re F, 2017)	Severe	Very common	Yes	Yes	NR	NR	NR	No	Intraluminal thrombus formation, medial elastin fragmentation, angiogenesis,	2

									leukocytes and MMPs	
Angiotensin (subcutaneous) and TGFβ-blocking antibody(Wang Y, 2010)	Severe	Very common	Yes	No	NR	NR	NR	NR	Intramural haematoma, aortic dissection, inflammation and extracellular matrix degradation	4
Elastase (luminal) and Angiotensin II (subcutaneous) (Yue J, 2020)	Severe	Very common	NR	No	NR	NR	NR	NR	Upregulation of cytokines	2
AngII (subcutaneous) and BAPN oral or subcutaneous(Cooper HA, 2020; Kanematsu Y, 2010)	Moderate	Common	NR	No	NR	NR	NR	NR	Medial elastin fragmentation, influx of macrophages, upregulation of markers of oxidative stress, inflammation and MMPs	6
Elastase (luminal) and flow restriction	Severe	NR	Limited	Yes	NR	NR	NR	NR	Presumed to be same as	4

(Busch A, 2018)									standard model	
Extended elastase (luminal)(Busch A, 2018)	Moderate	Rare	Limited	Extended to Juxta-renal aorta or iliac arteries	NR	NR	NR	NR	Presumed to be same as standard model	4

BAPN: ß3-aminopropionitrile fumarate salt; MMP: Matrix metalloproteinase; NR: not reported; TGF: transforming growth factor-β. Adapted from a previously published table.(Golledge, 2019)

Table 2: Methods of assessing outcomes in mouse models of AAA

Outcome assessment method	Adaptations	Outcome measures	Advantages	Disadvantages	Examples references
Histology and molecular biology techniques	Electron microscopy RNA sequencing Proteomics	Morphometry Aortic inflammation Aortic matrix degradation Expression of genes and proteins	Widely available Can assess wide range of molecular pathways	Require terminal samples	(Lareyre F, 2017; Xiong W, 2009; Yao Y, 2020)
Ultrasound	High resolution Four dimensional Pulse wave imaging	Maximum AAA diameter Circumferential strain	Assessment *in vivo* Easily repeated Rapid Suitable machines commonly available Used in clinical practice	Measurement error	(Cooper HA, 2020; Hiromi T, 2020; Li Z, 2020; Nandlall SD, 2016; Romary DJ, 2019; Sharma N; Shi X, 2020)
Magnetic resonance imaging	Elastin-specific probe Iron oxide particles Fibrin-specific probe MMP-specific probe Albumin-binding probe	Maximum AAA diameter Intra-luminal or mural hematoma Aortic inflammation Aortic matrix degradation Vascular permeability	Assessment *in vivo* High resolution Wide ranging assessments	Suitable machines not widely available Expensive Time consuming Not widely available	(Adams LC, 2020; Botnar RM, 2018; Brangsch J, 2019; Yao Y, 2020)
Computed tomography	Positron emission tomography CCR2-targeted Gold nanoparticles conjugated with an elastin antibody (EL-GNP)	Maximum AAA diameter Intra-luminal or mural hematoma Aortic inflammation Aortic matrix degradation	Assessment *in vivo* Rapid to complete Wide ranging assessments	Expensive Not widely available Require radiation protection	(English SJ, 2020; Gandhi R, 2020; Shannon AH, 2020)

MMP: matrix metalloproteinase; CCR-2: chemokine receptor type 2.

Table 3: Studies examining mechanisms involved in AAA progression in mouse models

AAA induction	Strain	N	AAA establishment period (days)*	Monitoring period (days)	Intervention	Outcome assessment	Effect on AAA growth	Mechanism	Reference
AngII	Cre-Lox system plus AAV-PCSK9 gain of function	18	28	28	HPβCD	US	Reduced	Activates TFEB, reduced elastin fragmentation and apoptosis	(Lu H, 2020)
AngII	ApoE$^{-/-}$	20	7	21	Kallistatin	NR	Reduced	Reduced Wnt pathway and ICAM-1 expression	(He Y, 2020)
AngII	LDLR$^{-/-}$	31	28	56	High fat diet	US Morphometry	Promotes	NR	(Liu J, 2020)
Elastase (luminal)	C57BL/6	17	3	11	Spermidine**	Morphometry	NSA	NA	(Liu S, 2020)
Elastase (luminal)	C57BL/6	20	14	14	pentagalloyl glucose (PGG)-loaded nanoparticles‡	US Morphometry	Reduced	Decreased macrophage infiltration & TGFß-1	(Dhital S, 2020)
Elastase (luminal)	C57BL/6	16	4	10	Ulinastatin	US	Reduced	Reduced elastin degradation, macrophages, T & B cells and angiogenesis	(Li G, 2020)
AngII	ApoE$^{-/-}$	33	14	4	Depletion of CD11c+	US, Morphometry	Reduced	Down-regulated circulatin	(Krishna, Moran,

					dendritic Cells			g effector T cells and attenuated matrix degradation	Jose, Lazzaroni, Huynh & Golledge, 2019)
AngII	ApoE$^{-/-}$	36	28	4	Notch inhibitor	US, Morphometry	Reduced	Reduced inflammatory response	(Sharma et al., 2019)
AngII	ApoE$^{-/-}$	36	28	8	Ramipril or Carvedilol	Micro-computed tomography	Reduced	Decreased MCP-1	(Park, Hong, Kim, Jung, Kim & Choi, 2019)
Ang II	ApoE$^{-/-}$	21	3	28	Chymase Inhibitor	Morphometry	Reduced	Decreased pro-MMP9	(Tomimori, Manno, Tanaka, Futamura-Takahashi, Muto & Nagahira, 2019)

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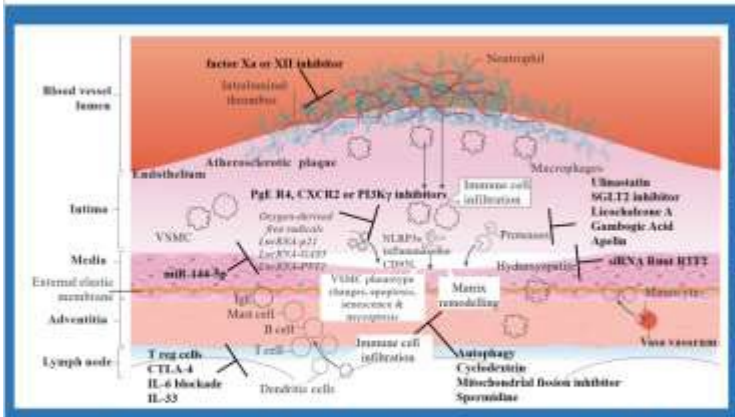
Mouse models for abdominal aortic aneurysm



Potential targets for an abdominal aortic aneurysm drug.

Innate and adaptive immunity, autophagy, protease-mediated extracellular matrix remodeling, hydroxyapatite-mediated micro-calcification, epigenetic changes, and intra-luminal thrombus are strongly implicated in abdominal aortic aneurysm (AAA). Immune response regulators, autophagy promoting agents, protease inhibitors, small interfering RNA inhibiting hydroxyapatite formation, microRNAs and coagulation cascade inhibitors have been reported to limit AAA development in mice.

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