Clinical Science (2020) **134** 1049–1061
https://doi.org/10.1042/CS20191020

PORTLAND PRESS

Research Article

Factor XII blockade inhibits aortic dilatation in angiotensin II-infused apolipoprotein E-deficient mice

Corey S. Moran[1], Sai-Wang Seto[2], Erik Biros[1], Smriti M. Krishna[1], Susan K. Morton[1], Christoph Kleinschnitz[3], Con Panousis[4] and Jonathan Golledge[1,5]

[1]Queensland Research Centre for Peripheral Vascular Disease, College of Medicine and Dentistry, James Cook University, Townsville, Australia; [2]Department of Applied Biology and Chemical Technology, The Hong Kong Polytechnic University, Hong Kong, China; [3]Department of Neurology, Essen University Hospital, Essen, Germany; [4]Research and Development, CSL Limited, Parkville, Australia; [5]Department of Vascular and Endovascular Surgery, The Townsville University Hospital, Townsville, Australia

Correspondence: Jonathan Golledge (jonathan.golledge@jcu.edu.au)

Abdominal aortic aneurysm (AAA) is an important cause of mortality in older adults. Chronic inflammation and excessive matrix remodelling are considered important in AAA pathogenesis. Kinins are bioactive peptides important in regulating inflammation. Stimulation of the kinin B2 receptor has been previously reported to promote AAA development and rupture in a mouse model. The endogenous B2 receptor agonist, bradykinin, is generated from the kallikrein–kinin system following activation of plasma kallikrein by Factor XII (FXII). In the current study whole-body FXII deletion, or neutralisation of activated FXII (FXIIa), inhibited expansion of the suprarenal aorta (SRA) of apolipoprotein E-deficient mice in response to angiotensin II (AngII) infusion. FXII deficiency or FXIIa neutralisation led to decreased aortic tumor necrosis factor-α-converting enzyme (TACE/a disintegrin and metalloproteinase-17 (aka tumor necrosis factor-α-converting enzyme) (ADAM-17)) activity, plasma kallikrein concentration, and epithelial growth factor receptor (EGFR) phosphorylation compared with controls. FXII deficiency or neutralisation also reduced Akt1 and Erk1/2 phosphorylation and decreased expression and levels of active matrix metalloproteinase (Mmp)-2 and Mmp-9. The findings suggest that FXII, kallikrein, ADAM-17, and EGFR are important molecular mediators by which AngII induces aneurysm in apolipoprotein E-deficient mice. This could be a novel pathway to target in the design of drugs to limit AAA progression.

Introduction

Abdominal aortic aneurysm (AAA) is an important cause of death in older adults due to aortic rupture or complications of surgical repair [1]. Currently the only treatment for AAA is surgical repair but previous clinical trials suggest this is not beneficial for small aneurysms or people that are deemed unfit [2,3]. AAA is frequently diagnosed when the aneurysm is small and at low risk of rupture [4]. The natural history of most small AAAs is expansion to a larger size when the risk of rupture is much greater. Drug therapies that are effective at halting AAA growth could be very beneficial in the management of people with early-stage AAA [5].

Kinins are potent bioactive peptides important in regulating vascular permeability and inflammation following tissue injury [6]. Administration of a kinin-receptor agonist has been reported to amplify the response to angiotensin II (AngII) within a mouse model of AAA and promote the formation of large AAAs that commonly rupture [7]. A kinin-receptor antagonist limited aneurysm growth and reduced aortic rupture in the same experimental model [7]. These findings suggested that blocking kinin signalling could

Received: 30 September 2019
Revised: 05 April 2020
Accepted: 20 April 2020

Accepted Manuscript online: 20 April 2020
Version of Record published: 04 May 2020

Clinical Science (2020) **134** 1049–1061
https://doi.org/10.1042/CS20191020

be a potential treatment strategy to limit AAA progression, although how to achieve this most effectively and safely remained unclear.

The contact system, initiated by Factor XII (FXII), can stimulate both prothrombotic and proinflammatory pathways, which are both implicated in AAA pathogenesis [1,7–11]. The inflammatory arm of the contact system is initiated when FXII activation leads to release of the the most well-known kinin, bradykinin, through activation of the plasma kallikrein–kinin system [12,13]. Plasma kallikrein released from prekallikrein by activated FXII (FXIIa) generates bradykinin from high molecular weight kininogen at the luminal surface of blood vessels [14]. It was hypothesised that FXII blockade would limit kinin activation and prevent progression of AAA pathological processes.

The current study investigated the effect of FXII deficiency and FXIIa neutralisation on the severity of aortic dilatation in apolipoprotein E-deficient ($ApoE^{-/-}$) mice in response to AngII.

Methods

FXIIa inhibitor

The FXIIa neutralising antibody 3F7 (ch3F7-mG1L-aFXII) and control peptide were kindly provided by Dr. Con Panousis of CSL Limited, Australia.

Mouse model and *in vivo* studies

The mouse studies were performed in accordance with institutional and ethical guidelines of James Cook University, Australia (AEC approvals A1883 and A2252), and conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, U.S.A.) and the ARRIVE criteria set by The National Centre for the Replacement, Refinement and Reduction of Animals in Research (London, U.K.).

Mice

The Animal Resources Centre, Western Australia, supplied male $ApoE^{-/-}$ mice. $ApoE^{-/-}$ mice deficient in FXII ($ApoE^{-/-}FXII^{-/-}$) were generated through crossbreeding with $FXII^{-/-}$ mice kindly provided by Professor Christoph Kleinschnitz, University Hospital Essen, Germany. All mice were housed under a 12:12-h light–dark cycle (relative humidity: 55–60%; temperature: 22 $\pm$ 1°C) and were given standard chow and water *ad libitum*.

Aortic dilatation and intervention

Aortic dilatation was induced in 6-month-old male $ApoE^{-/-}$ mice by subcutaneous infusion of AngII (1.0 μg/kg/min) for 28 days as previously described [7,15]. Briefly, an osmotic micro-pump (ALZET Model 1004, Durect Corporation) containing AngII (#A9525, Sigma–Aldrich) dissolved in sterile water was inserted into the subcutaneous space left of the dorsal midline under general anaesthesia (4% isoflurane) to administer AngII at a rate of 1.0 μg/kg/min over 28 days. Intraperitoneal administration of 3F7 commenced 24 h prior to placement of the AngII pumps, then every 2 days over the 28-day AngII infusion period. Sudden mouse fatality required necropsy within 24 h to confirm aortic rupture as cause of death. Mice completing the study protocol were isoflurane-sedated and CO_2-killed. Harvested aortas were phosphate buffered saline-perfused *in situ* and digitally photographed (Coolpix 4500, Nikon).

Studies

Two studies investigated the effect of FXIIa down-regulation on AngII-induced aortic dilatation:

(i) *FXII deficiency:* $ApoE^{-/-}FXII^{-/-}$ (n=26) and $ApoE^{-/-}$ (control, n=20) mice were infused with AngII over 28 days;
(ii) *FXIIa neutralisation:* $ApoE^{-/-}$ mice were allocated to receive the FXIIa-blocking antibody 3F7 (10 mg/kg; n=15) or isotype control (n=15) and infused with AngII over 28 days.

Assessment of suprarenal aorta dilatation

Ultrasound measurements of the suprarenal aorta (SRA) were obtained prior to AngII infusion (day 0) then at days 14 and 28 of the study period. Scans were performed in sedated mice (4% isoflurane) using yLab™ 70 VETXV platform (Esaote, Italy) with a 40-mm linear transducer at an operating frequency of 10 MHz (LA435; Esaote, Italy) to provide a sagittal image of the SRA. Maximum outer-wall SRA diameter (SRA_{Max}) was measured at peak systole using the calliper measurement feature. Good interobserver reproducibility of these measurements in our laboratory has been previously reported [15].

Protein analysis

Mouse serum samples were assayed using commercial ELISAs for plasma kallikrein (MBS705761, MyBioSource) and bradykinin (EK-009-01, Phoenix Pharmaceutical Inc). For aortic samples, protein was obtained by RIPA buffer (Sigma–Aldrich) homogenisation of tissue harvested at the end of the study and sample protein concentration determined using the Bradford reagent (Bio-Rad Laboratories) according to the manufacturer's recommendations. Protein samples were assayed using commercial kits for plasma kallikrein (MBS705761, MyBioSource), bradykinin (ADI-900-206, Enzo Life Sciences), phosphorylated epidermal growth factor receptor (pEGFR; MBS751770, MyBioSource), myeloperoxidase (MPO; HK210-02, Hycult Biotech), active matrix metalloproteinase (Mmp)-2 (QZBmmp2M, QuickZyme Biosciences) and Mmp-9 (QZBmmp9M, QuickZyme Biosciences), total and phosphorylated protein kinase B (Akt1, ab176657, SimpleStep ELISA™, Abcam), and total and phosphorylated extracellular signal-regulated kinase (Erk1/2, ab176660, SimpleStep ELISA™, Abcam). Tumor necrosis factor-α-converting enzyme (TACE/a disintegrin and metalloproteinase-17 (aka tumor necrosis factor-α-converting enzyme) (ADAM-17)) activity in SRA protein samples was measured using the SensoLyte® 520 Activity Assay (AS-72085, AnaSpec).

Real-time PCR

QuantiTect® Primer Assays were used to determine gene expression for kinin receptor B1 (*Bdkrb1;* QT00326886) and B2 (*Bdkrb2;* QT00111027) in mouse aortic tissue using quantitative real time (qPCR) as previously described [16]. The relative expression of these genes in experimental and control samples were calculated by using the concentration-C_t-standard curve method and normalised using the average expression of glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*; QT01658692) for each sample using the Rotor-Gene Q operating software version 2.0.24. The QuantiTect SYBR® Green one-step RT-PCR Kit (Qiagen) was used according to the manufacturer's instructions with 40 ng of total RNA as template. All reactions were independently repeated in duplicate to ensure the reproducibility of the results and the mean of the two values for each sample was used for analyses.

Statistical analysis

Data were analysed using GraphPad Prism (version 7) and TIBCO Spotfire S+ (version 8.2). D'Agostino and Pearson's test was used to assess data normality. Parametric or non-parametric tests were applied appropriately to data distribution. Data are presented as median and interquartile range with maximum and minimum points unless otherwise stated. Student's *t* or Mann–Whitney U tests were used for two-group comparisons of parametric and non-parametric data, respectively. Repeated measures one-way ANOVA followed by Tukey's multiple comparisons test (parametric), or Kruskal–Wallis test followed by Dunn's multiple comparisons test (non-parametric), were used to compare data within each group obtained as a function of time, with statistical significance between groups (control vs intervention) determined by mixed-effects linear regression. A contingency analysis applied Fisher's exact test. A *P*-value less than 0.05 was considered statistically significant in all analyses.

Results

FXII deficiency and FXIIa neutralisation reduced circulating and aortic levels of plasma kallikrein and bradykinin

Circulating and aortic levels of plasma kallikrein and bradykinin were determined in serum and SRA samples harvested from $ApoE^{-/-}FXII^{-/-}$ mice, $ApoE^{-/-}$ mice administered the FXIIa-neutralising antibody 3F7 (10 mg/kg/48 h, i.p.) and $ApoE^{-/-}$ control mice. $ApoE^{-/-}FXII^{-/-}$ mice and $ApoE^{-/-}$ mice receiving 3F7, had significantly lower serum concentration of plasma kallikrein and bradykinin compared with $ApoE^{-/-}$ controls (Figure 1). Concentrations of plasma kallikrein and bradykinin within the SRA of $ApoE^{-/-}$ mice were also significantly reduced by FXII deficiency in comparison with controls (Figure 2). FXIIa neutralisation significantly reduced SRA concentration of plasma kallikrein, but not bradykinin, compared with controls (Figure 2).

Factor XII deficiency and FXIIa neutralisation limited AngII-induced SRA dilatation

SRA_{Max} was measured by ultrasound prior to AngII pump insertion (baseline) and at days 14 and 28 of the experimental period. Baseline SRA_{Max} was comparable in $ApoE^{-/-}$ and $ApoE^{-/-}FXII^{-/-}$ mice and in $ApoE^{-/-}$ mice allocated to receive either 3F7 or as controls (Supplementary Figure S1). SRA_{Max} in all mice increased in response to

Clinical Science (2020) **134** 1049–1061
https://doi.org/10.1042/CS20191020

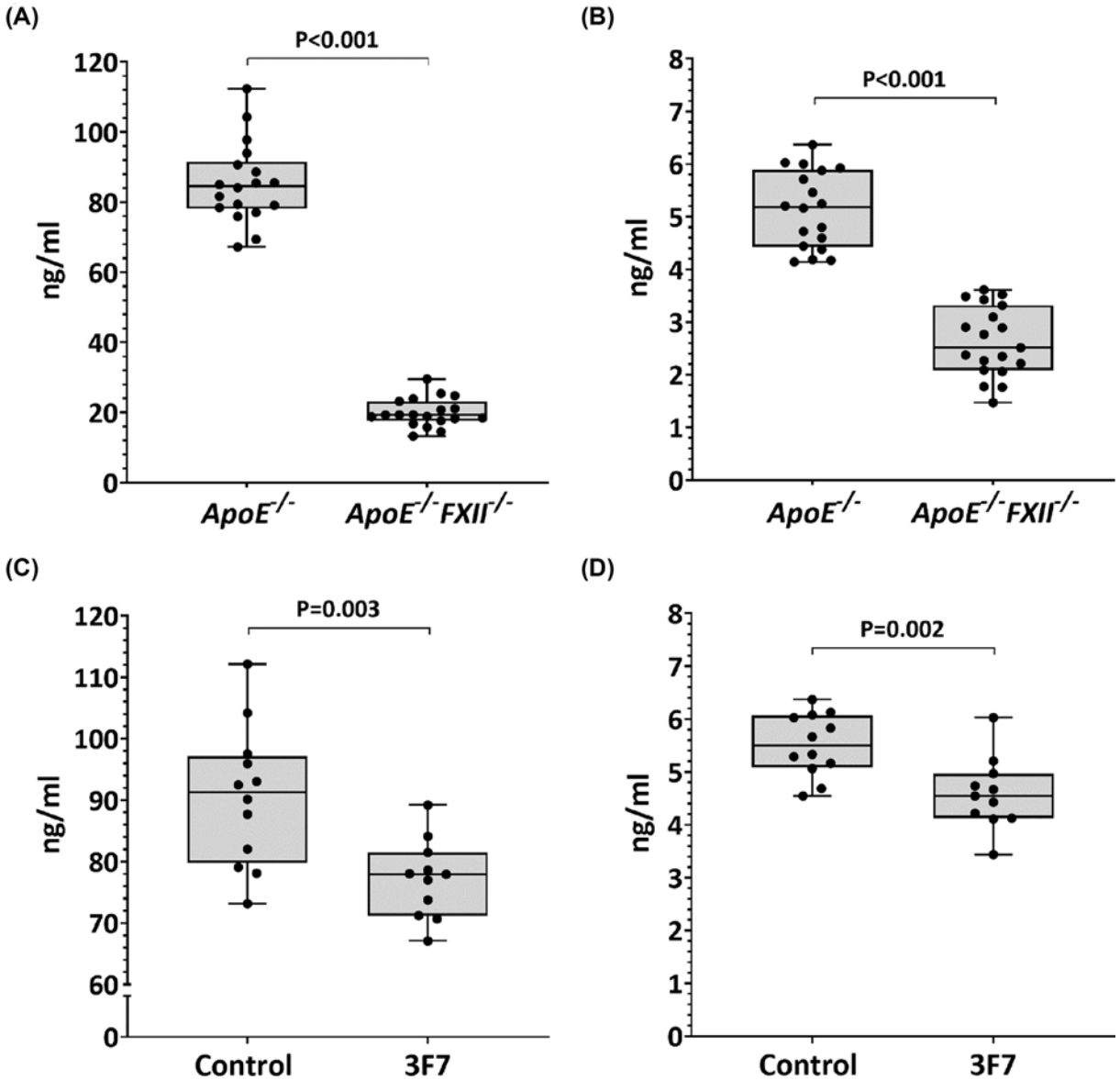

Figure 1. Circulating plasma kallikrein and bradykinin

Serum concentration of plasma kallikrein (**A**,**C**) and bradykinin (**B**,**D**) in AngII-infused *ApoE*$^{-/-}$ mice genetically deficient for FXII (ApoE$^{-/-}$FXII$^{-/-}$; A,B), or administered the FXIIa-neutralising antibody 3F7 (C,D), compared with control mice after 28 days. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for nanograms (ng) of protein per millilitre (ml) of serum.

AngII infusion over 28 days (Figure 3A,B). The degree of SRA dilatation was significantly less in *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice and *ApoE*$^{-/-}$ mice administered 3F7 compared with controls (Figure 3C,D and Supplementary Figures S2 and S3). Seven out of twenty-six *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice had aortic rupture compared with two out of twenty *ApoE*$^{-/-}$ control mice (P=0.262; Fisher's exact test). The incidence of aortic rupture in mice administered 3F7 was 4 out of 15 compared with 3 out of 15 in control mice (P>0.999; Fisher's exact test).

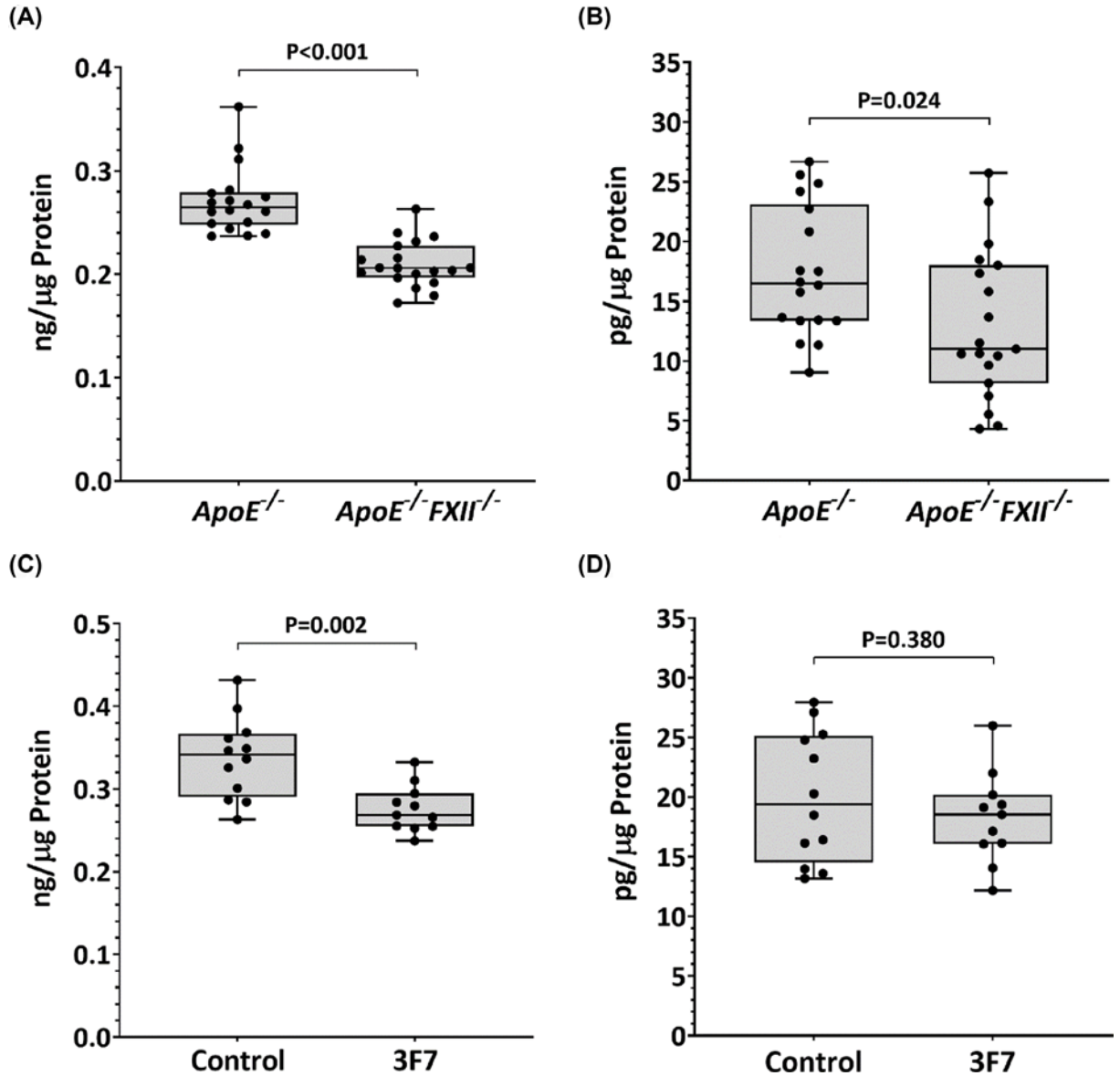

Figure 2. Plasma kallikrein and bradykinin within the suprarenal aorta (SRA)

Concentration after 28 days of plasma kallikrein (**A**) and bradykinin (**B**) within the SRA of AngII-infused *ApoE*$^{-/-}$ mice genetically deficient for FXII (*ApoE*$^{-/-}$*FXII*$^{-/-}$) compared with control mice. Concentration after 28 days of plasma kallikrein (**C**) and bradykinin (**D**) within the SRA of AngII-infused *ApoE*$^{-/-}$ mice administered the FXIIa-neutralising antibody 3F7 compared with control. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for nanograms (ng) of plasma kallikrein, or picograms (pg) of bradykinin, per microgram (µg) of SRA protein.

FXII deficiency and FXIIa neutralisation reduced ADAM-17 activity and EGFR phosphorylation

SRA protein samples obtained from *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice, *ApoE*$^{-/-}$ mice administered 3F7 and *ApoE*$^{-/-}$ controls at completion of the study (day 28) were assayed. ADAM-17 activity and levels of phosphorylated EGFR were significantly lower in *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice and *ApoE*$^{-/-}$ mice administered 3F7, compared with *ApoE*$^{-/-}$ controls (Figure 4).

Clinical Science (2020) **134** 1049–1061
https://doi.org/10.1042/CS20191020

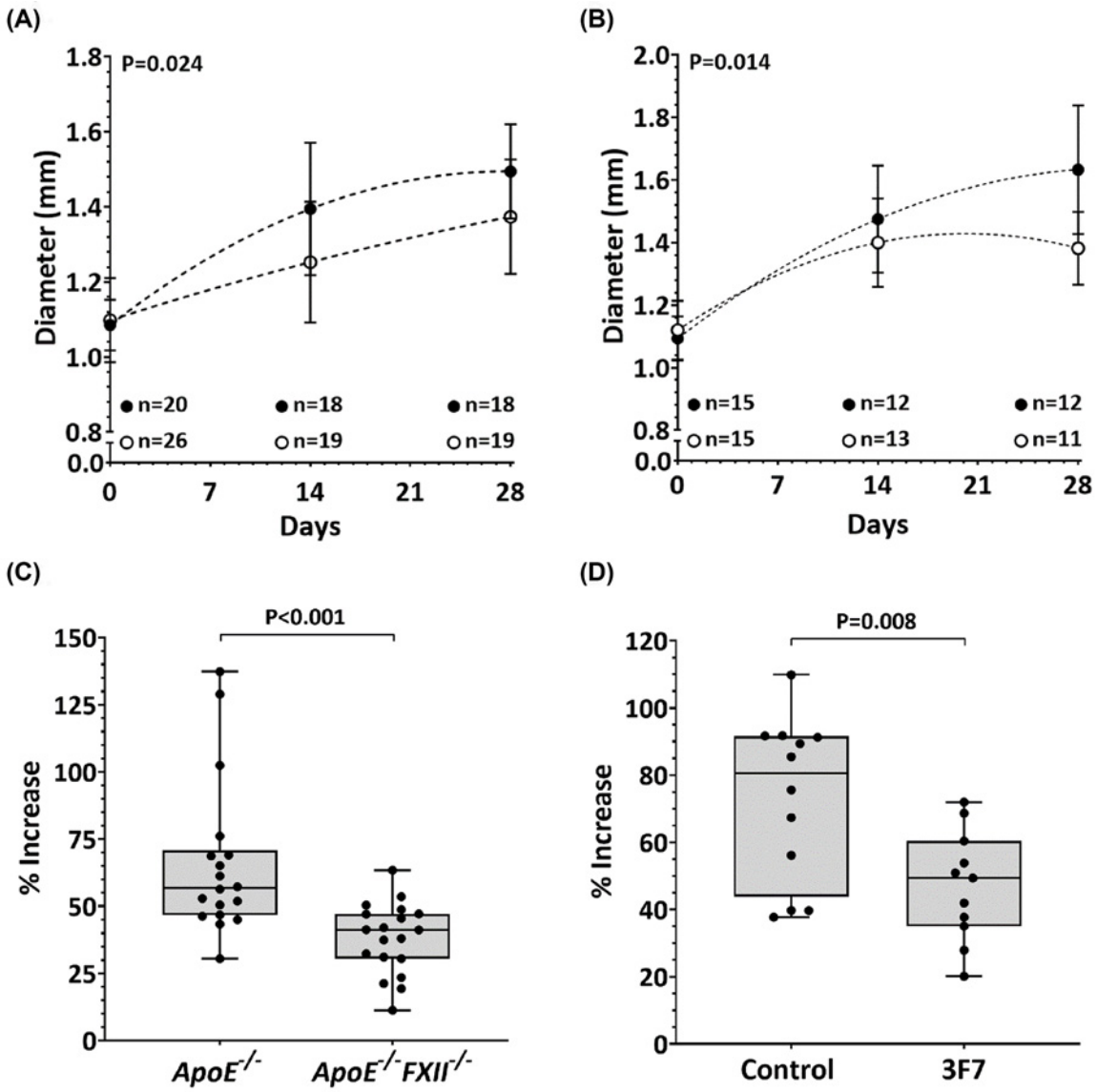

Figure 3. AngII-induced SRA dilatation

(**A**) Maximum SRA diameter by ultrasound over 28 days in AngII-infused *ApoE*$^{-/-}$ mice genetically deficient for FXII (*ApoE*$^{-/-}$*FXII*$^{-/-}$; open circle) compared with *ApoE*$^{-/-}$control mice (closed circle). (**B**) Maximum SRA diameter by ultrasound over 28 days in AngII-infused *ApoE*$^{-/-}$ mice administered the FXIIa-neutralising antibody 3F7 (open circle) compared with isotype control (closed circle). Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for maximum SRA diameter (mm); *P*-values calculated for difference between groups by mixed-effects linear regression. Severity of AngII-induced SRA dilatation in *ApoE*$^{-/-}$ mice genetically deficient for FXII (ApoE$^{-/-}$FXII$^{-/-}$; **C**), or administered the FXIIa-neutralising antibody 3F7 (**D**), compared with control mice after 28 days. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for percent increase in diameter relative to day 0 (baseline).

FXII deficiency and FXIIa neutralisation differentially regulated SRA kinin receptor gene expression and neutrophil activation

Gene expression was assessed in SRA samples from *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice, *ApoE*$^{-/-}$ mice administered 3F7 and *ApoE*$^{-/-}$ controls at completion of the study (day 28). FXII deficiency resulted in significant down-regulation in gene expression for both the kinin B1 and the B2 receptors (Figure 5A and Supplementary Figure S4). Gene expression for the kinin B2 receptor, but not the kinin B1 receptor, was also significantly lower within the SRA of *ApoE*$^{-/-}$ mice receiving the FXIIa neutralising antibody by comparison with controls (Figure 5A and Supplementary Figure

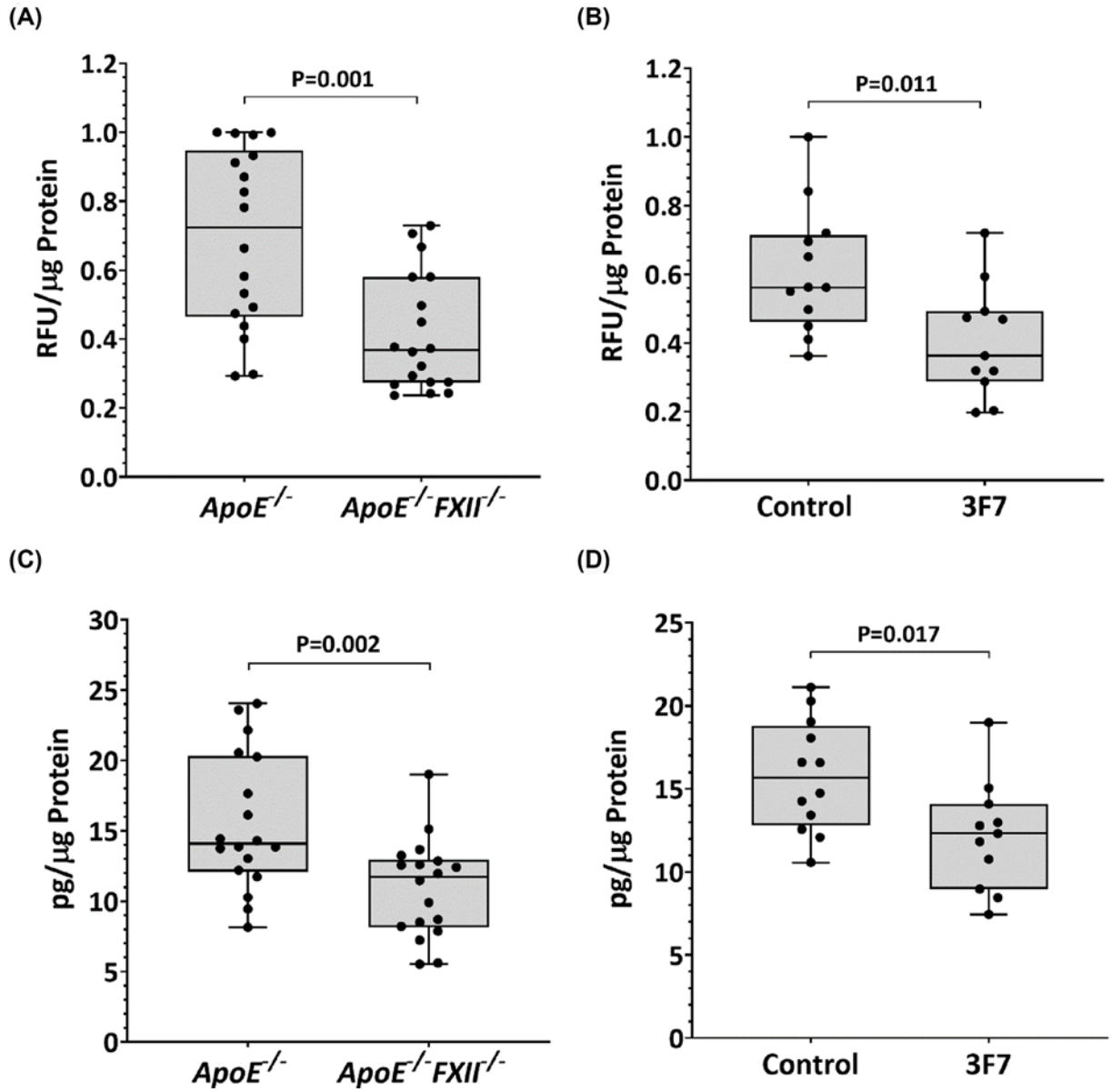

Figure 4. Aortic ADAM-17 and EGFR

ADAM-17 activity (**A**,**B**) and phosphorylated EGFR (**C**,**D**) within the SRA of AngII-infused *ApoE*$^{-/-}$ mice either genetically deficient for FXII (*ApoE*$^{-/-}$*FXII*$^{-/-}$) or administered the FXIIa-neutralising antibody 3F7, compared with control mice after 28 days. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for relative fluorescence units (RFUs; ADAM-17 activity), or picograms (pg) of phosphorylated EGFR per microgram (μg) SRA protein.

S4). SRA protein samples obtained from *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice, *ApoE*$^{-/-}$ mice administered 3F7 and *ApoE*$^{-/-}$ controls at completion of the study (day 28) were assayed for MPO, as a marker of neutrophil activation. The SRA concentration of MPO was significantly lower in *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice than *ApoE*$^{-/-}$ control mice (Figure 5B). Administration of FXIIa neutralising antibody did not significantly effect SRA MPO concentration (Figure 5C).

FXII deficiency and FXIIa neutralisation decreased Akt and Erk signalling and proteolytic enzyme activity within the SRA

The concentrations of total Akt1 and Erk1/2 kinases within the SRA tissue of *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice and mice receiving 3F7 were similar to concentrations in control mice (Supplementary Figure S5). In contrast, phosphorylated

Clinical Science (2020) **134** 1049–1061
https://doi.org/10.1042/CS20191020

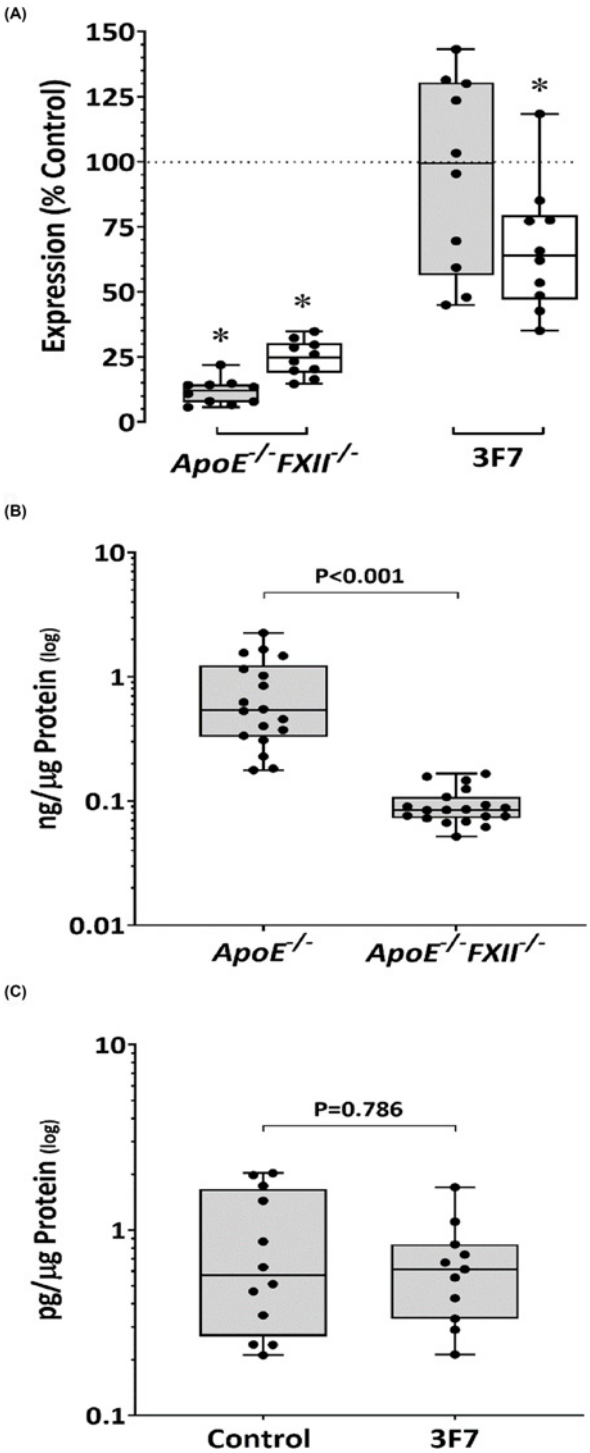

Figure 5. Aortic kinin receptor expression and neutrophil activation

(**A**) Kinin B1 (grey) and B2 (white) receptor gene expression within the SRA of AngII-infused *ApoE*$^{-/-}$ mice genetically deficient for FXII (*ApoE*$^{-/-}$*FXII*$^{-/-}$) mice or *ApoE*$^{-/-}$ mice administered the FXIIa-neutralising antibody 3F7, compared with control mice after 28 days. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for *Gapdh*-normalised gene expression relative to control; 100% relative gene expression (dotted line) means equivalent expression levels between experimental and control mice; *$P<0.05$ compared with control. Protein concentration of MPO within the SRA of AngII-infused *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice (**B**) or *ApoE*$^{-/-}$ mice administered 3F7 (**C**), compared with controls after 28 days. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for nanograms (ng) of MPO per microgram (µg) SRA protein.

Akt1 and Erk1/2 (and the ratio of phosphorylated-to-total kinase) were present at significantly lower concentrations in the SRA of mice with deficiency or neutralisation of FXII compared with controls (Figure 6 and Supplementary Figure S6). SRA protein concentrations for the active forms of Mmp-2 (Figure 6C,D) and Mmp-9 (Figure 6E,F) in $ApoE^{-/-}FXII^{-/-}$ mice and $ApoE^{-/-}$ mice administered 3F7 were significantly lower than in $ApoE^{-/-}$ controls.

Discussion

The current study demonstrated that blockade of the FXII/kallikrein pathway reduced AngII-induced aortic expansion. The contact system stimulates prothrombotic and proinflammatory pathways implicated in AAA pathogenesis [1,7–11]. The inflammatory arm of the contact system is initiated when FXII activation leads to generation of bradykinin through activation of the plasma kallikrein–kinin system [12,13]. Two separate approaches were used to investigate the role of the FXII/kallikrein pathway in aortic dilatation induced by AngII. There was whole body genetic deficiency of FXII and antibody-directed FXIIa neutralisation in the $ApoE^{-/-}$ mouse model.

AngII-induced SRA dilatation was inhibited in both $ApoE^{-/-}FXII^{-/-}$ mice and $ApoE^{-/-}$ mice administered FXIIa-neutralising antibody in the present study. This effect was associated with significant reductions in circulating concentrations of plasma kallikrein and bradykinin, consistent with a previous study demonstrating significantly lower plasma levels of bradykinin in FXII-deficient compared with wild-type mice [17]. In mice with whole body FXII deficiency there were reduced SRA concentrations of plasma kallikrein and bradykinin, down-regulation in expression of kinin B1 and B2 receptors, evidence of decreased Akt1/Erk1/2 signalling and lower concentrations of active Mmp2 and Mmp9 and MPO, compared with controls. These findings were similar, but less marked, in mice in which FXIIa was neutralised via 3F7, possibly due to less severe FXII blockade. Importantly, FXIIa neutralisation in contrast with FXII-deficiency had no significant effect on SRA bradykinin concentration despite reduced levels of aortic plasma kallikrein. Furthermore, the similarity in SRA levels of MPO and kinin B1 receptor expression between mice receiving 3F7 and controls suggested a more limited effect of FXIIa neutralisation on the inflammatory response to AngII than whole body FXII deficiency.

Reduction in plasma kallikrein within the SRA of mice administered 3F7 may have been due to the inhibition of FXIIa activity on prekallikrein within the artery wall. Following endothelial cell injury, which can be induced by AngII infusion, vascular smooth muscle cells become exposed to plasma constituents. Previous studies suggest that plasma-derived FXII binds to vascular smooth muscle cells and activates prekallikrein to plasma kallikrein at the cell surface [18]. Despite the observed reduction in plasma kallikrein within the SRA of mice receiving FXIIa neutralising antibody, bradykinin levels were not affected. It is known that infiltrating leucocytes are a significant source of kinins within the artery wall during inflammation. The proteases derived from these cells are important *non-kallikrein* mediators of bradykinin formation and their presence with the SRA may have accounted for the maintained levels of bradykinin [19–23].

Neutrophil kinin B2 receptors are believed to facilitate extravascular migration of inflammatory cells at the sites of inflammation where they participate in local release of bradykinin into inflamed tissue [24]. Bradykinin is the endogenous agonist for the kinin B2 receptor and it has been previously shown that stimulation of the kinin B2 receptor augments AngII-induced aortic dilatation and rupture in $ApoE^{-/-}$ mice [7]. This effect appeared to occur via neutrophil activation, stimulation of an inflammatory phenotype in vascular smooth muscle cells and production of proinflammatory proteins [7]. A recent study by Stavrou et al. [25] showed that human and mouse neutrophils express FXII following activation and that neutrophils from $FXII^{-/-}$ mice exhibit reduced adherence and migration compared with wild-type controls. Migration and chemotaxis in both wild-type and $FXII^{-/-}$ neutrophils were stimulated by FXII, an effect that did not require its catalytic activity (FXIIa) but rather was a zymogen FXII function [25]. It is plausible that the persistent neutrophil activation within the SRA of mice administered 3F7 was contributed to by activity of the FXII zymogen in promoting SRA leucocyte infiltration and activation independent of FXIIa neutralisation.

These findings suggested that the effect of FXII-deficiency to limit SRA dilatation in response to Ang II infusion was due to two main mechanisms. Firstly, the inhibition of the plasma kallikrein–kinin system and subsequent down-regulation of bradykinin generation within the SRA. Secondly, the inhibition of neutrophil infiltration and activation within the SRA in response to AngII. The cumulative effect of these two processes appeared to be responsible for limiting matrix remodelling and SRA dilatation in $ApoE^{-/-}FXII^{-/-}$ mice. SRA concentrations of bradykinin and MPO were not affected by FXIIa neutralisation. This finding suggested the need to investigate other potential mechanisms to explain how FXII/kallikrein blockade limited SRA dilatation.

Tumor necrosis factor (TNF)-α has been implicated in AAA pathogenesis [26,27]. Plasma and aortic concentrations of TNF-α have been reported to be elevated in AAA patients [26]. Inhibition of TNF-α limits experimental AAA

Clinical Science (2020) **134** 1049–1061
https://doi.org/10.1042/CS20191020

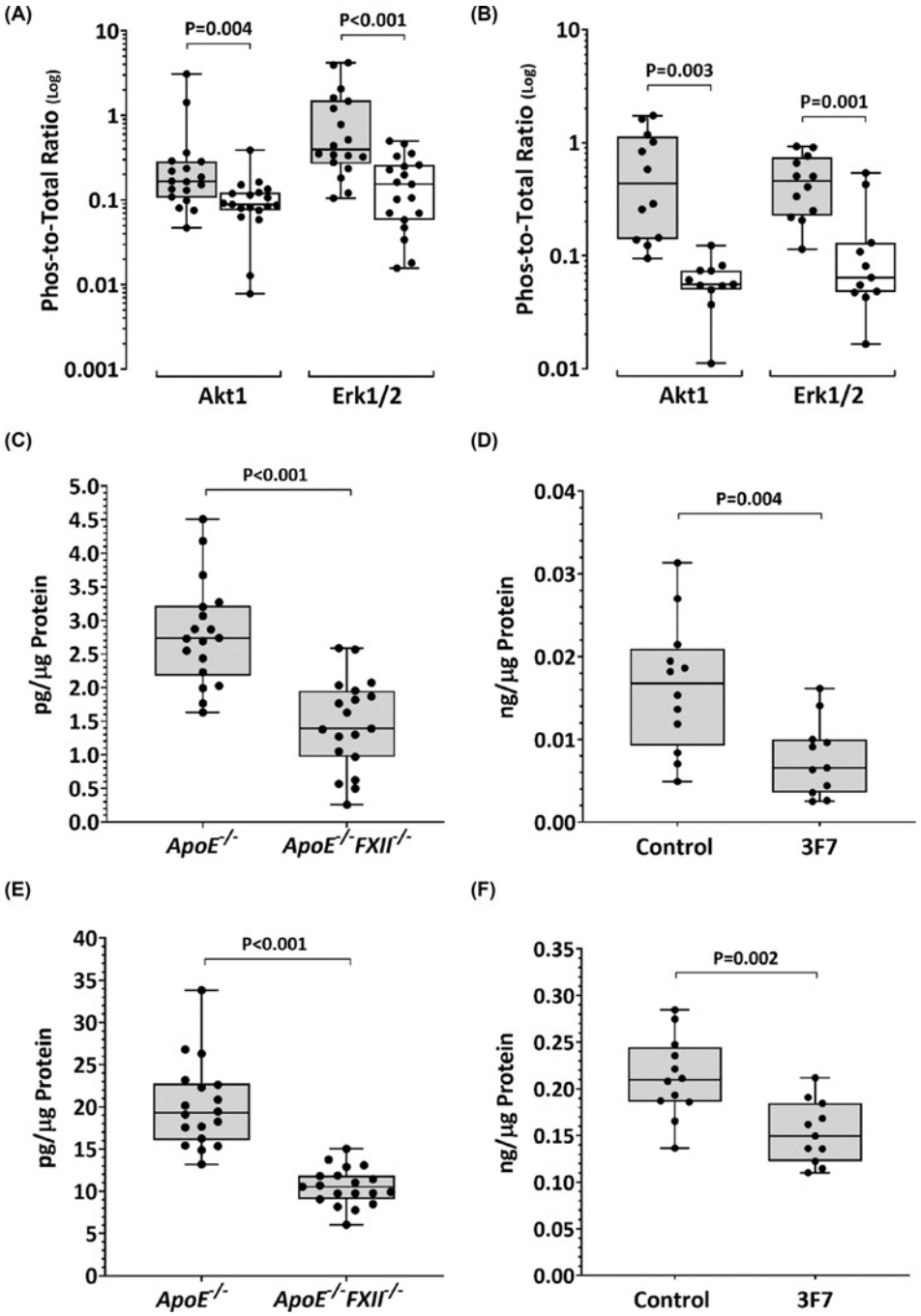

Figure 6. Aortic kinase signalling and proteolytic enzymes

(**A**) Levels of phosphorylated Akt1 and Erk1/2 within the SRA of AngII-infused *ApoE*$^{-/-}$ mice genetically deficient for FXII (white) compared with control mice (grey) after 28 days. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for phosphorylated-to-total kinase ratio. (**B**) Levels of phosphorylated Akt1 and Erk1/2 within the SRA of AngII-infused *ApoE*$^{-/-}$ mice administered the FXIIa-neutralising antibody 3F7 (white) compared with control (grey) after 28 days. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for phosphorylated-to-total kinase ratio. SRA concentration of active Mmp-2 in AngII-infused *ApoE*$^{-/-}$ mice genetically deficient for FXII (*ApoE*$^{-/-}$*FXII*$^{-/-}$; **C**) or administered 3F7 (**D**) compared with control mice after 28 days. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for picograms (pg) or nanograms (ng) of active Mmp per microgram (µg) SRA protein. Concentration of active Mmp-9 in AngII-infused *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice (**E**) or *ApoE*$^{-/-}$ mice administered 3F7 (**F**) compared with control mice after 28 days. Data expressed as median and interquartile range with maximum and minimum data points (whiskers) for picograms (pg) or nanograms (ng) of active Mmp per microgram (µg) SRA protein.

by limiting aortic inflammation and matrix remodelling [27]. TNF-α is generated via the action of TACE, also known as ADAM-17. Expression of ADAM-17 is elevated in human AAA tissue and its deletion inhibits experimental AAA [28]. Pharmacological inhibition of ADAM-17 or vascular smooth muscle cell deficiency of ADAM-17 attenuates AAA formation and rupture induced by AngII [29]. In the current study, both FXII deficiency and FXIIa neutralisation significantly reduced SRA ADAM-17 activity. This may have contributed to the inhibition of SRA dilatation found.

Previous *in vitro* and experimental *in vivo* studies have suggested that ADAM-17 plays a role in AngII-induced activation of the EGFR in vascular smooth muscle cells [30]. Evidence of EGFR activation has been reported in human AAA samples [31]. EGFR activation has been implicated in vascular remodelling in mice infused with AngII [32,33]. Deletion of EGFR in vascular smooth muscle cells prevents AngII-induced vascular remodelling [34], while pharmacological inhibition of EGFR limits development of experimental AAA [31]. It has been previously reported that plasma kallikrein stimulates ADAM-17 activity in vascular smooth muscle cells via a *bradykinin-independent* mechanism involving EGFR activation and EGFR-dependent Erk1 signalling [35]. In line with this, in the current study reduced plasma kallikrein concentration within the SRA of FXII-deficient and 3F7-administered $ApoE^{-/-}$ mice were accompanied by down-regulation in ADAM-17 and EGFR activity. These effects may have contributed to the ability of FXII deficiency and neutralisation to limited SRA expansion.

Several limitations of the current study should be acknowledged. First, only one rodent model of AAA was used. AngII-infused $ApoE^{-/-}$ mice were used since our prior findings implicating kinins in AAA were made in this model [7]. Also this model has a similar aortic gene profile to human AAA [36]. Second, histological analysis of the SRA was not performed as suitable samples were not available. The number of protein and mRNA-based assays performed in the present study required use of the entire SRA sample. We opted for quantitative measurement of inflammatory cell activation and inflammation markers over semi-quantitative histological assessments. Third, although ADAM-17 has a large substrate profile, its activation is typically only in response to stimuli that drive disease states [37]. The EGFR-ADAM-17 pathway was focussed on since this has previously been implicated in AAA in other experimental models. Other ADAM-17 substrates, such as Notch1 and angiotensin-converting enzyme 2, may also be important but these were not investigated [10,16,38]. Finally, conclusions regarding the effect of intervention using samples from the study end-point have limitations and analyses of serum and aortic tissue taken from earlier time points may have provided additional mechanistic insight.

In summary, the current study found that FXII/FXIIa blockade inhibited AngII-induced aortic dilatation in $ApoE^{-/-}$ mice. ADAM-17/EGFR and Akt1/Erk1/2 signalling and Mmp-2 and Mmp-9 activity were down-regulated within the SRA. The findings suggest a novel axis to target in the design of an aortic aneurysm medical therapy.

Clinical perspectives

- Currently there is no drug therapy for aortic aneurysm but past research has suggested that inflammation and the kallikrein–kinin system may be potential targets for a drug therapy.
- FXII is an important activator of the kallikrein–kinin system. Deficiency or neutralisation of FXII inhibited aortic expansion in a mouse model of aortic aneurysm through down-regulating the activity of a novel pathway generating the matrix remodelling enzymes Mmp-2 and -9.
- The findings suggest that FXII may be a novel target for a drug therapy for aortic aneurysm.

Competing Interests

CP is an employee of CSL Limited. The other authors declare there are no competing interests associated with the manuscript.

Funding

This work was supported by the National Health and Medical Research Council [grant numbers 1098717, 1079369]; the Queensland Government [grant number SCRF]; in part by a Cardiac Health Institute Fellowship (to S.W.S.); a Practitioner Fellowship from the National Health and Medical Research Council, Australia [grant number 1117061 (to J.G.)]; a Senior Clinical Research Fellowship from the Queensland Government, Australia [grant number SCRF (to J.G.)]; the Deutsche Forschnungsgemeinschaft (German

Clinical Science (2020) **134** 1049–1061
https://doi.org/10.1042/CS20191020

Research Foundation) [grant number SFB 688 (to C.K.)]; and employee of CSL Limited (Australia) (to C.P.). The funding bodies played no role in the production of this publication.

Open Access

Open access for this article was enabled by the participation of James Cook University in an all-inclusive *Read & Publish* pilot with Portland Press and the Biochemical Society under a transformative agreement with CAUL.

Author Contribution

C.S.M. designed and performed the mouse work, analysed the data, and drafted the manuscript. S.-W.S. and S.M.K. assisted with the animal model and ultrasound analysis. E.B. performed molecular analyses. S.K.M. performed protein assays. C.K. provided the FXII knockout mice to generate *ApoE*$^{-/-}$*FXII*$^{-/-}$ mice used in the study. C.P. provided the FXIIa neutralising antibody 3F7 (ch3F7-mG1L-aFXII) and control peptide. J.G. obtained funding and contributed to experimental design, data analysis and interpretation, and co-wrote the paper. All authors contributed to critical revision of the manuscript.

Abbreviations

AAA, abdominal aortic aneurysm; ADAM-17, a disintegrin and metalloproteinase-17 (aka tumor necrosis factor-α-converting enzyme); AngII, angiotensin II; *ApoE*$^{-/-}$, apolipoprotein E-deficient mouse; *ApoE*$^{-/-}$*FXII*$^{-/-}$, whole-body *FXII*-deficient *ApoE*$^{-/-}$ mouse; ARRIVE, Aanimal Research: Reporting of In Vivo Experiments; EGFR, epidermal growth factor receptor; FXII, factor XII; FXIIa, factor XIIa; IP, intra-peritoneal; Mmp, matrix metalloproteinase; MPO, myeloperoxidase; SRA, suprarenal aorta; TACE, tumor necrosis factor-α-converting enzyme.

References

1 Golledge, J. (2019) Abdominal aortic aneurysm: update on pathogenesis and medical treatments. *Nat. Rev. Cardiol.* **16**, 225–242, https://doi.org/10.1038/s41569-018-0114-9
2 Sweeting, M.J., Rajesh, P., Powell, J.T., Greenhalgh, R.M. and EVAR Trial Investigators (2017) Endovascular repair of abdominal aortic aneurysm in patients physically ineligible for open repair: Very long-term follow-up in the EVAR-2 randomized controlled trial. *Ann. Surg.* **266**, 713–719, https://doi.org/10.1097/SLA.0000000000002392
3 Filardo, G., Powell, J.T., Martinez, M.A. and Ballard, D.J. (2015) Surgery for small asymptomatic abdominal aortic aneurysms. *Cochrane Database Syst. Rev.* **2015**, CD001835
4 Powell, J.T., Brady, A.R., Brown, L.C., Fowkes, F.G.R., Greenhalgh, R.M., Ruckley, C.V. et al. (2002) Long-term outcomes of immediate repair compared with surveillance of small abdominal aortic aneurysms. *N. Engl. J. Med.* **346**, 1445–1452
5 Golledge, J. and Norman, P.E. (2011) Current status of medical management for abdominal aortic aneurysm. *Atherosclerosis* **217**, 57–63, https://doi.org/10.1016/j.atherosclerosis.2011.03.006
6 Leeb-Lundberg, L.M., Marceau, F., Müller-Esterl, W., Pettibone, D.J. and Zuraw, B.L. (2005) International union of pharmacology. XLV. Classification of the kinin receptor family: from molecular mechanisms to pathophysiological consequences. *Pharmacol. Rev.* **57**, 27–77, https://doi.org/10.1124/pr.57.1.2
7 Moran, C.S., Rush, C.M., Dougan, T., Jose, R.J., Biros, E., Norman, P.E. et al. (2016) Modulation of kinin B2 receptor signaling controls aortic dilatation and rupture in the angiotensin II-infused apolipoprotein E-deficient mouse. *Arterioscler. Thromb. Vasc. Biol.* **36**, 898–907, https://doi.org/10.1161/ATVBAHA.115.306945
8 Parr, A., McCann, M., Bradshaw, B., Shahzad, A., Buttner, P. and Golledge, J. (2011) Thrombus volume is associated with cardiovascular events and aneurysm growth in patients who have abdominal aortic aneurysms. *J. Vasc. Surg.* **53**, 28–35, https://doi.org/10.1016/j.jvs.2010.08.013
9 Moran, C.S., Jose, R.J., Moxon, J.V., Roomberg, A., Norman, P.E., Rush, C. et al. (2013) Everolimus limits aortic aneurysm in the apolipoprotein E-deficient mouse by downregulating C-C chemokine receptor 2 positive monocytes. *Arterioscler. Thromb. Vasc. Biol.* **33**, 814–821, https://doi.org/10.1161/ATVBAHA.112.301006
10 Moran, C.S., Biros, E., Krishna, S.M., Wang, Y., Tikellis, C., Morton, S.K. et al. (2017) Resveratrol inhibits growth of experimental abdominal aortic aneurysm associated with upregulation of angiotensin-converting enzyme 2. *Arterioscler. Thromb. Vasc. Biol.* **37**, 2195–2203, https://doi.org/10.1161/ATVBAHA.117.310129
11 Moran, C.S., Seto, S.W., Krishna, S.M., Sharma, S., Jose, R.J., Biros, E. et al. (2017) Parenteral administration of factor Xa/IIa inhibitors limits experimental aortic aneurysm and atherosclerosis. *Sci. Rep.* **7**, 43079, https://doi.org/10.1038/srep43079
12 Nickel, K.F., Long, A.T., Fuchs, T.A., Butler, L.M. and Renné, T. (2017) Factor XII as a therapeutic target in thromboembolic and inflammatory diseases. *Arterioscler. Thromb. Vasc. Biol.* **37**, 13–20, https://doi.org/10.1161/ATVBAHA.116.308595
13 Björkqvist, J., Nickel, K.F., Stavrou, E. and Renné, T. (2014) *In vivo* activation and functions of the protease factor XII. *Thromb. Haemost.* **112**, 868–875, https://doi.org/10.1160/th14-04-0311
14 Sainz, I.M., Pixley, R.A. and Colman, R.W. (2007) Fifty years of research on the plasma kallikrein-kinin system: from protein structure and function to cell biology and *in vivo* pathophysiology. *Thromb. Haemostasis* **98**, 77–83, https://doi.org/10.1160/TH07-04-0250
15 Krishna, S.M., Seto, S.W., Moxon, J.V., Rush, C., Walker, P.J., Norman, P.E. et al. (2012) Fenofibrate increases high-density lipoprotein and sphingosine 1 phosphate concentrations limiting abdominal aortic aneurysm progression in a mouse model. *Am. J. Pathol.* **181**, 706–718, https://doi.org/10.1016/j.ajpath.2012.04.015

16 Biros, E., Walker, P.J., Nataatmadja, M., West, M. and Golledge, J. (2012) Downregulation of transforming growth factor, beta receptor 2 and Notch signaling pathway in human abdominal aortic aneurysm. *Atherosclerosis* **221**, 383–386, https://doi.org/10.1016/j.atherosclerosis.2012.01.004
17 Iwaki, T., Cruz-Topete, D. and Castellino, F.J. (2008) A complete factor XII deficiency does not affect coagulopathy, inflammatory responses, and lethality, but attenuates early hypotension in endotoxemic mice. *J. Thromb. Haemost.* **6**, 1993–1995, https://doi.org/10.1111/j.1538-7836.2008.03142.x
18 Fernando, A.N., Fernando, L.P., Fukuda, Y. and Kaplan, A.P. (2005) Assembly, activation, and signaling by Kinin-forming proteins on human vascular smooth muscle cells. *Am. J. Physiol. Heart Circ. Physiol.* **289**, H251–H257, https://doi.org/10.1152/ajpheart.00206.2004
19 Sato, F. and Nagasawa, S. (1988) Mechanism of kinin release from human low molecular mass kininogen by the synergistic action of human plasma kallikrein and leukocyte elastase. *Biol. Chem. Hoppe Seyler.* **369**, 1009–1017, https://doi.org/10.1515/bchm3.1988.369.2.1009
20 Kozik, A., Moore, R.B., Potempa, J., Imamura, T., Rapala-Kozik, M. and Travis, J. (1998) A novel mechanism for bradykinin production at inflammatory sites. Diverse effects of a mixture of neutrophil elastase and mast cell tryptase versus tissue and plasma kallikreins on native and oxidized kininogens. *J. Biol. Chem.* **273**, 33224–33229, https://doi.org/10.1074/jbc.273.50.33224
21 Stuardo, M., Gonzalez, C.B., Nualart, F., Boric, M., Corthorn, J., Bhoola, K.D. et al. (2004) Stimulated human neutrophils form biologically active kinin peptides from high and low molecular weight kininogens. *J. Leukoc. Biol.* **75**, 631–640, https://doi.org/10.1189/jlb.1103546
22 Dobo, J., Major, B., Kekesi, K.A., Szabo, I., Megyeri, M., Hajela, K. et al. (2011) Cleavage of kininogen and subsequent bradykinin release by the complement component: mannose-binding lectin-associated serine protease (MASP)-1. *PLoS ONE* **6**, 20036, https://doi.org/10.1371/journal.pone.0020036
23 Lizama, A.J., Andrade, Y., Colivoro, P., Sarmiento, J., Matus, C.E., Gonzalez, C.B. et al. (2015) Expression and bioregulation of the kallikrein-related peptidases family in the human neutrophil. *Innate Immunity* **21**, 575–586, https://doi.org/10.1177/1753425914566083
24 Böckmann, S. and Paegelow, I. (2000) Kinins and kinin receptors: importance for the activation of leukocytes. *J. Leukoc Biol.* **68**, 587–592
25 Stavrou, E.X., Fang, C., Bane, K.L., Long, A.T., Naudin, C., Kucukal, E. et al. (2018) Factor XII and uPAR upregulate neutrophil functions to influence wound healing. *J. Clin. Invest.* **128**, 944–959, https://doi.org/10.1172/JCI92880
26 Newman, K.M., Jean-Claude, J., Li, H., Ramey, W.G. and Tilson, M.D. (1994) Cytokines that activate proteolysis are increased in abdominal aortic aneurysms. *Circulation* **90**, II224–II227
27 Xiong, W., MacTaggart, J., Knispel, R., Worth, J., Persidsky, Y. and Baxter, B.T. (2009) Blocking TNF-alpha attenuates aneurysm formation in a murine model. *J. Immunol.* **183**, 2741–2746, https://doi.org/10.4049/jimmunol.0803164
28 Kaneko, H., Anzai, T., Horiuchi, K., Kohno, T., Nagai, T., Anzai, A. et al. (2011) Tumor necrosis factor-α converting enzyme is a key mediator of abdominal aortic aneurysm development. *Atherosclerosis* **218**, 470–478, https://doi.org/10.1016/j.atherosclerosis.2011.06.008
29 Kawai, T., Takayanagi, T., Forrester, S.J., Preston, K.J., Obama, T., Tsuji, T. et al. (2017) Vascular ADAM17 (a Disintegrin and Metalloproteinase Domain 17) is required for angiotensin II/β-aminopropionitrile-induced abdominal aortic aneurysm. *Hypertension* **70**, 959–963, https://doi.org/10.1161/HYPERTENSIONAHA.117.09822
30 Ohtsu, H., Dempsey, P.J., Frank, G.D., Brailoiu, E., Higuchi, S., Suzuki, H. et al. (2006) ADAM17 mediates epidermal growth factor receptor transactivation and vascular smooth muscle cell hypertrophy induced by angiotensin II. *Arterioscler. Thromb. Vasc. Biol.* **26**, e133–e137, https://doi.org/10.1161/01.ATV.0000236203.90331.d0
31 Obama, T., Tsuji, T., Kobayashi, T., Fukuda, Y., Takayanagi, T., Taro, Y. et al. (2014) Epidermal growth factor receptor inhibitor protects abdominal aortic aneurysm in a mouse model. *Clin. Sci. (Lond.)* **128**, 559–565, https://doi.org/10.1042/CS20140696
32 Takayanagi, T., Forrester, S.J., Kawai, T., Obama, T., Tsuji, T., Elliott, K.J. et al. (2016) Vascular ADAM17 as a novel therapeutic target in mediating cardiovascular hypertrophy and perivascular fibrosis induced by angiotensin II. *Hypertension* **68**, 949–955, https://doi.org/10.1161/HYPERTENSIONAHA.116.07620
33 Takayanagi, T., Kawai, T., Forrester, S.J., Obama, T., Tsuji, T., Fukuda, Y. et al. (2015) Role of epidermal growth factor receptor and endoplasmic reticulum stress in vascular remodelling induced by angiotensin II. *Hypertension* **65**, 1349–1355, https://doi.org/10.1161/HYPERTENSIONAHA.115.05344
34 Schreier, B., Hünerberg, M., Mildenberger, S., Rabe, S., Bethmann, D., Wickenhauser, C. et al. (2018) Deletion of the EGF receptor in vascular smooth muscle cells prevents chronic angiotensin II-induced arterial wall stiffening and media thickening. *Acta Physiol. (Oxf.)* **222**, https://doi.org/10.1111/apha.12996
35 Abdallah, R.T., Keum, J.S., El-Shewy, H.M., Lee, M.H., Wang, B., Gooz, M. et al. (2010) Plasma kallikrein promotes epidermal growth factor receptor transactivation and signaling in vascular smooth muscle through direct activation of protease-activated receptors. *J. Biol. Chem.* **285**, 35206–35215, https://doi.org/10.1074/jbc.M110.171769
36 Rush, C., Nyara, M., Moxon, J.V., Trollope, A., Cullen, B. and Golledge, J. (2009) Whole genome expression analysis within the angiotensin II-apolipoprotein E deficient mouse model of abdominal aortic aneurysm. *BMC Genomics* **10**, 298, https://doi.org/10.1186/1471-2164-10-298
37 Sommer, A., Kordowski, F., Büch, J., Maretzky, T., Evers, A., Andrä, J. et al. (2016) Phosphatidylserine exposure is required for ADAM17 sheddase function. *Nat. Commun.* **7**, 11523, https://doi.org/10.1038/ncomms11523
38 Sachdeva, J., Mahajan, A., Cheng, J., Baeten, J.T., Lilly, B., Kuivaniemi, H. et al. Smooth muscle cell-specific Notch1 haploinsufficiency restricts the progression of abdominal aortic aneurysm by modulating CTGF expression. *PLoS ONE* **12**, e0178538, https://doi.org/10.1371/journal.pone.0178538