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# Thrombin-Activatable Fibrinolysis Inhibitor in Human Abdominal Aortic Aneurysm Disease

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**Keywords**

Abdominal aortic aneurysm, Carboxypeptidase B2, Fibrinolysis, Thrombin-activatable fibrinolysis inhibitor, Thrombosis,

**Essentials**

- Patients with abdominal aortic aneurysms (AAA) develop dense clots that are resistant to lysis.
- This study explores the role of thrombin activatable fibrinolysis inhibitor (TAFI) in human AAA.
- There is evidence of chronically increased TAFI activation in patients with AAA.
- TAFI may represent a pharmacological target for cardiovascular risk reduction in AAA.

**Abstract**

**Background**

Intra-luminal thrombosis is a key factor in Abdominal Aortic Aneurysms (AAA) growth. Patients with AAA form dense clots that are resistant to fibrinolysis. Thrombin-activatable fibrinolysis inhibitor (TAFI) has been shown to influence AAA development in murine models.

**Objective**

The aim of this study is to characterise the role of TAFI in human AAA.

**Methods**

Plasma levels of TAFI, TAFI activation peptide (TAFI-AP), activated/inactivated TAFI (TAFIa/ai) and plasmin-α2-antiplasmin complex were measured by ELISAs in patients with AAA (n=202) and controls (n=188).

**Results**

TAFIa/ai and TAFI-AP levels were higher in patients than controls (median (IQR): 20.3 (14.6-32.8) vs. 14.2 (11.2-19.3) and 355.0 (232.4-528.1) vs. 248.6 (197.1-328.1) ng/ml). TAFIa/ai was positively correlated with TAFI-AP (r=0.164). Intact TAFI levels were not different between patients and controls (13.4 (11.2-16.1) vs. 12.8 (10.6-15.4) µg/ml). Plasmin-α2-

antiplasmin was higher in AAA patients than controls (690.0 (489.1-924.3) vs. 480.7 (392.6-555.3) ng/ml).

**Conclusions**

The increase in TAFIa/ai and TAFI-AP suggest an increased TAFI activation in patients with AAA. Prospective studies are required to further elucidate the role of TAFI and fibrinolysis in AAA pathogenesis.

## Introduction

Abdominal aortic aneurysm (AAA) is a permanent dilatation of the descending abdominal aorta. The aorta is described as aneurysmal when the inner-to-inner diameter is >3cm, or >1.5 times the width of the 'normal' aorta [1]. Risk factors for AAA include smoking, hypertension, male gender and increasing age, with AAA disease being most common in men over 65 years, affecting up to 1 in 25 in this group [2]. Unlike in ischaemic heart disease or stroke where diabetes increases risk, diabetes is protective against AAA. The exact mechanism for this has not been elucidated, but may result from a number of factors, including increased glycation of the extracellular matrix resulting in wall stiffening, and the inhibition of MMP secretion in the aortic media [3].

The sequelae that result in AAA are unknown. At the disease end stage there is degradation of the tunica media, with loss of elastin, disordered collagen deposition, vascular smooth muscle cell apoptosis [4], infiltration of lymphocytes and macrophages [5], and neovascularisation [6]. Large AAA are often accompanied by the presence of intra-luminal thrombus (ILT) [7]. Whilst it was assumed that ILT serves to protect against rupture [8], its increasing size is strongly associated with both the AAA expansion rate and the risk of rupture [9, 10]. Aortic wall covered by ILT is thinner and has decreased tensile strength, with reduced elastin and smooth muscle cells [11]. ILT is constantly remodelled at the luminal surface, resulting in the release of proteolytic factors which contribute to the breakdown of the aortic wall [12].

The natural history of AAA is expansion with eventual rupture, which is associated with a mortality of up to 80% [13, 14]. There are no pharmacological treatments which can prevent AAA, or slow growth. Surgical intervention remains the only treatment option available, although this continues to carry a significant risk of morbidity and mortality.

Previous studies have shown that there are systemic coagulation changes in AAA. Ex-vivo, patients with AAA develop denser clots with smaller pores that are more resistant to lysis compared with controls [15]. There is evidence of increased fibrinolysis even in the early stages of disease, with increased levels of D-dimer, Thrombin-antithrombin complexes and prothrombin F1+2 [16]. Coagulation and fibrinolysis activation is maintained in patients undergoing endovascular repair, but not open AAA repair [17], suggesting that the presence of ILT, is in some way responsible for fuelling the hypercoagulability in AAA.

Activated Thrombin-activatable fibrinolysis inhibitor (TAFIa), or plasma carboxypeptidase B2, is a metallocarboxypeptidase involved in the regulation of fibrinolysis and the maintenance of thrombus stability [18]. TAFI zymogen is activated by cleavage at Arg92 by thrombin, thrombin-thrombomodulin and plasmin, with the release of the activation peptide, TAFI-AP [19]. Activated TAFI (TAFIa) has a short half-life at 37ºC of 8-15 minutes, before it undergoes conformational change and becomes inactivated (TAFIai). TAFIai is further cleaved by thrombin [20] before being cleared from the circulation. TAFIa cleaves the C-terminal lysines from partially degraded fibrin, thus removing binding sites for tPA and plasminogen, and reducing plasmin formation [21]. TAFIa acts via a threshold mechanism; once TAFIa reaches threshold level, plasmin generation is interrupted, and fibrinolysis essentially ceases [22]. TAFIa also possesses anti-inflammatory properties, by cleaving C3a, C5a, thrombin-cleaved osteopontin, and bradykinin [23-25], and therefore regulates inflammatory properties of thrombin.

There are only two previous studies on TAFI in human AAA disease. In a small cohort study, Dubis et al. demonstrated decreased TAFI activity in AAA patients [26]. In another study, TAFIa levels were high in patients with ruptured and non-ruptured AAA, although no control group was included [27]. Both studies, however, were based on small sample sizes, and only measured TAFIa. The aim of our study was to elucidate the role of TAFI in AAA, by measuring plasma levels of TAFI, TAFIa/ai and TAFI-AP.

## Materials and Methods

### Patient Recruitment

Patients were recruited for the Leeds Aneurysm Development Study, the inclusion and exclusion criteria of which have been published [15]. The LEADS study is a prospective observational study, recruiting patients with AAA from Leeds Teaching Hospitals NHS trust. Control subjects were recruited via various measures, and included friends/relatives of patients, volunteers from surgical outpatient departments who did not have a diagnosis of AAA, and volunteers from the general public who were aware of the study due to media coverage. The inclusion criteria were presence/absence of AAA, Caucasian, age >55years, and the ability to provide informed consent. Exclusion criteria included the presence of any ongoing inflammatory, coagulatory or malignant disease, or patients taking anti-coagulant medication. At recruitment, in addition to collecting a blood sample, all participants had an abdominal ultrasound scan, which confirmed the presence (or absence) of AAA, and its size. They also completed a full health questionnaire, including an up to date medication history. A sample of free-flowing blood was collected from the antecubital vein. Blood was collected onto cold 0.1 M sodium citrate (9 parts of blood to 1 part of citrate), centrifuged at 4℃ at 2400g for 20 minutes, frozen in liquid nitrogen and stored at -40℃. Plasma CRP and creatinine levels were measured using clinical laboratory methods in the Leeds Teaching Hospitals Trust.

### TAFIa/TAFIai and PAP

Commercially available ELISAs were used to measure plasma levels of activated and inactivated TAFI (TAFIa/TAFIai), and plasmin-α2-antiplasmin complexes (PAP) (Asserachrom® TAFIa/TAFIai, Diagnostica Stago and PAP micro ELISA, DRG®).

### Intact TAFI

Intact TAFI levels were measured using ELISAs developed at KU Leuven [28]. In brief, Nunc-Immuno™ Maxisorp™ ELISA plates (Thermo Fisher Scientific Inc.) were coated with 200 µl of MA-T12D11 at a concentration of 4µg/ml diluted in 0.01 M phosphate buffered saline, PBS, pH 7.4. Plates were incubated for 72 hours at 4℃. After 72 hours, the wells were emptied and blocked with 300 µl per well of PBS containing 1% Bovine Serum Albumin (BSA). The plates were incubated for two hours at room temperature. The wells were

emptied, and washed four times with 300 µl of PBS buffer containing 0.002% TWEEN®-20. Plasma was diluted in PTAE buffer (0.01 M PBS containing 0.002% TWEEN®-20, 0.1% BSA and 5 mM Ethylenediaminetetraacetic acid, EDTA). Four dilutions of 1/160, 1/320, 1/640 and 1/1280 were prepared for each sample. Two standard samples were included on each plate (NPP and a single patient standard). Standard curves were created using purified TAFI, with serial dilutions in the range of 3.125-100 ng/ml. On all plates, equal numbers of samples from patients with AAA and control subjects were included, with 180 µl of sample added to each well. Plates were incubated overnight at 4℃. After washing, 170 µl of secondary antibody (MA-T30E5A2-HRP, diluted 1/8000 in PTA buffer (0.01 M PBS containing 0.1% BSA and 0.002% TWEEN®-20), was added, plates were incubated for 2 hours at room temperature, emptied and washed. To produce a colorimetric reaction, 160 µl of a substrate solution containing o-Phenylenediamine (OPD) was added to each well. The reaction was stopped using 50 µl of 4 M $H_2SO_4$ and the absorbance was measured at 492 nm OD.

TAFI AP

TAFI-AP levels were measured using ELISAs developed at KU Leuven [28]. For this ELISA, plates were coated, and blocked as described above. To produce the TAFI-AP standard, purified TAFI (final concentration 3.75 µg/ml) was incubated along with calcium (final concentration 5 mM), thrombin, (final concentration 16 nM) and thrombomodulin (final concentration 15 nM), in a water bath at 37℃ for exactly 13 minutes. The dilution buffer contained 25 mM HEPES, 137 mM NaCl, 3.5 mM KCl, 3 mM $CaCl_2$ and 0.1% BSA, pH 7.4. After 13 minutes, D-phenylalanyl-L-prolyl-L-arginine chloromethyl ketone (PPACK) was added to a final concentration of 30 µM. A standard curve was produced using 0.65-20 ng/ml of this TAFI-AP. Plasma samples were diluted at 1/40, 1/80, 1/160 and 1/320. The secondary antibody for this ELISA was MA-T18A8-HRP, which was diluted as described above. OPD was used to produce the colorimetric reaction.

Fibrinogen Levels

Fibrinogen levels were determined using the Clauss method with a Start 4 Heamostasis analyzer (Diagnostica Stago,) and the Fibri-prest automated kit (Diagnostica Stago) according to the manufacturer's instructions.

Sample size and Statistical Analysis

Samples sizes were determined using pilot data. For the intact TAFI ELISA, with a standard deviation in each group of 4 μg/ml with 90% power at $p<0.01$, 23 per group were required to detect a significant difference in the mean values. As the variation was higher in the pilot data for the TAFIa/TAFIai (standard deviation up to 19 ng/ml) and TAFI-AP ELISAs (standard deviation up to 250 ng/ml), 105 and 163 samples respectively were required per group. Normality of data distribution was tested using Shapiro-Wilk. A normal distribution could not be achieved by transforming data using $Log_{10}$ or natural Log for the results of TAFI plasma levels, and so data was analysed as non-parametric data. Non-parametric data was analysed using Mann-Whitney U, and parametric data was analysed using Student's t-testing. Non-parametric data is presented as median (Inter-quartile range), and parametric data as mean±standard deviation. For patient demographics, continuous data is presented as median (Inter-quartile range) and categorical data as number of subjects (% of total), with differences between groups analysed using T-test for continuous data and Pearsons Chi-Squared for categorical data.

Given the heterogeneous and complex nature of AAA, it was likely that a number of confounding factors would be present. As a result, any differences in plasma levels between groups were adjusted for confounders using multivariate analysis. Factors included in the models were age, aspirin and statin use, history of MI, BP, smoking status, gender, fibrinogen concentration and CRP.

**Results**

In total, 202 AAA patients and 188 control subjects were included in this study. Their basic demographics are shown in Table 1. The two groups were compared for compounding risk factors, such as cardiovascular disease and smoking. As expected, there were some marked differences in the incidence of myocardial infarction (AAA 27.7 vs. controls 6.4%, $p<0.0001$), hypertension (61.9 vs. 31.6%, $p<0.0001$) and smoking (ever smoked, 89.6 vs. 67.0%, $p<0.0001$), which remained even after correcting for multiple testing (Benjamini and Hochberg). Patients with AAA were further classified into large AAA (>5 cm) and small AAA (3-5 cm). The demographics for these groups are included in Table 1.

The levels of intact TAFI were comparable between groups; AAA 13.36 (11.21-16.12) vs. controls 12.76 (10.62-15.35) µg/ml, Mann Whitney U test p=0.13, Figure 1A. There was no difference with size of AAA; large AAA 13.27 (11.24-15.53) vs. small AAA 13.56 (11.04-16.98) µg/ml. There was an increase in the plasma levels of TAFIa/TAFIai measured in AAA patients compared with controls (AAA 20.32 (14.57-32.79) vs. controls 14.19 (11.20-19.25) ng/ml, Mann Whitney U test p<0.0001), and this effect was correlated with increasing size of AAA (Small AAA 18.72 (13.63-30.38), Large AAA 21.55 (14.80-36.50) ng/ml, Kruskal Wallis controls/small AAA/large AAA p<0.0001), Figure 1B and 1E. The same was true for plasma levels of TAFI-AP; AAA 355.0 (232.4-528.1) vs. controls 248.6 (197.1-328.1) ng/ml, Mann Whitney U test p<0.0001, Figure 1C. The differences between patients and controls in TAFIa/TAFIai and TAFI AP levels remained significant after adjusting for confounding factors in a multivariate model (p=0.03 and p=0.001 respectively).

Plasma levels of TAFIa/TAFIai and TAFI-AP showed a positive correlation (r=0.164, p=0.001), indicating that the results of these ELISAs are both indicators of the amount of TAFI that has been activated (Figure 1D). Plasma levels of TAFIa/ai were divided into quartiles ($1^{st}$ <12.49, $2^{nd}$ 12.5-17.05, $3^{rd}$ 17.06-26.26, $4^{th}$ >26.26 ng/ml) in order to establish if a dose effect was present for TAFIa/ai and AAA. As TAFIa/ai levels increased, the percentage of subjects in each set with AAA increased (30.9, 44.3, 59.8 and 72.4% respectively), Figure 1F. Plasmin-α2-antiplasmin complex levels were also significantly increased in patients with AAA (AAA 690.0 (489.1-924.3) vs. controls 480.7 (392.6-555.3) ng/ml p<0.0001), Figure 2, indicating a possible role for plasmin in TAFI activation in AAA.

Previous work from our group has shown that there was no difference in the distribution of the Thr325Ile polymorphism between AAA patients and controls [30]. Despite this, levels of TAFIa/ai and TAFI-AP still correlated with genotype across the whole population, with a reduction in TAFIa/ai and TAFI-AP in Ile/Ile homozygotes (Figure 3).

**Discussion**

We investigated aspects of TAFI pathophysiology in a large group of patients with AAA and compared these with a control population. While TAFI total antigen levels were similar between patients and controls, TAFI activation peptide and TAFIa/TAFIai were both increased in AAA patients compared with controls. These data indicate increased activation

of TAFI in-vivo in AAA patients, and these changes correlated with AAA size. PAP levels were higher in patients versus controls, suggesting an increased activation of TAFI by plasmin in AAA patients, and that TAFI activation represents a marker of inflammation and coagulation in these patients. Future prospective studies are warranted to further investigate the importance of TAFI in AAA formation and development.

TAFI levels in the plasma of patients with AAA were studied using a number of different ELISA systems. Historically, there has been some difficulty in measuring TAFI using ELISAs, as a number of antibodies were specific only for one of two alleles of the Thr325Ile polymorphisms [19]. In addition, the difference in half-life between the two forms of TAFI may have led to falsely high or low results in patients who are homozygous for either form of TAFI. The ELISAs used for this study were not specific for either isoform, and detect both forms with equal specificity. Although the half-life of the two TAFIa forms is different, by measuring TAFIa/TAFIai in combination, any TAFIa already inactivated in the sample should have still been detected in equal measure, thus correcting for the difference in half-life between the two forms. Furthermore, we previously investigated TAFI Thr325Ile in patients with AAA and found no difference in genotype distribution of this polymorphism in patients and controls [29].

Whilst there was no difference between the levels of TAFI zymogen between patients with AAA and controls, the levels of TAFIa/TAFIai and the TAFI activation peptide were significantly higher in patients compared with control subjects, indicating increased TAFI activation in AAA. AAA is a chronic disease, and this increased TAFI activation could be due to the continuous plasmin generation within the ILT and the aortic wall [30], or caused by the increased thrombin generation previously observed in AAA [16,17]. It would have been interesting to be able to correlate TAFI levels with ILT itself. In our study, all patients underwent abdominal ultrasound scanning, the resolution of which can make it difficult to accurately quantify ILT. Further studies with magnetic resonance imaging or similar would be able to explore the association between activated TAFI and ILT in more detail.

Only a fraction of the TAFI zymogen needs to be activated to have a full effect (in the order of 1%),[22] and so only in the case of massive TAFI consumption would there be a detectable decrease in the total zymogen levels, which was not seen in our cohort. The

relative increase in activation of TAFI results in higher levels of TAFIa/TAFIai and the TAFI-AP. Although the TAFIa/TAFIai ELISA aims to measure both active and inactive TAFI in the plasma samples, it is likely that due to the very short half-life of active TAFI, the majority of what is measured by this ELISA is actually inactivated TAFI.

While the thrombin/thrombomodulin complex is generally considered to be the main physiological activator of TAFI, plasmin has been shown to also be capable of activating TAFI both in vitro and in vivo [31]. Certain anionic molecules, including dermatan sulphates and polyphosphates, have been shown to enhance plasmin-mediated activation of TAFI [32]. Polyphosphates are released by activated platelets [33], and in view of the increased prothrombotic activity in AAA [17], plasma concentrations of polyphosphates are likely increased in AAA patients. Alongside the higher PAP levels, this seems to suggest that in patients with AAA, plasmin-mediated TAFI activation may play an important role. It is known that the levels of D-dimer (the plasmin mediated fibrin breakdown product) are higher in patients with AAA compared with control subjects [16,17]. Also, high levels of D-dimer and plasmin-antiplasmin complexes have been shown within the ILT of patients with AAA [34]. However, increased thrombin generation through increased levels of F1+2 and TAT have also been demonstrated in AAA [16,17], and could therefore also contribute to increased TAFI activation. Further studies are needed to investigate whether TAFI activation in AAA is due to increased plasmin or thrombin generation.

This study has several limitations. The control group was not matched with the patient group for age and gender. However, since TAFI correlates with age only in women and not in men [35] and our study consisted largely of men, this unlikely influences the results in our current study. There were differences between the AAA and the control groups in terms of past medical history, the majority of which are known to be associated with AAA disease. Whilst it is impossible to correct for all possible confounding factors, even when correcting for many (including age, gender, past medical history of MI, aspirin, statin, smoking status), the significant differences in the levels of TAFIa/ai and TAFI-AP remained. Finally, we analysed TAFI using different in-house assays, each measuring a different aspect of TAFI biology. Using different assays may provide differing results, but ultimately provide a more comprehensive picture of the changes occurring to TAFI in AAA. Plasma samples were not taken in the presence of a stabilizer that could stabilize TAFIa activity which would allow measurement of TAFIa activity. We did not include an assay that measures TAFIa activity.

In conclusion, we observed evidence of altered in-vivo TAFI activation in patients with AAA, as shown by the increased plasma levels of TAFIa/TAFIai and TAFI-AP. Together, these data indicate a possible role for TAFI in AAA formation and progress, and warrant prospective studies to investigate this further.

**Addendum**

K. I. Bridge designed the study, optimised all assays, performed the TAFI and TAFI-AP ELISAs, activity assays and the turbidity lysis studies, analysed and interpreted the data, and wrote the manuscript; M. Hesketh performed the TAFIa/TAFIai ELISA and assisted with the TAFI activity assays; J. Zhong performed the PAP ELISAs and critically reviewed the manuscript; L. Bollen optimised the TAFI and TAFI-AP ELISAs and critically reviewed the manuscript; F. L. Macrae processed patient blood samples and critically reviewed the manuscript; A. B. Johnson enrolled the patients into the LEADS study and collected the blood samples; H. Philippou assisted in the study design and critically reviewed the manuscript; D. Julian Scott enrolled the patients into the LEADS study, assisted in the study design and critically reviewed the manuscript; A. Gils developed the TAFI and TAFI-AP ELISAs and critically reviewed the manuscript; and R. A. S. Ariens designed and supervised the study, assisted in the analysis and interpretation of the data, and wrote the manuscript.

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## Disclosure of Conflict of Interests

A. Gils has a patent *diabody anti-TAFI/anti-PAI-1* pending. The other authors state that they have no conflict of interest.

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**Table 1. Demographics of patients and control subjects**

**Figure 1. Plasma levels of TAFIa/TAFIai and TAFI-AP are increased in patients with AAA, and are associated with aneurysm size**

**Figure 2. Plasma levels of PAP are increased in patients with AAA.**

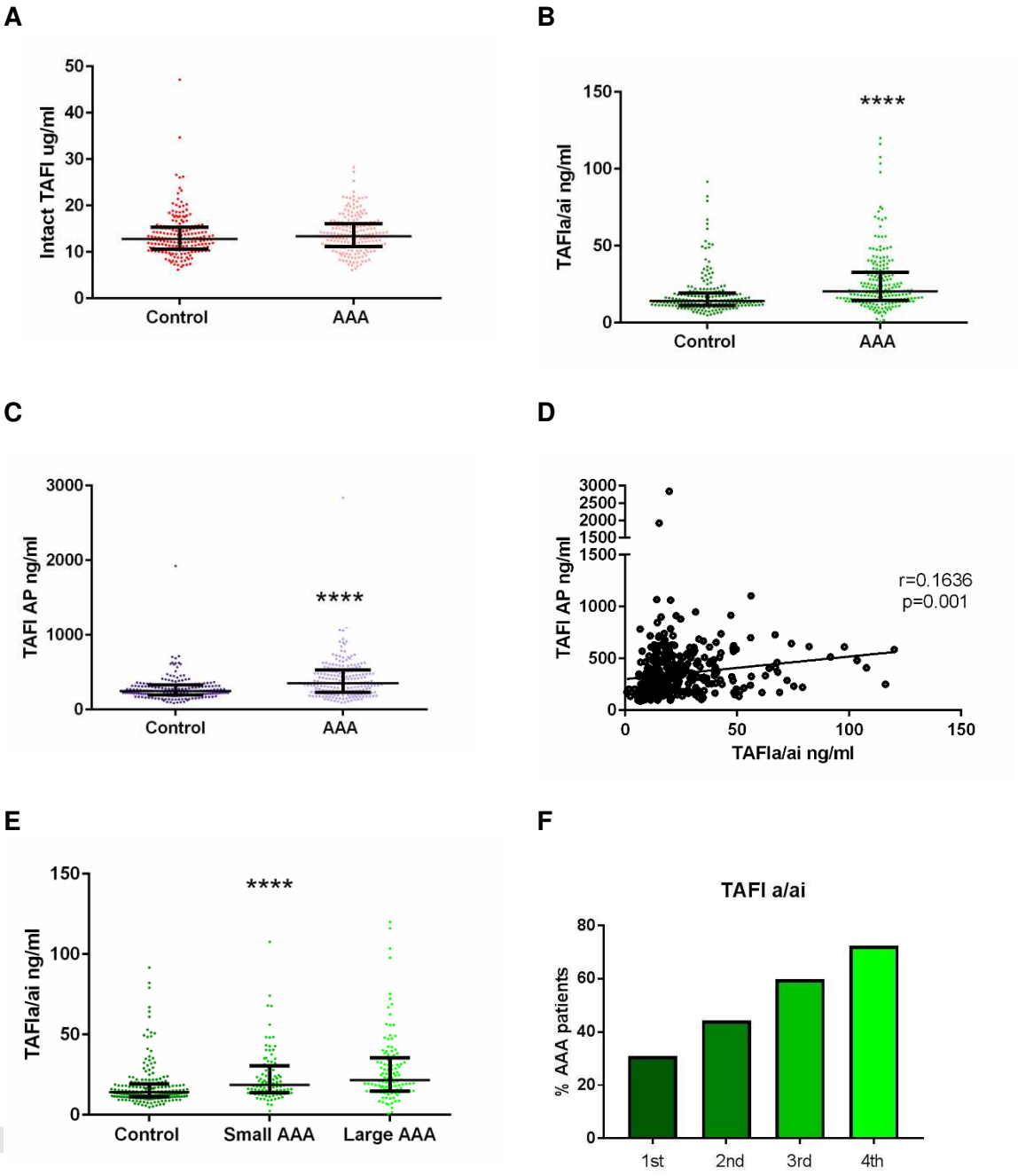
**Figure 3. Impact of TAFI Thr325Ile polymorphism on TAFIa/ai and AP levels.**

**Table 1. Demographics of patients and control subjects**

| | | Controls (no AAA) **n=188** | Patients (AAA) **n=202** | Large AAA (>5cm) **n=110** | Small AAA (3-5cm) **n=92** | p-value AAA vs. controls |
|---|---|---|---|---|---|---|
| **Aorta size (mm)** | | 1.8 (1.6-2.0) | 5.2 (4.0-6.3) | 6.3 (5.7-7.0) | 4.0 (3.5-4.5) | <0.0001 |
| **Age (years)** | | 69.0 (64.0-75.8) | 74.0 (68.0-80.0) | 75.0 (68.0-80.9) | 73.0 (68.0-79.0) | <0.0001 |
| **Male n(%)** | | 132 (70.2%) | 169 (83.7%) | 92 (88.5%) | 77 (78.6%) | 0.002 |
| **BMI (weight kg/height $m^2$)** | | 27.8 (24.7-30.2) | 27.4 (24.2-30.6) | 27.4 (23.9-30.5) | 27.4 (24.9-30.7) | 0.68 |
| **Drug history n(%)** | Aspirin | 64 (34%) | 145 (71.8%) | 73 (70.2%) | 72 (73.5%) | <0.0001 |
| | Statin | 84 (44.7%) | 168 (83.2%) | 86 (82.7%) | 82 (83.7%) | <0.0001 |
| **Past Medical History n(%)** | DM | 21 (11.2%) | 26 (12.9%) | 13 (12.5%) | 13 (13.3%) | 0.432 |
| | MI | 12 (6.4%) | 56 (27.7%) | 31 (29.8%) | 25 (25.5%) | <0.0001 |
| | Angina | 17 (9.0%) | 51 (25.2%) | 34 (32.7%) | 17 (17.3%) | <0.0001 |
| | CVA/TIA | 25 (13.3%) | 27 (13.4%) | 13 (12.5%) | 14 (14.3%) | 0.552 |
| | ↑BP | 67 (35.6%) | 125 (61.9%) | 55 (52.9%) | 70 (71.4%) | <0.0001 |
| **Smoking Status n(%)** | Current | 22 (11.7%) | 45 (23.9%) | 25 (24.0%) | 20 (20.4%) | 0.012 |
| | Ever | 126 (67.0%) | 181 (89.6%) | 96 (92.3%) | 85 (86.7%) | <0.0001 |

| | | | | | |
|---|---|---|---|---|---|
| **Plasma Fibrinogen (g/L)** | 3.4 (3.1-4.0) | 3.8 (3.3-4.5) | - | - | <0.0001 |
| **C-reactive protein (ng/ml)** | <5 (<5-5) | <5 (<5-9.5) | - | - | 0.0002 |
| **Creatinine (µmol/L)** | 87 (77-103) | 101.5 (89-116.3) | - | - | <0.0001 |

DM – Diabetes Mellitus, MI – Myocardial Infarction, CVA/TIA Cerebrovascular Accident or Transient Ischaemic Attack, ↑BP – hypertension, AAA – abdominal aortic aneurysm, BMI – body mass index. Results are shown as median (interquartile range) for continuous data and number (percentage of patients) for discrete data. Differences between groups were analysed using *Pearsons Chi squared and #Mann Whitney U Test, with $p < 0.05$ taken as being statistically significant after correcting for multiple testing.




| | Control | AAA | Large AAA | Small AAA |
|---|---|---|---|---|
| **Intact TAFI** µg/ml | **12.8** (10.6-15.4) | **13.4** (11.2-16.1) | **13.3** (11.2-15.5) | **13.6** (11.0-17.0) |
| **TAFIa/TAFIai** ng/ml | **14.2** (11.2-19.3) | **20.3** (14.6-32.8) | **21.6** (14.8-35.6) | **18.7** (13.7-30.4) |
| **TAFI AP** ng/ml | **248.6** (197.1-328.1) | **355.0** (232.4-528.1) | **324.6** (204.5-504.7) | **416.0** (262.5-548.2) |

**Figure 1. Plasma levels of TAFIa/TAFIai and TAFI-AP are increased in patients with AAA, and are associated with aneurysm size.** Levels of TAFI, TAFIa/TAFIai and TAFI-AP were measured in the plasma of patients with AAA (n=202) and control subjects (n=188) using ELISAs. There was no difference in the plasma levels of total TAFI zymogen in patients with AAA compared with controls (Mann Whitney U Test, p=0.13, Panel A). The levels of TAFIa/TAFIai and TAFI-AP were significantly increased in patients with AAA compared with control subjects; TAFIa/TAFIai Mann Whitney U Test p<0.0001, Panel B, and TAFI-AP Mann Whitney U Test p<0.0001, Panel C. Plasma levels of TAFIa/TAFIai and TAFI-AP are positively correlated, Panel D. The increase in plasma TAFIa/TAFIai levels are associated with AAA size, Panel E, Kruskal Wallis p<0.0001. The percentage of patients with AAA increases with levels of TAFIa/ai expressed as quartiles, Panel F.

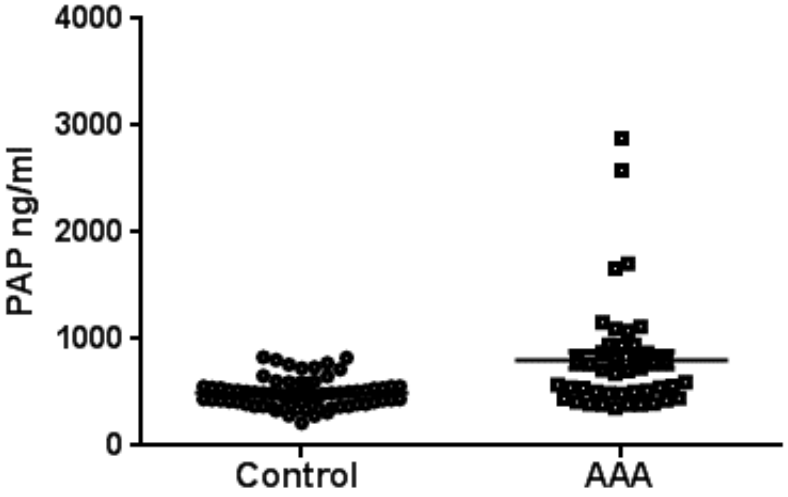



**Figure 2. Plasma levels of PAP are increased in patients with AAA.** Plasma levels of plasmin-α2-antiplasmin complexes (PAP) were measured in the plasma of patients and controls using an ELISA. Plasma levels were higher in patients compared with controls (AAA 690.0(489.1-924.3) vs. Controls 480.7(392.6-555.3) ng/ml p<0.0001).

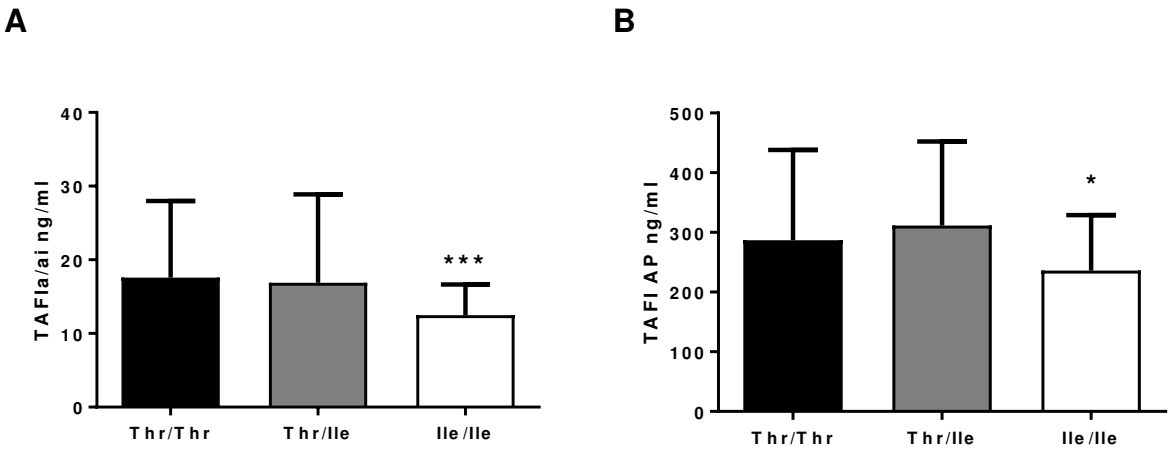



**Figure 3. Impact of TAFI Thr325Ile polymorphism on TAFIa/ai and AP levels.** Levels of TAFIa/ai (panel A) and TAFI-AP (panel B) were correlated with Thr325Ile polymorphism status across all participants (AAA and controls). Plasma levels of both TAFIa/ai and TAFI-AP were lower in participants who were homozygote for the isoleucine allele. Results are displayed as median (IQR), analysed using Mann Whitney-U test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 vs Thr/Thr homozygotes.