Integrated Plasma and Tissue Proteomics Reveals Attractin Release by Intraluminal Thrombus of Abdominal Aortic Aneurysms and Improves Aneurysm Growth Prediction in Humans

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Abdominal aortic aneurysms (AAAs) are pathological dilatation of the abdominal aorta to larger than 30mm in diameter. Left untreated, it eventually results in AAA rupture and high mortality. Methods for the prediction of AAA growth is considered as a priority for research in the opinions of our peers[1]. It can guide different aspects of clinical management in terms of the frequency of monitoring of AAAs and the optimal timing for surgery.

The Oxford Abdominal Aortic Aneurysm (OxAAA) Study has previously reported a method of AAA growth prediction by incorporating 9 circulating proteins (derived using a commercially available protein array), flow mediated dilatation of brachial artery (FMD, a physiological marker of systemic endothelial function), and AAA diameter[2]. We had also observed that systemic endothelial function (measured by brachial artery FMD) deteriorates during the natural history of AAA growth and is reversed by AAA repair[3].

Most AAAs contain intra-luminal thrombus (ILT)[4]. Since ILT is either removed or excluded from circulation after successful repair of AAAs, we hypothesise that ILT is the source of mediators that contribute to AAA growth. In this report, we utilised mass spectrometry analyses on blood, thrombus tissue, and tissue supernatant collected from patients during the natural history of AAA progression to discover novel predictors of AAA growth in humans.

Details regarding the OxAAA study cohort and recruitment process have been published[3]. In brief, this prospective study (Ethics Ref: 13/SC/0250) recruited patients in the National Health Service setting. Baseline assessments were performed. In addition to the measurement of AAA antero-posterior diameter by ultrasound imaging, fasting blood sample was collected and Platelet-poor plasma was prepared using two-staged centrifugation as previously described[5] and stored at -80□C for subsequent analysis.

Prospective AAA annual growth rates were calculated based on the diameter measurements in the subsequent AAA monitoring ultrasound scans.

Plasma samples were collected at baseline and at 1 year from each patient (n=59). Based on the prospectively recorded aneurysm growth rates, we selected a subset of patients [fastest (n=10) vs slowest (n=10)]. These were pooled for the initial discovery analysis. Plasma samples were also collected before and at 10-12 weeks after surgery from each patient (n=29). *Paired* aneurysm wall, ILT, omental biopsies were collected intra-operatively during open surgical repair (n=3). In addition to analyses of the tissue, supernatant was obtained from *ex vivo* culture of these paired tissue samples. We utilised a similar approach for plasma biomarker discovery as recently described[6]. Samples were subjected to Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) proteomic analysis to discover protein level differences[6]. LC-MS/MS data were analysed using the Progenesis QI software (NonLinear Dynamics), and included only proteins with at least two matched peptide sequences.

The median AAA size at baseline was 48 mm. The median growth rate of AAA was 3.8%/year (IQR 1.9% to 6.8%). Comparison between patients with the fastest vs the slowest AAA growth showed 116 proteins (listed by the UniProt Protein IDs in Figure Panel 1) to be differentially expressed in their plasma (Figure panel 2-A). Among these proteins, 35 also changed significantly before and after AAA repair (Figure panel 2-B), suggesting their origin from the AAA complex. Comparison of the proteomics profile of aneurysm tissue, ILT, and omental artery show 128 proteins to be uniquely present in ILT (Figure panel 2-C).

Analyses of the tissue culture supernatant further revealed 3 proteins that were: (i) uniquely present in ILT; (ii) released by ILT; (iii) reduced in systemic circulation after AAA surgery; (iv)

different between fast and slow growth AAAs (Figure panel 2-D). These are: attractin (UniProt ID O75882), complement C8 (UniProt ID P07360), heat shock protein AA5P (UniProt ID Q58FG0). To technically validate the LC-MS/MS data, attractin level in individual patient was measured by ELISA (R&D Quantikine DATRN0). Consistent with the LC-MS/MS data, plasma attractin level is significantly higher in patients with fast AAA growth (Figure panel 3, median 28.5 vs 21.9 ng/ml, $P<0.001$). Plasma attractin level correlates significantly with future AAA growth rate (Figure panel 4, Spearman $r=0.35$, $P<0.005$).

We tested the utility of a generalised linear model to predict aneurysm growth in these patients. We regressed the measured values of attractin in combination with the measurements of AAA diameter against a categorical response with levels of 'Slow/no' growth (0%) or growth (>0% growth) for outcomes at 12 months. Using attractin and AAA diameter as input variables, the area under receiver operating characteristics (AUROC) for predicting no growth of AAA at 12 months is 85% (Figure panel 5, asymptotic $P<0.001$) as compared to 76% with AAA diameter alone.

This report is a significant breakthrough from our previous work[2]. By focusing on the role of thrombus as a source of systemic mediator release, we discover novel proteins that are released from thrombus and drive AAA growth in humans, tested in a prospectively recruited cohort. This is the first report in which novel proteins that correlate to future AAA growth have been discovered through a mass spectrometry workflow. The validity of the mass spectrometry discovery workflow is demonstrated by the precise replication of the LC-MS/MS data by ELISA measurements on individual patient samples.

Since the first description of attractin in 1998[7], there has been little in the reported literature regarding its biological role in disease. Evidence points toward its release by

activated T-cell. It is involved in the initial immune cell clustering during inflammatory response and it regulates chemotactic activity of chemokines[7]. There is mounting evidence of T-cells being active in AAA ILT and that it plays a role in AAA pathophysiology[8]. This report is the first to implicate attractin in human AAA progression and warrants further mechanistic investigations.

It is important for external cohorts to replicate the efficacy of our biomarker panel. We hope this work serves as a primer to generate interest in the vascular surgical community and stimulates future efforts to validate the prediction algorithm.

References

1. Lee R, Jones A, Cassimjee I and Handa A. International opinion on priorities in research for small abdominal aortic aneurysms and the potential path for research to impact clinical management. *Int J Cardiol*. 2017;245:253-255.

2. Lee R, Charles PD, Lapolla P, Cassimjee I, Huang H, Kessler B, Fischer R and Handa A. Integrated Physiological and Biochemical Assessments for the Prediction of Growth of Abdominal Aortic Aneurysms in Humans. *Ann Surg*. 2018.

3. Lee R, Bellamkonda K, Jones A, Killough N, Woodgate F, Williams M, Cassimjee I and Handa A. Flow Mediated Dilatation and Progression of Abdominal Aortic Aneurysms. *Eur J Vasc Endovasc Surg*. 2017;53:820-829.

4. Whaley ZL, Cassimjee I, Novak Z, Rowland D, Lapolla P, Chandrashekar A, Pearce BJ, Beck AW, Handa A and Lee R. The Spatial Morphology of Intraluminal Thrombus Influences Type II Endoleak After Endovascular Repair of Abdominal Aortic Aneurysms. *Ann Vasc Surg*. 2019.

5. Lee R, Antonopoulos AS, Alexopoulou Z, Margaritis M, Kharbanda RK, Choudhury RP, Antoniades C and Channon KM. Artifactual elevation of plasma sCD40L by residual platelets in patients with coronary artery disease. *Int J Cardiol*. 2013;168:1648-50.

6. Lee R, Fischer R, Charles PD, Adlam D, Valli A, Di Gleria K, Kharbanda RK, Choudhury RP, Antoniades C, Kessler BM and Channon KM. A novel workflow combining plaque imaging, plaque and plasma proteomics identifies biomarkers of human coronary atherosclerotic plaque disruption. *Clin Proteomics*. 2017;14:22.

7. Duke-Cohan JS, Tang W and Schlossman SF. Attractin: a cub-family protease involved in T cell-monocyte/macrophage interactions. *Adv Exp Med Biol*. 2000;477:173-85.

8. Ismail Cassimjee RLaJP. Inflammatory Mediators in Abdominal Aortic Aneurysms. 2017.

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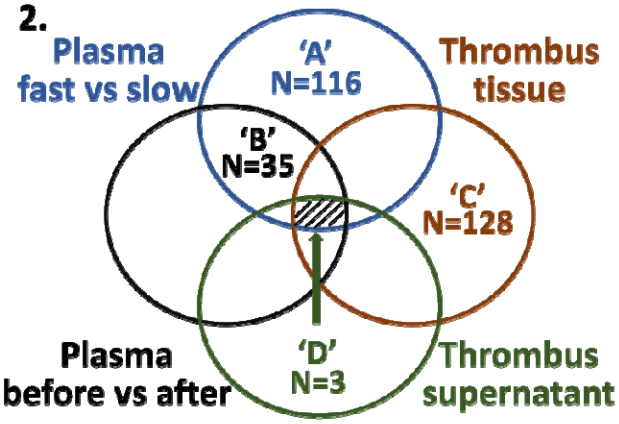
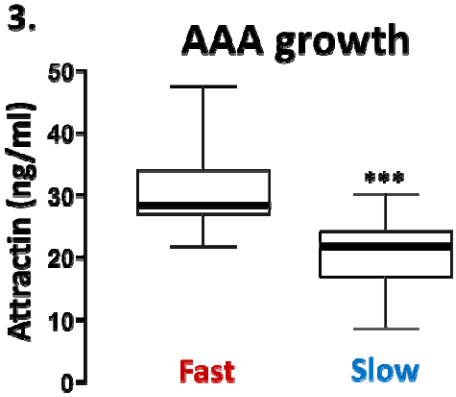
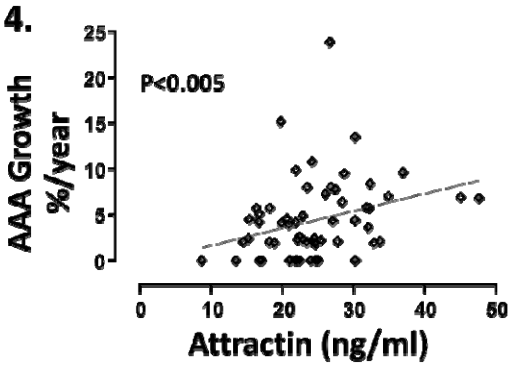
Figure

1. UniProt ID of proteins that significantly differ between patients with fast vs slow AAA growth

O00533	P00746	P04271	P0DOX6	P15311	P30086	P55884	Q14315	Q7Z3D4	Q9H361
O00555	P00915	P05091	P0DP01	P18085	P31948	P57721	Q15084	Q7Z4W1	Q9NQH7
O15144	P01034	P06576	P10643	P20774	P32119	P61769	Q15847	Q8N436	Q9NVI1
O43488	P01764	P06727	P10809	P20851	P33981	P61916	Q16363	Q8WZ75	Q9UNM6
O60641	P01860	P07099	P11142	P21810	P35908	P62306	Q4VNC0	Q96SN8	Q9Y5C1
O75882	P01871	P07195	P11168	P21980	P42166	P78371	Q58FG0	Q96TC7	Q9Y5Z4
O75891	P02655	P07360	P11217	P23470	P46821	Q04637	Q5MJ70	Q99497	A0A0C4DH68
O76013	P02656	P07858	P11309	P24592	P48643	Q12906	Q5VST9	Q99879	A0A0C4DH31
O95568	P07741	P07988	P13500	P27348	P49458	Q13093	Q6IMN6	Q99943	
O95782	P02771	P08133	P13645	P27797	P50990	Q13162	Q6UX71	Q9BSJ8	
O95810	P02775	P08567	P13796	P27816	P51884	Q14019	Q6ZN28	Q9BZA8	
P00390	P02776	P08729	P14151	P27824	P54727	Q14204	Q702N8	Q9BZK3	







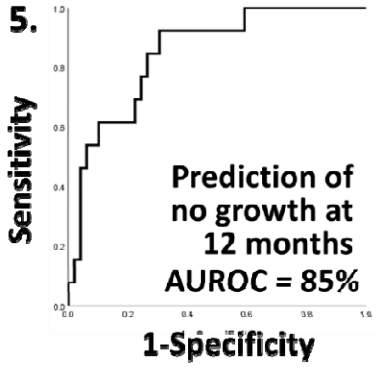


Figure: Integrated Plasma and Tissue Proteomics Reveals Attractin Release by Intraluminal Thrombus of Abdominal Aortic Aneurysms and Improves Aneurysm Growth Prediction in Humans. AAA growth rates were prospectively recorded in 59 patients. Based on the growth rate in the subsequent 12 months, we selected a subset of patients (fastest vs slowest, n=10 each) for the initial discovery analysis. Plasma samples were also collected from patients before and after AAA repair (n=29). Paired aneurysm wall, ILT, omental biopsies were collected intra-operatively during open surgical repair (n=3). In addition to analyses of the tissue, supernatant was obtained from *ex vivo* culture of these paired tissue samples. Samples were subjected to Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) proteomic analysis to discover protein level differences. Plasma samples were pooled in their respective groups for analysis. Other samples were analysed individually. LC-MS/MS data were analysed using the Progenesis QI software (NonLinear Dynamics) and included only proteins with at least two matched peptide sequences. Comparison between patients with the fastest vs the slowest aneurysm growth showed 116 proteins (listed by the UniProt Protein IDs in Panel 1) to be differentially expressed in their plasma (2-A). Among these proteins, 35 also changed significantly before and after AAA repair (2-B), suggesting their origin from the AAA complex. Comparison of the proteomics profile of aneurysm tissue, ILT, and omental artery showed 128 proteins to be uniquely present in ILT (2-C). Analyses of the tissue culture supernatant further revealed 3 proteins that were: (i) uniquely present in ILT; (ii) released by ILT; (iii) systemic levels reduced after AAA surgery; (iv) different between fast and slow growth AAAs (2-D). These are: attractin (UniProt ID O75882), complement C8 (UniProt ID P07360), heat shock protein AA5P (HSPAA5P, UniProt ID Q58FG0). Attractin level in individual patient was further measured by ELISA (R&D Quantikine DATRN0). Plasma attractin level is significantly higher in patients with fast AAA growth (panel 3, median 28.5 vs 21.9 ng/ml, $P<0.001$). Plasma attractin level correlates significantly with future AAA growth rate (Figure panel 4, Spearman $r=0.35$, $P<0.005$). We regressed the measured values of attractin in combination with AAA diameter against a categorical response with levels of 'Slow/no' growth (0%) or growth (>0% growth) for outcomes at 12 months. Using attractin and AAA diameter as input variables, the AUROC for predicting no growth of AAA at 12 months is 85% (panel 5, asymptotic $P<0.001$).