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Plasma Low-density Lipoprotein Receptor-related Protein 1 Concentration is not Associated with Human Abdominal Aortic Aneurysm Presence☆

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WHAT THIS PAPER ADDS

Polymorphisms within *LRP1* have been suggested to contribute to the risk of abdominal aortic aneurysm (AAA). This study demonstrates that plasma concentrations of low-density lipoprotein receptor-related protein 1 (LRP1) are similar in men with and without AAA, suggesting that this protein is an unsuitable marker with which to screen for in at-risk populations. This study confirms that *LRP1* expression is reduced in aortic biopsies collected from patients with AAA compared with nonaneurysmal controls, and demonstrates that inhibition of LRP1 reduces the ability of vascular smooth muscle cells to internalize matrix metalloprotease 9 *in vitro*. These findings suggest that localized LRP1 dysregulation may be important in AAA pathogenesis but is an unsuitable marker with which to screen at-risk populations.

Objective/Background: Recent genetic data suggest that a polymorphism of *LRP1* is an independent risk factor for abdominal aortic aneurysm (AAA). The aims of this study were to assess whether plasma and aortic concentrations of low-density lipoprotein receptor-related protein 1 (LRP1) are associated with AAA, and to investigate the possible relevance of LRP1 to AAA pathophysiology.

Methods: Three analyses were conducted. First, plasma LRP1 concentrations were measured in community-dwelling men with and without AAA ($n = 189$ and $n = 309$, respectively) using enzyme-linked immunosorbent assay. Second, Western blotting analyses were employed to compare the expression of LRP1 protein in aortic biopsies collected from patients with AAA and nonaneurysmal postmortem donors ($n = 6$/group). Finally, the effect of *in vitro* LRP1 blockade on matrix metalloprotease 9 (MMP9) clearance by vascular smooth muscle cells was assessed by zymography.

Results: Plasma LRP1 concentrations did not differ between groups of men with and without AAA (median concentration 4.56 μg/mL [interquartile range {IQR} (3.39–5.96)] and 4.43 μg/mL [IQR 3.44–5.84], respectively; $p = .48$), and were not associated with AAA after adjusting for other risk factors (odds ratio 1.10 [95% confidence interval: 0.91–1.32]; $p = 0.35$). In contrast, LRP1 expression was approximately 3.4-fold lower in aortic biopsies recovered from patients with AAA compared with controls (median [IQR] expression 1.72 [0.94–3.14] and 5.91 [4.63–6.94] relative density units, respectively; $p < .01$). *In vitro* LRP1 blockade significantly reduced the ability of vascular smooth muscle cells to internalize extracellular MMP9.

Conclusions: These data suggest that aortic but not circulating LRP1 is downregulated in patients with AAA and indicates a possible role for this protein in clearing an aneurysm-relevant ligand.

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☆ Sadly, Professor Philip Walker passed away during the production of this publication. The authors are deeply indebted to his contribution to this and other vascular research.

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INTRODUCTION

Abdominal aortic aneurysm (AAA) affects approximately 2% of men and roughly 1% of women aged > 65 years, and significantly increases the risk of mortality through aortic rupture and associated cardiovascular events.[1,2] The aetiology of AAA remains unclear; however, inflammation, extracellular matrix degeneration, and vascular smooth muscle cell (VSMC) loss appear to be important in the pathogenesis.[3,4] AAA risk factors include advanced age, male sex, and smoking.[1] A positive family history roughly doubles the risk of AAA, suggesting that genetic factors are important in the development of AAA.[5,6] A genome-wide association study identified a positive association of the rs1466535 major (C) allele within *LRP1* with the presence of AAA (odds ratio [OR] ~1.2).[7] This association appeared to be AAA specific and was maintained after adjusting for cardiovascular risk factors, leading to the suggestion that the *LRP1* rs1466535 C allele might significantly contribute to the risk of AAA.[8,9] However, this is contradicted by recent reports of a positive association between AAA and the *LRP1* rs1466535 T (minor) allele in a geographically distinct patient population.[10]

Low-density lipoprotein receptor-related protein 1 (LRP1) is structurally related to the low-density lipoprotein receptor and comprises a 100-amino acid cytoplasmic domain, a membrane-spanning region, and an extracellular loop with four ligand-binding regions.[11,12] LRP1 is predominantly expressed by VSMCs and facilitates the internalization and clearance of bound ligands.[12–14] Previous studies suggest that LRP1 has functions beyond lipid metabolism, and known LRP1 ligands include growth factors, matrix metalloproteases (MMPs), protease inhibitor complexes, and extracellular matrix components.[8,11,12] Conditional LRP1 knockout rodent models demonstrate that LRP1 deficiency results in abnormal VSMC proliferation and migration, increased extracellular matrix turnover and aneurysm formation within the mesenteric arteries.[14–16] Currently, only one study has directly investigated whether LRP1 expression is altered in human AAA.[17] In this study, Chan et al. employed Western blotting and immunohistochemistry to demonstrate significant downregulation of the LRP1 protein in aortic biopsies of Chinese patients with AAA compared with nonaneurysmal controls. It was hypothesized in the present study that reduced aortic LRP1 concentrations may result from release of this protein from the aneurysm wall, which may increase circulating LRP1 concentrations in patients with AAA. In this study the association of circulating and aortic concentrations of LRP1 with AAA were assessed. The functional role of LRP1 in clearing MMP9 in VSMCs was also examined *in vitro*.

MATERIALS AND METHODS

Detailed materials and methods are provided in Supplementary File 1.

Participants

Samples collected from three Australian cohorts were used: (i) plasma samples collected from participants of the Health in Men Study (HIMS); (ii) aortic biopsies from patients undergoing open surgery to repair large AAA; (iii) aortic biopsies from nonaneurysmal heart-beating, brain-dead organ donors.[18] For HIMS participants, an infrarenal aortic (IRA) diameter of 30–55 mm was defined as a small AAA. An IRA diameter >55 mm was considered as a large AAA. Clinical information collected during the HIMS included age, medical history, and smoking status. For patients undergoing AAA repair, risk factors were recorded as previously described (Supplementary File 1).[19,20] Maximum IRA diameter was measured from axial computed tomography angiography images as previously described.[21] No clinical information other than age and sex was available for organ donor participants. In all instances, written informed consent and institutional ethics approval was provided.

Measurement of plasma LRP1

Plasma LRP1 concentrations were measured using a commercially available enzyme-linked immunosorbent assay (ELISA) (#E91010Hu, USCN Life Sciences, Wuhan, China). This ELISA employs antibodies against a soluble extracellular LRP1 immunogen suggesting suitability for plasma analysis. Reported inter- and intra-assay variabilities are < 12%. Owing to limited plasma volumes, single measurements were taken for each patient as previously described.[19,22]

In vitro *experiments*

Human aortic VSMC (CC-2571; Lonza, Walkersville, MD, USA) were maintained in growth medium in a humidified 5% CO_2 atmosphere at 37 °C and passaged when 70–80% confluent. After five passages, LRP1-blocking antibodies or isotype control antibodies (#MA1-27198 and MA5-14453, respectively; Thermo Fisher Scientific, Scoresby, Australia) were added to the culture media at concentrations of 30 μg/mL. Cell culture supernatants were decanted after 24 hours and replaced with media containing 20 ng/mL recombinant human MMP9 (#911-MP-010; R&D Systems, Minneapolis, MN, USA). Cell culture supernatants were harvested at 24 and 48 hours, and stored at −80 °C. Data presented are from three independent culture experiments.

Protein extraction and Western blotting

Isolation of aortic proteins and Western blotting was performed as previously described.[23] Full-thickness human abdominal aortic samples were homogenized in the presence of protease inhibitors. Samples (30 μg protein/lane) were loaded onto a 10% sodium dodecyl sulfate–polyacrylamide gel, electrophoresed and transferred onto a polyvinylidene difluoride membrane. The membrane was cut at ~60 kDa and each half separately blocked with 5% nonfat dry milk at 4 °C overnight. The 60–250-kDa proteins were incubated with anti-LRP1 antibody (MAB6360; R&D Systems), while the proteins < 60 kDa were incubated with anti-β actin antibody (#AB75186; Abcam, Cambridge, UK) for 1 hour (room temperature). Membranes were washed and incubated with antimouse horseradish peroxidase-conjugated (LRP1 blot) or antirabbit (β-actin)-conjugated