



HAL
open science

Proteomic profiles of left atrial volume and its influence on response to spironolactone: Findings from the HOMAGE trial and STANISLAS cohort

Masatake Kobayashi, João Pedro Ferreira, Duarte Kevin, Emmanuel Bresso, Olivier Huttin, Erwan Bozec, Hans-peter Brunner La Rocca, Christian Delles, Andrew L Clark, Frank Edelmann, et al.

► To cite this version:

Masatake Kobayashi, João Pedro Ferreira, Duarte Kevin, Emmanuel Bresso, Olivier Huttin, et al.. Proteomic profiles of left atrial volume and its influence on response to spironolactone: Findings from the HOMAGE trial and STANISLAS cohort. *European Journal of Heart Failure*, 2024, 10.1002/ejhf.3202 . hal-04525185

HAL Id: hal-04525185

<https://hal.univ-lorraine.fr/hal-04525185>

Submitted on 28 Mar 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Proteomic profiles of left atrial volume and its influence on response to spironolactone: Findings from the HOMAGE trial and STANISLAS cohort

Masatake Kobayashi^{1,2}, João Pedro Ferreira^{1,3}, Duarte Kevin¹, Emmanuel Bresso¹, Olivier Huttin¹, Erwan Bozec¹, Hans-Peter Brunner La Rocca⁴, Christian Delles⁵, Andrew L. Clark⁶, Frank Edelmann⁷, Arantxa González^{8,9}, Stephane Heymans⁴, Pierpaolo Pellicori⁵, Johannes Petutschnigg¹⁰, Job A.J. Verdonschot⁴, Patrick Rossignol^{1,11}, John G.F. Cleland⁵, Faiez Zannad¹, and Nicolas Girerd^{1*}, on behalf of the HOMAGE Trial Committees and Investigators

¹Université de Lorraine, INSERM, Centre d'Investigations Cliniques Plurithématique 1433, Inserm U1116, CHRU de Nancy and F-CRIN INI-CRCT, Nancy, France; ²Department of Cardiology, Tokyo Medical University Hospital, Tokyo, Japan; ³Cardiovascular Research and Development Center, Department of Surgery and Physiology, Faculty of Medicine of the University of Porto, Porto, Portugal; ⁴Department of Cardiology, Maastricht University Medical Center, Maastricht, The Netherlands; ⁵School of Cardiovascular and Metabolic Health, University of Glasgow, Glasgow, UK; ⁶Department of Cardiology, University of Hull, Castle Hill Hospital, Yorkshire, UK; ⁷Department of Internal Medicine and Cardiology Campus Virchow Klinikum, Charité University Medicine Berlin and German Centre for Cardiovascular Research (DZHK), Partner Site Berlin, Berlin, Germany; ⁸CIMA Universidad de Navarra, Department of Pathology, Anatomy and Physiology Universidad de Navarra and IdiSNA, Pamplona, Spain; ⁹CIBERCV, Carlos III Institute of Health, Madrid, Spain; ¹⁰Department of Internal Medicine and Cardiology, Campus Virchow Klinikum, Charité University Medicine Berlin, and German Heart Center Berlin, and Berlin Institute of Health (BIH), and German Centre for Cardiovascular research (DZHK), Berlin, Germany; and ¹¹Medical Specialties and Nephrology Dialysis Departments, Monaco Princess Grace Hospital and Monaco Private Hemodialysis Centre, Monaco, Monaco

Received 11 December 2023; revised 21 February 2024; accepted 7 March 2024

Aims

High left ventricular filling pressure increases left atrial volume and causes myocardial fibrosis, which may decrease with spironolactone. We studied clinical and proteomic characteristics associated with left atrial volume indexed by body surface area (LAVi), and whether LAVi influences the response to spironolactone on biomarker expression and clinical variables.

Methods and results

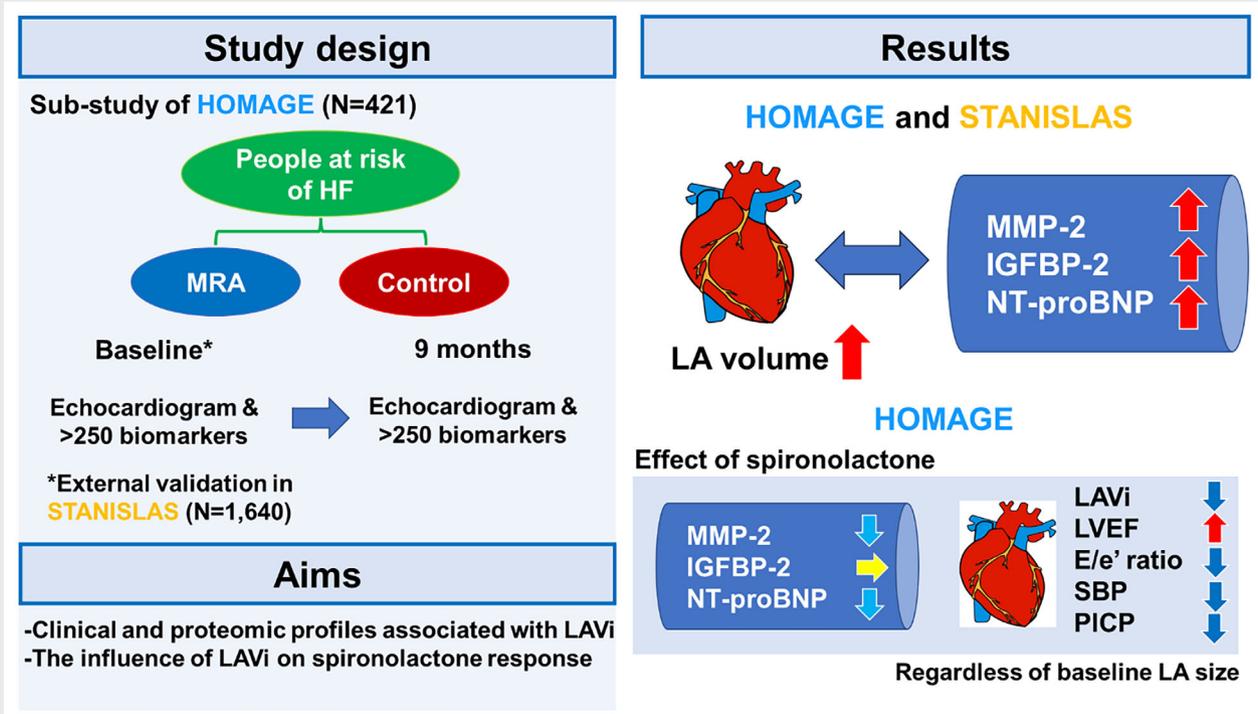
In the HOMAGE trial, where people at risk of heart failure were randomized to spironolactone or control, we analysed 421 participants with available LAVi and 276 proteomic measurements (Olink) at baseline, month 1 and 9 (mean age 73 ± 6 years; women 26%; LAVi 32 ± 9 ml/m²). Circulating proteins associated with LAVi were also assessed in asymptomatic individuals from a population-based cohort (STANISLAS; $n = 1640$; mean age 49 ± 14 years; women 51%; LAVi 23 ± 7 ml/m²). In both studies, greater LAVi was significantly associated with greater left ventricular masses and volumes. In HOMAGE, after adjustment and correction for multiple testing, greater LAVi was associated with higher concentrations of matrix metalloproteinase-2 (MMP-2), insulin-like growth factor binding protein-2 (IGFBP-2) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) (false discovery rates [FDR] < 0.05). These associations were externally replicated in STANISLAS (all FDR < 0.05). Among these biomarkers, spironolactone decreased concentrations of MMP-2 and NT-proBNP, regardless of baseline LAVi ($p_{\text{interaction}} > 0.10$). Spironolactone also significantly reduced LAVi, improved left ventricular ejection fraction, lowered E/e', blood pressure and serum procollagen type I C-terminal propeptide (PICP) concentration, a collagen synthesis marker, regardless of baseline LAVi ($p_{\text{interaction}} > 0.10$).

*Corresponding author. Centre d'Investigation Clinique 1433 Module Plurithématique, CHRU Nancy – Hopitaux de Brabois, Institut Lorrain du Coeur et des Vaisseaux Louis Mathieu, Nancy 54500, France. Email: n.girerd@chru-nancy.fr

Conclusion

In individuals without heart failure, LAVi was associated with MMP-2, IGFBP-2 and NT-proBNP. Spironolactone reduced these biomarker concentrations as well as LAVi and PICP, irrespective of left atrial size.

Graphical Abstract



In individuals at risk of developing heart failure (HF) (HOMAGE), left atrial volume index (LAVi) showed associations with matrix metalloproteinase-2 (MMP-2), insulin-like growth factor binding protein-2 (IGFBP-2), and N-terminal pro-B-type natriuretic peptide (NT-proBNP). These associations were validated in an independent community-based cohort (STANISLAS). Among these biomarkers, spironolactone reduced concentrations of MMP-2 and NT-proBNP over a 9-month period in HOMAGE participants, irrespective of baseline LAVi. Additionally, spironolactone significantly decreased LAVi, improved left ventricular ejection fraction (LVEF), reduced E/e', blood pressure, and serum procollagen type I C-terminal propeptide (PICP) concentration, regardless of baseline LAVi. LA, left atrial; MRA, mineralocorticoid receptor antagonist; SBP, systolic blood pressure.

Keywords

Heart failure • Spironolactone • Left atrial volume • Echocardiogram • Biomarkers

Introduction

In patients with higher left ventricular (LV) diastolic pressure, the left atrium gradually dilates and develops fibrosis; hence, left atrial (LA) volume may be a surrogate measure of the chronicity and severity of increased LV filling pressure.^{1–3} LA enlargement is a key echocardiographic feature in the diagnosis of heart failure (HF) with preserved ejection fraction (HFpEF),^{4,5} and is a well-known marker of a heightened risk of morbidity and/or mortality in the general population as well as in patients with HFpEF.^{6–8}

Mineralocorticoid receptor antagonists (MRAs) reduce LA volume by decreasing LV filling pressure, extracellular matrix turnover and myocardial collagen.⁹ In the Heart OMics in AGEing (HOMAGE) trial, spironolactone (vs. standard care) significantly reduced LA volume indexed to body surface area (LAVi) over 9 months in individuals at high risk of developing HF.¹⁰ However, other studies did not find similarly significant reverse LA remodelling with MRA treatment.^{11–13} Circulating proteins whose domains are likely to be involved in congestion, thrombosis, inflammation, and fibrotic pathways may provide insights into the

biological pathways underlying LA enlargement^{14,15} and LA reverse remodelling in response to spironolactone.

The aim of the present study was to explore the clinical and proteomic characteristics associated with LAVi in patients at risk of HF in the HOMAGE trial. These associations with LAVi were externally validated in asymptomatic individuals in the Suivi Temporaire Annuel Non-Invasif de la Santé des Lorrains Assurés Sociaux (STANISLAS) cohort (ClinicalTrials.gov identifier: NCT01391442).¹⁶ In addition, we studied whether the responses of circulating biomarker concentrations, cardiac structure and function, and blood pressure to spironolactone were influenced by baseline LA size.

Methods

Trial design and population

The HOMAGE trial was a prospective, randomized, open-label, blinded-endpoint, multicentre design, in which people at a high risk of developing HF were randomized to receive either spironolactone or standard of care, and explored the effect of spironolactone on circulating markers of fibrosis, natriuretic peptides, blood pressure and cardiac structure and function over 9 months. The rationale, design and main results have been described previously.^{10,17} The main inclusion criteria were age ≥ 65 years (amended to ≥ 60 years during the course of the trial), established coronary artery disease or at least two criteria indicating high cardiovascular risk (i.e. hypertension, diabetes, microalbuminuria, or an abnormal electrocardiogram), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) between 125 and 1000 pg/ml (or B-type natriuretic peptide [BNP] between 35 and 280 pg/ml). Patients with a pre-existing diagnosis of HF, a LV ejection fraction (LVEF) $< 45\%$, atrial fibrillation (AF), an estimated glomerular filtration rate (eGFR¹⁸) < 30 ml/min/1.73 m² or serum potassium > 5.0 mmol/L were excluded.

The trial was approved by the relevant ethics committees and regulatory bodies. Participants provided written informed consent (ClinicalTrials.gov Identifier: NCT02556450).

The STANISLAS cohort study is a single-centre familial longitudinal population-based cohort from the Nancy region in France in which participants with a history of AF were excluded.¹⁶ Among 1705 participants undergoing the fourth cohort visit, 1613 participants had both available echocardiographic and proteomic biomarker data.

Echocardiogram

In both HOMAGE and STANISLAS, LA volume, LV volumes and LVEF were measured using the biplane method of disks summation (modified Simpson's rule). Diastolic function was assessed from the pattern of mitral inflow by pulsed-wave Doppler. Mitral annular early diastolic velocity (e') was assessed at the septal and lateral sites of the mitral annulus using tissue Doppler imaging. E/A ratio, average e' and average E/e' ratio were calculated.¹⁹ Tricuspid annular plane systolic excursion measurement was obtained.²⁰

Left ventricular hypertrophy was defined as LV mass indexed to body surface area > 115 g/m² in men or > 95 g/m² in women, and abnormal e' by a septal $e' < 7$ cm/s or lateral $e' < 10$ cm/s.^{19,21}

In the Lorraine university, in which all echocardiographic variables were measured in both HOMAGE and STANISLAS, intra- and interobserver intraclass correlation coefficients of each

echocardiographic variable provided good reproducibility as previously reported.^{22,23}

Proteomic biomarkers

Baseline, month 1 and 9 plasma samples in HOMAGE and baseline samples in STANISLAS were analysed for 276 protein biomarkers by the TATAA-biocyte using the Olink Proseek[®] Multiplex cardiovascular (CVD) II, CVD III, and inflammation panels. The proteins were determined using high-throughput Olink Proseek[®] Multiplex 96 \times 96 kits, which measure 92 selected proteins simultaneously in 1 μ l of plasma per kit. It is based on the proximity extension assay technology with dual-recognition DNA-coupled readout, where 92 oligonucleotide-labelled antibody probe pairs bind to their respective target in the sample allowing hybridization of the DNA-tag sequences and proximity extension with subsequent amplification by a Fluidigm BioMark[™] HD real-time polymerase chain reaction platform. The platform provides log₂ normalized protein expression (NPX) values with relative quantification. A detailed description of the Olink[®] technology is provided on the website: <https://www.olink.com/>. The abbreviations, full names and respective Olink[®] multiplex panels of the studied proteins are described in online supplementary Table S1. In addition, serum procollagen type I C-terminal propeptide (PICP) and procollagen type III N-terminal propeptide (PIIINP) as fibrotic markers associated with histologically proven myocardial fibrosis,²⁴ were measured by enzyme immunoassay (METRA; Quidel Corporation[®]), and by radio-immunoassay (Orion Diagnostica[®]), respectively. In HOMAGE, plasma NT-proBNP was measured by electro-chemiluminescent assays (Roche Diagnostics) as previously reported.¹⁰

All biomarkers were available in 98.3% and 98.4% of participants in HOMAGE and STANISLAS, respectively. Biomarkers with 75% or more values below the limit of detection (LOD) were excluded from candidate variables. When the proportion of below LOD value was 75% or less, the below limit of detection values was replaced by the LOD. Totally, 25 and 24 protein biomarkers were not included in the current analysis in HOMAGE and STANISLAS, respectively.

Complex network analysis

Pathway and gene ontology term overrepresentation was calculated for proteins selected by the statistical analysis. Protein annotations were extracted from the FHF graph knowledge box (FHF-GKBox).²⁵ In the FHF-GKBox, protein-pathway relationships were extracted from Reactome (version 69)²⁶ and gene ontology annotation from AmiGO.²⁷ Only annotations with a p -value lower than 5% linked to at least two selected proteins and proteins linked to these pathways were considered to construct the graph. The network was displayed using Cytoscape (version 3.10.1).²⁸

Statistical analysis

Categorical variables are presented as frequencies (percentages) and continuous variables as means \pm standard deviations or medians (25th and 75th percentiles) depending on distribution. Comparisons of the patient characteristics across LAVi tertiles were analysed using analysis of variance, Kruskal-Wallis and χ^2 tests, as appropriate.

In HOMAGE and STANISLAS, 276 protein biomarkers plus PICP and PIIINP were used to assess whether circulating proteins were associated with the extent of LAVi. Linear regression analyses with LAVi as an outcome variable were performed with each circulating

biomarker as an independent variable plus age, sex, body mass index, prevalence of hypertension and diabetes, and eGFR as adjustment covariates, followed by p -values corrected for the multiplicity of tests using the Benjamini–Hochberg method. To better visualize the associations of biomarkers with LAVi in multivariable models, we performed a volcano plot using the beta coefficients of models having LAVi as an outcome.

For those biomarkers in both HOMAGE and STANISLAS with differential expression by LAVi, we then tested whether spironolactone changed the levels of biomarker during follow-up, using analysis of covariance (ANCOVA) to compare the difference in changes between spironolactone and control groups in HOMAGE.

ANCOVA was used with a treatment-by-LAVi interaction term to study the relation between LAVi and the effect of spironolactone on clinical variables (i.e. natriuretic peptide, cardiac function, and collagen synthesis marker). The effect of spironolactone on LAVi was assessed using a mixed effect model with LAVi as dependent variable (measured at baseline, month 1 and month 9) and treatment (spironolactone vs. control) as independent fixed-effects variable; the random intercepts were set at the patient 'ID' level with an unstructured covariance matrix.

Statistical analyses were performed using R version 4.2.1 (R Development Core Team, Vienna, Austria). A two-sided p -value <0.05 was considered statistically significant.

Results

Patient characteristics

Among 421 HOMAGE patients with available baseline LAVi, mean age was 73 ± 6 years, 26% were women, one-third (33%) had a body mass index ≥ 30 kg/m², 77% had hypertension, 41% had a history of myocardial infarction, and median plasma concentration of NT-proBNP was 207 pg/ml (25th and 75th percentiles 134–354 pg/ml). Mean LVEF was $62 \pm 7\%$, 28% had LV hypertrophy and 86% had abnormal e' .

The respective LAVi tertiles were: tertile 1: <28 ml/m² ($n = 141$), tertile 2: $28–34$ ml/m² ($n = 140$), and tertile 3: >34 ml/m² ($n = 140$). A greater LAVi was significantly associated with lower heart rate, a greater LV volume, LV mass indexed to body surface area, and higher plasma NT-proBNP (all p -values <0.01) (Table 1). There were no significant differences in measures of LA strain (i.e. reservoir strain, conduit strain, contractile strain) across the LAVi tertiles (Table 1).

Similar baseline echocardiographic features associated with LAVi were observed in STANISLAS (online supplementary Table S2).

Patients having a greater baseline LAVi were more likely to decrease the levels of systolic blood pressure, eGFR, LV end-diastolic volume index and LAVi from baseline to month 1 and month 9 (all $p < 0.01$) (online supplementary Table S3).

Circulating biomarkers associated with left atrial volume index in HOMAGE and STANISLAS

In HOMAGE, among 253 analysed biomarkers, 33 biomarkers were significantly associated with the degrees of LAVi after adjustment

for covariates. A volcano plot showing all differentially expressed biomarkers for LAVi is demonstrated in Figure 1. After accounting for multiple testing, six biomarkers were selected (Figure 1 and Table 2). Notably, higher levels of matrix metalloproteinase-2 (MMP-2), insulin-like growth factor binding protein-2 (IGFBP-2), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) were significantly associated with a greater LAVi. These associations were externally replicated in STANISLAS as shown in Figure 1 and Table 2.

The list of biomarkers that were significantly associated with LAVi in both studies is shown in online supplementary Tables S4 and S5.

Pathways linked to left atrial volume index-associated biomarkers

When incorporating 14 biomarkers significantly associated with LAVi in complex network analysis, extracellular matrix remodelling and collagen synthesis/degradation were over-represented pathways, which were linked to the highest number of biomarkers (i.e. MMP-2, MMP-3, decorin, and cathepsin L1), highlighting the fibrotic pathways as the most central biological feature (online supplementary Figure S1).

Effect of spironolactone on top proteins associated with left atrial volume index

Among biomarkers associated with LAVi in both HOMAGE and STANISLAS, spironolactone reduced MMP-2 and NT-proBNP without significant treatment-by-LAVi heterogeneity ($p_{\text{interaction}} > 0.10$). Spironolactone did not significantly change the circulating levels of IGFBP-2 (Table 3).

These effects of spironolactone were consistent, regardless of baseline use of statin therapy or angiotensin-converting enzyme inhibitor or angiotensin receptor blocker (ACEi/ARB) (all $p_{\text{interaction}} > 0.10$).

Effects of spironolactone on clinical variables by baseline left atrial size

During a 9-month period, spironolactone decreased LAVi by -1.32 ml/m² versus standard care ($p = 0.01$). The effect of spironolactone on LAVi was consistently observed across LAVi tertiles ($p_{\text{interaction}} = 0.23$) (Table 4).

There was no interaction between baseline LA size and the effects of spironolactone (vs. control) on increasing LVEF, reducing E/e' , lowering NT-proBNP, systolic blood pressure and serum PICP concentrations ($p_{\text{interaction}} > 0.10$ for all) (Table 4).

Discussion

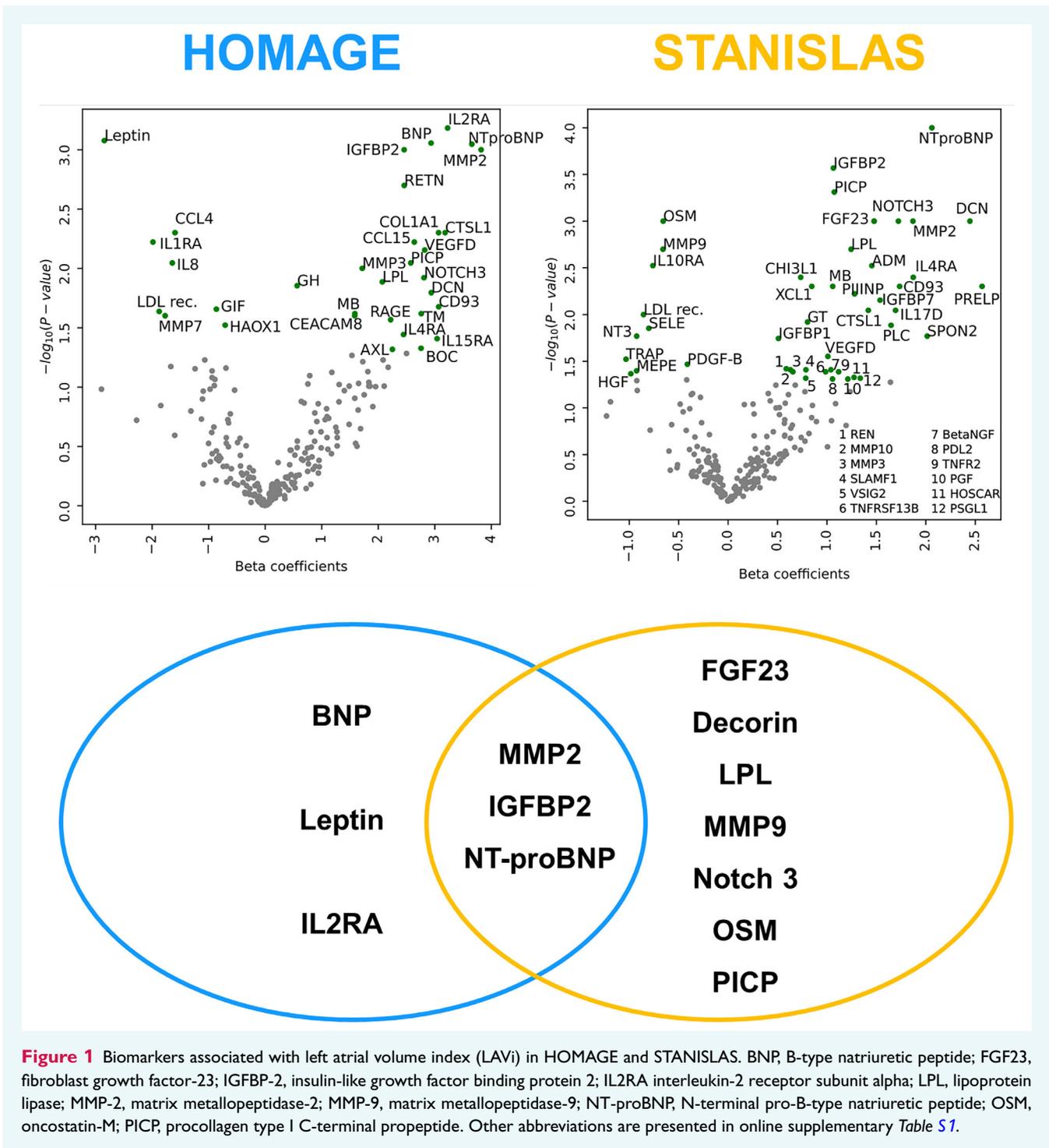
This analysis reveals that an increase in LA volume is consistently associated with elevated levels of NT-proBNP, MMP-2, and IGFBP-2, which were mainly linked to fibrotic pathways as revealed by complex network analysis. These associations were evident in

Table 1 Baseline characteristics according to left atrial volume index tertiles in HOMAGE

	Left atrial volume index				p-value
	Overall (n = 421)	T1 <28 ml/m ² (n = 141)	T2 28–34 ml/m ² (n = 140)	T3 >34 ml/m ² (n = 140)	
Age, years	73 ± 6	73 ± 6	73 ± 6	74 ± 7	0.238
Female sex, n (%)	108 (25.7)	34 (24.1)	30 (21.4)	44 (31.4)	0.140
Body mass index, kg/m ²	29 ± 5	29 ± 5	28 ± 5	29 ± 5	0.814
Smoker, n (%)	36 (8.6)	16 (11.3)	6 (4.3)	14 (10.0)	0.080
Medical history, n (%)					
Hypertension	323 (76.7)	100 (70.9)	108 (77.1)	115 (82.1)	0.083
Diabetes	170 (40.4)	58 (41.1)	58 (41.4)	54 (38.6)	0.866
Coronary artery disease	307 (72.9)	102 (72.3)	108 (77.1)	97 (69.3)	0.329
Myocardial infarction	171 (40.6)	60 (42.6)	59 (42.1)	52 (37.1)	0.590
Stroke	23 (5.5)	1 (0.7)	12 (8.6)	10 (7.1)	0.008
COPD	29 (6.9)	14 (9.9)	8 (5.7)	7 (5.0)	0.211
Medications, n (%)					
ACEi or ARB	331 (78.6)	107 (75.9)	104 (74.3)	120 (85.7)	0.041
Beta-blocker	299 (71.0)	104 (73.8)	99 (70.7)	96 (68.6)	0.629
Calcium channel blockers	85 (20.2)	23 (16.3)	31 (22.1)	31 (22.1)	0.372
Statin	355 (84.3)	121 (85.8)	120 (85.7)	114 (81.4)	0.514
Anti-platelet therapy	335 (79.6)	109 (77.3)	115 (82.1)	111 (79.3)	0.600
Physical assessment					
Systolic BP, mmHg	143 ± 21	140 ± 20	142 ± 22	146 ± 21	0.070
Heart rate, bpm	61 ± 9	63 ± 8	61 ± 10	60 ± 10	0.004
Laboratory data					
Haemoglobin, g/dl	14.0 ± 1.4	14.3 ± 1.4	14.0 ± 1.2	13.7 ± 1.5	0.002
Sodium, mmol/L	139.3 ± 2.8	139.1 ± 2.3	139.0 ± 3.0	139.9 ± 3.0	0.010
Potassium, mmol/L	4.3 ± 0.4	4.3 ± 0.4	4.4 ± 0.4	4.3 ± 0.3	0.640
eGFR, ml/min/1.73 m ²	71 ± 16	70 ± 15	71 ± 16	72 ± 16	0.697
NT-proBNP, pg/ml	207 (134–354)	176 (126–256)	209 (134–380)	249 (141–489)	0.003
NT-proBNP (NPX)	5.4 ± 0.8	5.1 ± 0.7	5.4 ± 0.8	5.6 ± 0.8	<0.001
Echocardiographic variables					
LVEDVi, ml/m ²	43 ± 11	38 ± 9	43 ± 11	48 ± 12	<0.001
LVEF, %	62 ± 7	62 ± 6	62 ± 7	62 ± 7	0.999
GLS, % (n = 76)	−17.8 ± 2.5	−18.1 ± 2.4	−17.1 ± 2.6	−18.2 ± 2.2	0.235
LVMi, g/m ²	97 ± 26	90 ± 21	96 ± 24	106 ± 29	<0.001
Men	98 ± 25	91 ± 22	97 ± 25	106 ± 27	<0.001
Women	96 ± 28	87 ± 19	92 ± 20	106 ± 35	0.006
LV hypertrophy, n (%)	110 (27.6%)	27 (20.0%)	35 (25.9%)	48 (37.2%)	0.007
E wave, cm/s	69 ± 18	65 ± 15	69 ± 17	73 ± 20	0.001
A wave, cm/s	81 ± 20	80 ± 19	80 ± 19	83 ± 22	0.483
E/A ratio	0.9 ± 0.3	0.8 ± 0.3	0.9 ± 0.3	0.9 ± 0.3	0.071
Lateral e', cm/s	8.7 ± 2.7	8.5 ± 2.4	8.6 ± 2.6	9.1 ± 3.1	0.121
Septal e', cm/s	6.0 ± 1.6	6.0 ± 1.6	6.1 ± 1.6	5.9 ± 1.6	0.837
Septal e' <7 cm/s or lateral e' <10 cm/s, n (%)	335 (85.7)	112 (84.8)	113 (85.6)	110 (86.6)	0.921
E/e' mean	9.9 ± 3.2	9.5 ± 3.2	10.0 ± 3.1	10.2 ± 3.4	0.213
E/e' mean >14	40 (10.3%)	10 (7.7%)	15 (11.5%)	15 (11.8%)	0.483
TAPSE, mm	22.3 ± 6.4	21.9 ± 6.1	23.0 ± 6.4	22.0 ± 6.7	0.310
LA variables					
LA volume, ml	61 ± 17	45 ± 7	60 ± 7	79 ± 14	<0.001
LA volume/height, ml/m	36 ± 10	27 ± 4	35 ± 3	47 ± 8	<0.001
LA reservoir strain, % (n = 104)	24.8 ± 4.9	25.3 ± 5.0	25.0 ± 5.1	23.8 ± 4.6	0.441
LA conduit strain, % (n = 104)	10.5 ± 3.4	10.6 ± 3.2	10.5 ± 3.8	10.3 ± 3.4	0.947
LA contractile strain, % (n = 104)	14.3 ± 3.8	14.7 ± 4.3	14.4 ± 3.6	13.4 ± 3.3	0.404
LA emptying fraction, % (n = 104)	56.1 ± 9.4	56.2 ± 9.9	58.3 ± 9.4	53.0 ± 8.0	0.071

Values are expressed as mean ± standard deviation, n (%), or median (25th–75th percentile).

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BP, blood pressure; BSA, body surface area; COPD, chronic obstructive pulmonary disease; eGFR, estimated glomerular filtration rate; GLS, global longitudinal strain; LA, left atrial; LV, left ventricular; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVMi, left ventricular mass index; NT-proBNP, N-terminal B-type natriuretic peptide; TAPSE, tricuspid annular plane systolic excursion.



asymptomatic individuals with relatively low cardiovascular risk, and in those with high cardiovascular risk (i.e. coronary artery disease) and a risk for developing HF. Notably, the use of spironolactone led to a significant reduction in LA volume and levels of natriuretic peptide and MMP-2. The treatment with spironolactone also resulted in improvements in LVEF, changes in diastolic function, lowered systolic blood pressure, and reduced markers of collagen synthesis, with these beneficial effects observable regardless

of baseline LA size (*Graphical Abstract*). These findings provide new insights into the biological processes driving LA enlargement and highlight the potential of spironolactone in reversing this structural abnormality, offering valuable implications for the treatment, and understanding of HF development.

In the current analysis of the HOMAGE trial, participants had a similar LA volume to those with symptomatic HFpEF included in prior clinical trials,^{7,11,29} supporting a HFpEF-like remodeled LA

Table 2 Top proteins associated with left atrial volume index in HOMAGE and STANISLAS

	HOMAGE			STANISLAS		
	Beta (95% CI)	p-value	FDR	Beta (95% CI)	p-value	FDR
MMP-2	3.82 (1.53 to 6.11)	0.001	0.036	1.87 (0.72 to 3.02)	0.001	0.028
IGFBP-2	2.46 (1.03 to 3.89)	0.001	0.036	1.07 (0.49 to 1.64)	<0.001	<0.001
NT-proBNP	3.66 (2.69 to 4.62)	<0.001	<0.001	2.06 (1.66 to 2.46)	<0.001	<0.001
BNP	2.93 (2.23 to 3.64)	<0.001	<0.001	–	–	–
Leptin	–2.85 (–3.93 to –1.76)	<0.001	<0.001	–	–	–
IL2RA	3.23 (1.55 to 4.91)	<0.001	<0.001	–	–	–
FGF23	–	–	–	1.48 (0.61 to 2.34)	0.001	0.028
Decorin	–	–	–	2.45 (0.97 to 3.92)	0.001	0.028
Lipoprotein lipase	–	–	–	1.24 (0.46 to 2.03)	0.002	0.046
MMP-9	–	–	–	–0.66 (–1.08 to –0.24)	0.002	0.046
Notch 3	–	–	–	1.72 (0.72 to 2.73)	0.001	0.028
Oncostatin-M	–	–	–	–0.66 (–1.04 to –0.27)	0.001	0.028
PICP	–	–	–	1.07 (0.51 to 1.64)	<0.001	<0.001

BNP, B-type natriuretic peptide; CI, confidence interval; FDR, false discovery rate; FGF23, fibroblast growth factor-23; IGFBP-2, insulin-like growth factor binding protein-2; IL2RA interleukin-2 receptor subunit alpha; LAVi, left atrial volume index; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PICP, procollagen type I C-terminal propeptide. Proteins measured by Olink are presented with log₂NPX. PICP was transformed by natural logarithm.

Table 3 Effect of spironolactone on the top proteins associated with left atrial volume index in HOMAGE

	Treatment-group-difference		Treatment-by-LAVi P _{interaction}
	Month 1	Month 9	
MMP-2	–0.104 (–0.170 to –0.037) p = 0.002	–0.118 (–0.185 to –0.051) p < 0.001	0.697
IGFBP-2	–0.068 (–0.150 to 0.015) p = 0.110	–0.014 (–0.106 to 0.078) p = 0.766	0.327
NT-proBNP	–0.294 (–0.436 to –0.152) p < 0.001	–0.144 (–0.299 to –0.012) p = 0.072	0.582

IGFBP-2, insulin-like growth factor binding protein-2; LAVi, left atrial volume index; MMP-2, matrix metalloproteinase-2; NT-proBNP, N-terminal pro-B-type natriuretic peptide.

size. Compared to individuals in the lower LAVi tertile (<28 ml/m²), those in the upper tertile of LAVi (>34 ml/m²) had greater LV volume, greater LV mass, and higher natriuretic peptide level, without significant differences in the various LA strains.

Matrix metalloproteinase-2 is involved in a variety of processes, including extracellular matrix, tissue repair, angiogenesis, and inflammation, and is thought to be secreted by cardiomyocytes and myofibroblasts.^{30,31} MMP-2 may also promote the occurrence of AF and LA fibrosis via connexin-43,³² and was correlated with LA volume as shown previously.³³ MMP-2 was associated with a risk of incident HF and AF in the general population³⁴ as well as with worsening HF in those with established disease.³⁵ In pre-clinical studies, an inhibition of MMP mitigated LV remodelling and improved cardiac function^{36,37}; however, clinical trials showed that MMP inhibitors failed to attenuate LV remodelling following myocardial infarction, possibly due to the enhanced MMP phenotype.³⁸ Nonetheless, in HOMAGE, spironolactone significantly reduced MMP-2 levels over 9 months, suggesting its potential MMP inhibitory properties. This finding further supports the notion that MMP could serve as a target for pharmacological approach in the management of HF, which should be worthy of testing in dedicated studies.

Insulin-like growth factor binding protein-2 modulates metabolic and inflammation pathways in the heart, and counteracts the effect of IGFBP-1, which was related to the progression of myocardial remodelling and fibrosis.³⁹ In previous studies, IGFBP-2 was suggested as a marker to diagnose acute HF as well as predict prognosis in chronic HF.^{40–42} In an interesting analysis using public biological databases, IGFBP-2 had many pathways linking to HF, which did not overlap with BNP.²⁵ In HOMAGE, IGFBP-2 was not significantly changed with spironolactone treatment over 9 months. Along with well-known antifibrotic effect of spironolactone, our data suggest that IGFBP-2 might be related to metabolic and inflammatory rather than fibrotic pathways as confirmed by complex network analysis.^{1,43}

In the present study, spironolactone significantly reduced LAVi from baseline to month 9, regardless of baseline LAVi. However, surprisingly, in prior studies, MRAs had no impact on LA size in patients with HFpEF. In the Aldosterone Receptor Blockade in Diastolic Heart Failure (Aldo-DHF) trial, in which 422 patients with symptomatic HFpEF were randomized to either spironolactone or placebo, over 12 months, spironolactone versus placebo significantly improved diastolic function reflected by decreases in E/e' and e', but did not change LAVi.¹¹ In a post-hoc analysis of

Table 4 Effect of spironolactone on outcomes across left atrial volume index tertiles in HOMAGE

	Month 1		Month 9		Overall joint p-value	Treatment-by-LAVi p _{interaction}
	Treatment-group-difference (95% CI)	p-value	Treatment-group-difference (95% CI)	p-value		
LAVi change (mL/m ²)						
Overall	-0.91 (-1.91 to 0.08)	0.072	-1.32 (-2.32 to -0.32)	0.010	0.096	
LAVi <28 ml/m ²	-0.58 (-1.79 to 0.64)	0.355	-1.10 (-2.31 to 0.10)	0.076		0.234
LAVi 28–34 ml/m ²	-1.18 (-2.74 to 0.38)	0.141	-0.26 (-2.04 to 1.52)	0.778		
LAVi >34 ml/m ²	-0.85 (-3.26 to 1.55)	0.489	-2.68 (-4.77 to -0.6)	0.013		
LVEF change (%)						
Overall	-0.49 (-1.65 to 0.66)	0.402	1.17 (0.17 to 2.18)	0.026	0.325	
LAVi <28 ml/m ²	0.89 (-1.24 to 3.03)	0.415	1.09 (-0.57 to 2.75)	0.202		0.939
LAVi 28–34 ml/m ²	-1.64 (-3.61 to 0.33)	0.107	1.13 (-0.54 to 2.81)	0.188		
LAVi >34 ml/m ²	-1.62 (-3.77 to 0.52)	0.142	0.98 (-1.27 to 3.23)	0.396		
E/e' change						
Overall	-0.23 (-0.71 to 0.25)	0.342	-0.48 (-0.94 to -0.02)	0.041	0.119	
LAVi <28 ml/m ²	0.41 (-0.26 to 1.07)	0.234	-0.30 (-1.13 to 0.52)	0.474		0.859
LAVi 28–34 ml/m ²	-0.73 (-1.52 to 0.06)	0.072	-0.73 (-1.44 to -0.03)	0.043		
LAVi >34 ml/m ²	-0.28 (-1.31 to 0.76)	0.601	-0.41 (-1.30 to 0.48)	0.371		
NT-proBNP change (pg/ml)						
Overall	-9.41 (-17.05 to -1.77)	0.016	-5.81 (-10.11 to -1.51)	0.008	0.033	
LAVi <28 ml/m ²	-4.31 (-7.73 to -0.89)	0.015	-4.40 (-10.09 to 1.30)	0.133		0.082
LAVi 28–34 ml/m ²	-3.42 (-7.92 to 1.07)	0.138	0.38 (-6.36 to 7.13)	0.912		
LAVi >34 ml/m ²	-19.92 (-42.25 to 2.41)	0.082	-12.82 (-21.69 to -3.94)	0.005		
SBP change (mmHg)						
Overall	-6.01 (-8.84 to -3.18)	<0.001	-10.58 (-13.62 to -7.53)	<0.001	<0.001	
LAVi <28 ml/m ²	-5.23 (-9.75 to -0.72)	0.025	-7.04 (-12.35 to -1.73)	0.010		0.182
LAVi 28–34 ml/m ²	-7.29 (-12.57 to -2.01)	0.008	-13.16 (-18.71 to -7.61)	<0.001		
LAVi >34 ml/m ²	-5.69 (-10.58 to -0.81)	0.024	-11.81 (-16.72 to -6.90)	<0.001		
PICP change (µg/L)						
Overall	-5.74 (-9.15 to -2.32)	0.001	-9.13 (-13.35 to -4.91)	<0.001	0.004	
LAVi <28 ml/m ²	-4.46 (-10.10 to 1.17)	0.123	-11.08 (-17.88 to -4.28)	0.002		0.978
LAVi 28–34 ml/m ²	-7.19 (-12.88 to -1.49)	0.015	-4.87 (-12.36 to 2.62)	0.205		
LAVi >34 ml/m ²	-5.85 (-12.36 to 0.66)	0.081	-11.66 (-19.33 to -3.99)	0.003		

CI, confidence interval; LAVi, left atrial volume index; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PICP, procollagen type I C-terminal propeptide; SBP, systolic blood pressure.

the Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist (TOPCAT) trial, which included 239 patients with HFpEF who were randomized to spironolactone or placebo, there was no significant difference between changes in any echocardiographic variables between treatment groups during a median follow-up of 24 months.¹² The neutral effects of MRAs on LAVi were also reported in several smaller trials in HFpEF.^{13,44,45} The differences in baseline LA size are likely to be responsible for differences in the response to MRA treatment among these studies. However, it should be noted that, in a meta-analysis of HOMAGE, Aldo-DHF and TOPCAT trials, spironolactone significantly reduced LAVi compared with placebo or usual care.⁴⁶ Additionally, the HOMAGE trial was uniquely designed to exclude people who had a history or presence of AF, which may theoretically impact LA size and the response to treatments.⁹ Our data suggest that spironolactone may potentially help reverse LA remodelling regardless of LA size in people with sinus rhythm and prevent symptomatic HFpEF.

Left atrial enlargement is recognized as an indicator of either comorbidity burden or increased risk of HF.^{47,48} The established risk marker is likely due to the underlying fibrotic pathways in a population prior to HF onset. Although statin therapy and ACEi/ARB may have the potential to have anti-fibrotic effects,^{49,50} spironolactone significantly and consistently decreased these biomarker expressions, irrespective of the baseline use of anti-fibrotic treatments. Moreover, spironolactone reduced LAVi and improved outcomes such as diastolic dysfunction, natriuretic peptide levels, and biomarkers of collagen synthesis, irrespective of baseline LA size. These results support the use of MRA therapy as a preventive measure against HF development, going beyond just LAVi assessment. Moreover, a comprehensive cardiac remodelling assessment, like that offered by the e'VM algorithm, may be more apt than a singular focus on LA volume, providing a better framework for identifying those who will most benefit from MRA therapy.^{51,52}

Strengths and limitations

The presented results have several potential strengths and limitations. The strengths include the robust quality of echocardiographic and biomarker variables. One experienced examiner centrally measured all echocardiographic images, blinded to treatment allocation, demographic and clinical data. Additionally, these echocardiographic variables and >250 circulating proteins were sequentially measured at baseline, month 1 and month 9.

Our results should be regarded as hypothesis-generating given the post-hoc nature of our study, although we showed the external replication of the main findings. This study, additionally, included the relatively moderate sample size of LAVi, and the lack of mechanistic confirmation at the cellular level. HOMAGE and STANISLAS included only individuals who had no prior history of AF by design, which may limit the generalizability of our findings. However, AF may greatly confound clinical and treatment associations with LA size. In the present analysis, we did not use large unbiased screens, but rather pre-selected protein biomarkers based on mechanistic hypotheses and previous literature. The Olink panels (CVD II, III, and inflammation) contain circulating proteins previously found to be associated with cardiovascular and inflammatory diseases. Other pathways are missing, for example, metabolism and nutrition, that could enrich our results. Although we did not observe any interaction between spironolactone treatment and baseline treatment use on changes in biomarker expression, several treatments (i.e. statin therapy) might influence the changes in such biomarkers.⁴⁹

Conclusions

In individuals without manifest HF, LAVi was associated with MMP-2, IGFBP-2 and NT-proBNP. Notably, spironolactone was effective in reducing these biomarker concentrations as well as LAVi and serum PICP, a marker of collagen synthesis. This beneficial effect was consistent, regardless of baseline LA size. These findings highlight the potential of spironolactone in modulating key pathological processes in early HF stages, suggesting its broader applicability in preemptive cardiac care.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Funding

The research leading to these results has received funding from the European Union Commission's Seventh Framework programme under grant agreement N° 305507 (HOMAGE). S.H. acknowledges the support from the Netherlands Cardiovascular Research Initiative, an initiative with support of the Dutch Heart Foundation, Dutch Cardiovascular Alliance CVON2016-Early HFPEF, 2015-10, CVON She-PREDICTS, grant 2017-21. Furthermore, we acknowledge the support of FWO G091018N and FWO G0B5930N to S.H. A.G. was supported by the Instituto de Salud Carlos III (PI18/01469 co-financed by FEDER funds). S.H. receives personal

fees for scientific advice to AstraZeneca, Cellprothera and CSL Behring; unrestricted research grant from Pfizer.

Conflict of interest: none declared.

References

- Kobayashi M, Girerd N, Ferreira JP, Kevin D, Huttin O, González A, et al. The association between markers of type I collagen synthesis and echocardiographic response to spironolactone in patients at risk of heart failure: Findings from the HOMAGE trial. *Eur J Heart Fail* 2022;**24**:1559–1568. <https://doi.org/10.1002/ehjhf.2579>
- Thomas L, Marwick TH, Popescu BA, Donal E, Badano LP. Left atrial structure and function, and left ventricular diastolic dysfunction: JACC state-of-the-art review. *J Am Coll Cardiol* 2019;**73**:1961–1977. <https://doi.org/10.1016/j.jacc.2019.01.059>
- Abhayaratna WP, Seward JB, Appleton CP, Douglas PS, Oh JK, Tajik AJ, et al. Left atrial size: Physiologic determinants and clinical applications. *J Am Coll Cardiol* 2006;**47**:2357–2363. <https://doi.org/10.1016/j.jacc.2006.02.048>
- McDonagh TA, Metra M, Adamo M, Gardner RS, Baumach A, Böhm M, et al. 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). With the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2022;**24**:4–131. <https://doi.org/10.1002/ehjhf.2333>
- Baumgartner H, Hung J, Bermejo J, Chambers JB, Edvardsen T, Goldstein S, et al. Recommendations on the echocardiographic assessment of aortic valve stenosis: A focused update from the European Association of Cardiovascular Imaging and the American Society of Echocardiography. *J Am Soc Echocardiogr* 2017;**30**:372–392. <https://doi.org/10.1016/j.echo.2017.02.009>
- Inciardi RM, Claggett B, Minamisawa M, Shin SH, Selvaraj S, Gonçalves A, et al. Association of left atrial structure and function with heart failure in older adults. *J Am Coll Cardiol* 2022;**79**:1549–1561. <https://doi.org/10.1016/j.jacc.2022.01.053>
- Shah AM, Claggett B, Sweitzer NK, Shah SJ, Anand IS, O'Meara E, et al. Cardiac structure and function and prognosis in heart failure with preserved ejection fraction: Findings from the echocardiographic study of the Treatment of Preserved Cardiac Function Heart Failure With an Aldosterone Antagonist (TOPCAT) trial. *Circ Heart Fail* 2014;**7**:740–751. <https://doi.org/10.1161/CIRCHEARTFAILURE.114.001583>
- Pellicori P, Zhang J, Lukaschuk E, Joseph AC, Bourantas CV, Loh H, et al. Left atrial function measured by cardiac magnetic resonance imaging in patients with heart failure: Clinical associations and prognostic value. *Eur Heart J* 2015;**36**:733–742. <https://doi.org/10.1093/eurheartj/ehu405>
- Inciardi RM, Bonelli A, Biering-Sorensen T, Cameli M, Pagnesi M, Lombardi CM, et al. Left atrial disease and left atrial reverse remodelling across different stages of heart failure development and progression: A new target for prevention and treatment. *Eur J Heart Fail* 2022;**24**:959–975. <https://doi.org/10.1002/ehjhf.2562>
- Cleland JGF, Ferreira JP, Mariottoni B, Pellicori P, Cuthbert J, Verdonschot JA, et al.; HOMAGE Trial Committees and Investigators. The effect of spironolactone on cardiovascular function and markers of fibrosis in people at increased risk of developing heart failure: The Heart 'OMics' in AGEing (HOMAGE) randomized clinical trial. *Eur Heart J* 2021;**42**:684–696. <https://doi.org/10.1093/eurheartj/ehaa758>
- Edelmann F, Wachter R, Schmidt AG, Kraigher-Krainer E, Colantonio C, Kamke W, et al.; Aldo-DHF Investigators. Effect of spironolactone on diastolic function and exercise capacity in patients with heart failure with preserved ejection fraction: The Aldo-DHF randomized controlled trial. *JAMA* 2013;**309**:781–791. <https://doi.org/10.1001/jama.2013.905>
- Shah AM, Claggett B, Sweitzer NK, Shah SJ, Deswal A, Anand IS, et al. Prognostic importance of changes in cardiac structure and function in heart failure with preserved ejection fraction and the impact of spironolactone. *Circ Heart Fail* 2015;**8**:1052–1058. <https://doi.org/10.1161/CIRCHEARTFAILURE.115.002249>
- Deswal A, Richardson P, Bozkurt B, Mann DL. Results of the Randomized Aldosterone Antagonism in Heart Failure with Preserved Ejection Fraction trial (RAAM-PEF). *J Card Fail* 2011;**17**:634–642. <https://doi.org/10.1016/j.cardfail.2011.04.007>
- Kobayashi M, Ferreira MB, Costa RQ, Fonseca T, Oliveira JC, Marinho A, et al. Circulating biomarkers and cardiac structure and function in rheumatoid arthritis. *Front Cardiovasc Med* 2021;**8**:754784. <https://doi.org/10.3389/fcvm.2021.754784>
- Hage C, Michaëlsson E, Linde C, Donal E, Daubert JC, Gan LM, et al. Inflammatory biomarkers predict heart failure severity and prognosis in patients with heart failure with preserved ejection fraction: A holistic proteomic approach. *Circ Cardiovasc Genet* 2017;**10**:e001633. <https://doi.org/10.1161/CIRCGENETICS.116.001633>

16. Ferreira JP, Girerd N, Bozec E, Mercklé L, Pizard A, Bouali S, et al. Cohort profile: Rationale and design of the fourth visit of the STANISLAS cohort: A familial longitudinal population-based cohort from the Nancy region of France. *Int J Epidemiol* 2018;**47**:395–395j. <https://doi.org/10.1093/ije/dyx240>
17. Pellicori P, Ferreira JP, Mariottoni B, Brunner-La Rocca HP, Ahmed FZ, Verdonchot J, et al. Effects of spironolactone on serum markers of fibrosis in people at high risk of developing heart failure: Rationale, design and baseline characteristics of a proof-of-concept, randomised, precision-medicine, prevention trial. The Heart OMics in AGing (HOMAGE) trial. *Eur J Heart Fail* 2020;**22**:1711–1723. <https://doi.org/10.1002/ehjhf.1716>
18. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, et al. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006;**145**:247–254. <https://doi.org/10.7326/0003-4819-145-4-200608150-00004>
19. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF 3rd, Dokainish H, Edvardsen T, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2016;**29**:277–314. <https://doi.org/10.1016/j.echo.2016.01.011>
20. Rudski LG, Lai WW, Afilalo J, Hua L, Handschumacher MD, Chandrasekaran K, et al. Guidelines for the echocardiographic assessment of the right heart in adults: A report from the American Society of Echocardiography endorsed by the European Association of Echocardiography, a registered branch of the European Society of Cardiology, and the Canadian Society of Echocardiography. *J Am Soc Echocardiogr* 2010;**23**:685–713. <https://doi.org/10.1016/j.echo.2010.05.010>
21. Lang RM, Badano LP, Mor-Avi V, Afilalo J, Armstrong A, Ernande L, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2015;**28**:1–39.e14. <https://doi.org/10.1016/j.echo.2014.10.003>
22. Coiro S, Huttin O, Bozec E, Selson-Suty C, Lamiral Z, Carluccio E, et al. Reproducibility of echocardiographic assessment of 2D-derived longitudinal strain parameters in a population-based study (the STANISLAS cohort study). *Int J Cardiovasc Imaging* 2017;**33**:1361–1369. <https://doi.org/10.1007/s10554-017-1117-z>
23. Frikha Z, Girerd N, Huttin O, Courand PY, Bozec E, Olivier A, et al. Reproducibility in echocardiographic assessment of diastolic function in a population based study (the STANISLAS cohort study). *PLoS One* 2015;**10**:e0122336. <https://doi.org/10.1371/journal.pone.0122336>
24. López B, González A, Ravassa S, Beaumont J, Moreno MU, San José G, et al. Circulating biomarkers of myocardial fibrosis: The need for a reappraisal. *J Am Coll Cardiol* 2015;**65**:2449–2456. <https://doi.org/10.1016/j.jacc.2015.04.026>
25. Girerd N, Bresso E, Devignes MD, Rossignol P. Insulin-like growth factor binding protein 2: A prognostic biomarker for heart failure hardly redundant with natriuretic peptides. *Int J Cardiol* 2020;**300**:252–254. <https://doi.org/10.1016/j.ijcard.2019.11.100>
26. Fabregat A, Korninger F, Viteri G, Sidiropoulos K, Marin-Garcia P, Ping P, et al. Reactome graph database: Efficient access to complex pathway data. *PLoS Comput Biol* 2018;**14**:e1005968. <https://doi.org/10.1371/journal.pcbi.1005968>
27. Carbon S, Ireland A, Mungall CJ, Shu S, Marshall B, Lewis S. AmiGO: Online access to ontology and annotation data. *Bioinformatics* 2009;**25**:288–289. <https://doi.org/10.1093/bioinformatics/btn615>
28. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Res* 2003;**13**:2498–2504. <https://doi.org/10.1101/gr.1239303>
29. Shah AM, Cikes M, Prasad N, Li G, Getchevski S, Claggett B, et al. Echocardiographic features of patients with heart failure and preserved left ventricular ejection fraction. *J Am Coll Cardiol* 2019;**74**:2858–2873. <https://doi.org/10.1016/j.jacc.2019.09.063>
30. Martignetti JA, Aqeel AA, Sewairi WA, Boumah CE, Kambouris M, Mayouf SA, et al. Mutation of the matrix metalloproteinase 2 gene (MMP2) causes a multicentric osteolysis and arthritis syndrome. *Nat Genet* 2001;**28**:261–265. <https://doi.org/10.1038/90100>
31. Lindsey ML, Goshorn DK, Squires CE, Escobar GP, Hendrick JW, Mingoia JT, et al. Age-dependent changes in myocardial matrix metalloproteinase/tissue inhibitor of metalloproteinase profiles and fibroblast function. *Cardiovasc Res* 2005;**66**:410–419. <https://doi.org/10.1016/j.cardiores.2004.11.029>
32. Nagibin V, Egan Benova T, Vicenczova C, Vicenczova C, Szeiffova Bacova B, Dovinova I, et al. Ageing related down-regulation of myocardial connexin-43 and up-regulation of MMP-2 may predict propensity to atrial fibrillation in experimental animals. *Physiol Res* 2016;**65**:S91–s100. <https://doi.org/10.33549/physiolres.933389>
33. Zile MR, Jhund PS, Baicu CF, Claggett BL, Pieske B, Voors AA, et al.; Prospective Comparison of ARNI With ARB on Management of Heart Failure With Preserved Ejection Fraction (PARAMOUNT) Investigators. Plasma biomarkers reflecting profibrotic processes in heart failure with a preserved ejection fraction: Data from the Prospective Comparison of ARNI with ARB on Management of Heart Failure with Preserved Ejection Fraction study. *Circ Heart Fail* 2016;**9**:e002551. <https://doi.org/10.1161/CIRCHEARTFAILURE.115.002551>
34. Buckley LF, Agha AM, Dorbala P, Claggett BL, Yu B, Hussain A, et al. MMP-2 associates with incident heart failure and atrial fibrillation: The ARIC study. *Circ Heart Fail* 2023;**16**:e010849. <https://doi.org/10.1161/CIRCHEARTFAILURE.123.010849>
35. George J, Patal S, Wexler D, Roth A, Sheps D, Keren G. Circulating matrix metalloproteinase-2 but not matrix metalloproteinase-3, matrix metalloproteinase-9, or tissue inhibitor of metalloproteinase-1 predicts outcome in patients with congestive heart failure. *Am Heart J* 2005;**150**:484–487. <https://doi.org/10.1016/j.ahj.2004.11.016>
36. Peterson JT, Hallak H, Johnson L, Li H, O'Brien PM, Sliskovic DR, et al. Matrix metalloproteinase inhibition attenuates left ventricular remodeling and dysfunction in a rat model of progressive heart failure. *Circulation* 2001;**103**:2303–2309. <https://doi.org/10.1161/01.cir.103.18.2303>
37. Li H, Simon H, Bocan TM, Peterson JT. MMP/TIMP expression in spontaneously hypertensive heart failure rats: The effect of ACE- and MMP-inhibition. *Cardiovasc Res* 2000;**46**:298–306. [https://doi.org/10.1016/s0008-6363\(00\)00028-6](https://doi.org/10.1016/s0008-6363(00)00028-6)
38. Hudson MP, Armstrong PW, Ruzyllo W, Brum J, Cusmano L, Krzeski P, et al. Effects of selective matrix metalloproteinase inhibitor (PG-116800) to prevent ventricular remodeling after myocardial infarction: Results of the PREMIER (Prevention of Myocardial Infarction Early Remodeling) trial. *J Am Coll Cardiol* 2006;**48**:15–20. <https://doi.org/10.1016/j.jacc.2006.02.055>
39. Cittadini A, Grossman JD, Napoli R, Katz SE, Strömer H, Smith RJ, et al. Growth hormone attenuates early left ventricular remodeling and improves cardiac function in rats with large myocardial infarction. *J Am Coll Cardiol* 1997;**29**:1109–1116. [https://doi.org/10.1016/s0735-1097\(97\)00010-7](https://doi.org/10.1016/s0735-1097(97)00010-7)
40. Berry M, Galinier M, Delmas C, Fournier P, Desmoulin F, Turkieh A, Mischak H, Mullen W, Barutaut M, Eurlings LW, van Wijk S, Brunner-la Rocca HP, Caubere C, Butler J, Roncalli J, Evaristi MF, Cohen-Solal A, Seronde MF, Escamilla R, Ferrières J, Koukoui F, Smih F, Rouet P. Proteomics analysis reveals IGFBP2 as a candidate diagnostic biomarker for heart failure. *IJC Metab Endocr* 2015;**6**:5–12. <https://doi.org/10.1016/j.ijcme.2014.11.003>
41. Barutaut M, Fournier P, Peacock WF, Evaristi MF, Caubère C, Turkieh A, et al. Insulin-like growth factor binding protein 2 predicts mortality risk in heart failure. *Int J Cardiol* 2020;**300**:245–251. <https://doi.org/10.1016/j.ijcard.2019.09.032>
42. Ravassa S, Beaumont J, Cediell G, Lupón J, López B, Querejeta R, et al. Cardiorenal interaction and heart failure outcomes. A role for insulin-like growth factor binding protein 2? *Rev Esp Cardiol (Engl Ed)* 2020;**73**:835–843. <https://doi.org/10.1016/j.rec.2019.10.012>
43. Zannad F, Alla F, Dousset B, Perez A, Pitt B. Limitation of excessive extracellular matrix turnover may contribute to survival benefit of spironolactone therapy in patients with congestive heart failure: Insights from the Randomized Aldactone Evaluation Study (RALES). *Circulation* 2000;**102**:2700–2706. <https://doi.org/10.1161/01.cir.102.22.2700>
44. Kurrelmeyer KM, Ashton Y, Xu J, Nagueh SF, Torre-Amione G, Deswal A. Effects of spironolactone treatment in elderly women with heart failure and preserved left ventricular ejection fraction. *J Card Fail* 2014;**20**:560–568. <https://doi.org/10.1016/j.cardfail.2014.05.010>
45. Mak GJ, Ledwidge MT, Watson CJ, Phelan DM, Dawkins IR, Murphy NF, et al. Natural history of markers of collagen turnover in patients with early diastolic dysfunction and impact of eplerenone. *J Am Coll Cardiol* 2009;**54**:1674–1682. <https://doi.org/10.1016/j.jacc.2009.08.021>
46. Ferreira JP, Cleland JG, Girerd N, Bozec E, Rossignol P, Pellicori P, et al. Spironolactone effect on cardiac structure and function of patients with heart failure and preserved ejection fraction: A pooled analysis of three randomized trials. *Eur J Heart Fail* 2023;**25**:108–113. <https://doi.org/10.1002/ehjhf.2726>
47. Gupta S, Matulevicius SA, Ayers CR, Berry JD, Patel PC, Markham DW, et al. Left atrial structure and function and clinical outcomes in the general population. *Eur Heart J* 2013;**34**:278–285. <https://doi.org/10.1093/eurheartj/ehs188>
48. Thomas L, Abhayaratna WP. Left atrial reverse remodeling: Mechanisms, evaluation, and clinical significance. *JACC Cardiovasc Imaging* 2017;**10**:65–77. <https://doi.org/10.1016/j.jcmg.2016.11.003>
49. Tousoulis D, Andreou I, Tentolouris C, Antoniadou C, Pappageorgiou N, Gounari P, et al. Comparative effects of rosuvastatin and allopurinol on circulating levels of matrix metalloproteinases and tissue inhibitors of metalloproteinases in patients with chronic heart failure. *Int J Cardiol* 2010;**145**:438–443. <https://doi.org/10.1016/j.ijcard.2009.05.051>
50. Yu CM, Tipoe GL, Wing-Hon Lai K, Lau CP. Effects of combination of angiotensin-converting enzyme inhibitor and angiotensin receptor antagonist on inflammatory cellular infiltration and myocardial interstitial fibrosis after acute

- myocardial infarction. *J Am Coll Cardiol* 2001;**38**:1207–1215. [https://doi.org/10.1016/s0735-1097\(01\)01518-2](https://doi.org/10.1016/s0735-1097(01)01518-2)
51. Kobayashi M, Huttin O, Magnusson M, Ferreira JP, Bozec E, Huby AC, *et al.*; STANISLAS Study Investigators. Machine learning-derived echocardiographic phenotypes predict heart failure incidence in asymptomatic individuals. *JACC Cardiovasc Imaging* 2022;**15**:193–208. <https://doi.org/10.1016/j.jcmg.2021.07.004>
52. Kobayashi M, Huttin O, Ferreira JP, Duarte K, González A, Heymans S, *et al.*; HOMAGE Trial Committees and Investigators. A machine learning-derived echocardiographic algorithm identifies people at risk of heart failure with distinct cardiac structure, function, and response to spironolactone: Findings from the HOMAGE trial. *Eur J Heart Fail* 2023;**25**:1284–1289. <https://doi.org/10.1002/ejhf.2859>