Latent Transforming Growth Factor Beta Binding Protein 4 as a Biomarker of Arrhythmia-Induced Cardiomyopathy in Atrial Fibrillation: Insights from a High-Throughput Proteomic Analysis

Yuhi Hasebe (MD, PhD)[1, 2], Kotaro Nochioka (MD, PhD, FESC)[1], Makoto Nakano (MD, PhD)[1], Takahiko Chiba (MD, PhD)[1], Hiroyuki Sato (MD, PhD)[1], Nobuhiko Yamamoto (MD, PhD)[1], Tomohiro Ito (MD)[1], Takashi Noda (MD, PhD, FESC)[1, 3], Koji Kumagai (MD, PhD)[2], Aika Udagawa (PhD)[4], Shintaro Kato (PhD)[4], Katsunori Horii (PhD)[4], Iwao Waga (PhD)[4], Satoshi Yasuda (MD, PhD, FESC)[1]

1. Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, Sendai, Japan
2. First Division of Internal Medicine (Cardiovascular Medicine), Tohoku Medical and Pharmaceutical University Graduate School of Medicine, Sendai, Japan
3. Department of Cardiovascular Center, Kindai University, Osaka, Japan
4. NEC Solution Innovators, Ltd., Tokyo, Japan

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*Corresponding author:

Satoshi Yasuda, MD, PhD.

Department of Cardiovascular Medicine,

Tohoku University Graduate School of Medicine

1-1, Seiryomachi, Aoba-ku, Sendai 980-8574, Japan

E-mail: satoshi.yasuda.c8@tohoku.ac.jp

Introduction

Among patients with atrial fibrillation (AF) and left ventricular (LV) systolic dysfunction, catheter ablation (CA) leads to two clinical courses: recovery of LV function, indicating arrhythmia-induced cardiomyopathy (AIC), or persistent impairment consistent with non-AIC. However, this distinction can only be made retrospectively after sinus rhythm (SR) restoration and reassessment of LV function. Despite proposed clinical predictors, reliable blood-based biomarkers identifying patients who will recover LV function are lacking.[1]

High-throughput proteomics using modified aptamers has enabled comprehensive profiling of circulating proteins from minimal sample volumes.[2] The SomaScan assay enables high-throughput proteomic profiling and has emerged as an unbiased biomarker discovery platform in cardiovascular disease.[3]

Leveraging this approach, we aimed to identify proteins associated with AF-related LV dysfunction and validate candidate biomarkers distinguishing AIC from non-AIC.

Methods

This study included discovery and validation cohorts. All procedures were approved by the local ethics committee of Tohoku University School of Medicine (2025-1-046 and 2021-1-752), and all participants provided written informed consent.

The discovery cohort included 687 patients with stage B/C chronic heart failure (CHF). Plasma samples were analyzed using the SomaScan Version 3 assay (4,746 proteins; 4,572 after quality control). Patients were divided into AF (n=204) and SR (n=483), and among AF patients, reduced LVEF (<50%; n=56) and preserved LVEF (≥50%; n=148). Candidate biomarkers were identified through a three-step approach: (1) exclusion of proteins influenced by cardiovascular medications, including warfarin, β-blockers, ACE inhibitors, ARBs, amiodarone, and sotalol; (2) identification of AF-related proteins by comparing AF with SR; (3) detection of proteins differentiating AF with reduced versus preserved LVEF. Logistic regression adjusted for age and sex was performed with Bonferroni correction.

The validation cohort included 16 AF patients with LVEF<50% undergoing CA. Pulmonary vein isolation and additional ablation procedures were performed using standard radiofrequency techniques. Blood sampling was performed during CA, and proteomic profiling used SomaScan Version 4.1. At 3 months after CA, AIC classification was performed in patients maintaining SR, defined as an absolute LVEF increase >20 percentage points or normalization to ≥50%; others were classified as non-AIC.[4] Rhythm status was confirmed by scheduled 12-lead electrocardiograms and 24-hour Holter monitoring. Protein levels were compared using the Wilcoxon rank-sum test. Odds ratios per 1-SD increase in log10-transformed protein concentrations were estimated using Firth's penalized logistic regression. Because different SomaScan assay versions were used, protein concentrations were not directly compared between cohorts. Each cohort was analyzed independently using version-specific normalized data.

Results

In the discovery cohort (204 patients with AF; age 71.5±11.2 years; 74.5% male; **Figure A**), patients with reduced LVEF had larger LV end-diastolic diameter (LVEDD) (57.4±9.0 mm vs. 47.2±6.1 mm, P<0.001) and higher BNP levels (131 [77–324] vs. 67 [26–141] pg/ml, P<0.001) compared with preserved LVEF. Among 4,572 proteins, 38 differed significantly between AF and SR patients. Of these proteins, latent transforming growth factor-β binding protein 4 (LTBP4) and carbohydrate sulfotransferase 15 (CHST15) were significantly higher in AF patients with reduced versus preserved LVEF (P = 8.93e-05 and 6.33e-04, respectively). The elevation of LTBP4 across LVEF strata was more pronounced in AF patients than in SR patients (**Figure B and C**).

In the validation cohort (age 65.9±7.7 years; 75% male; LVEF 34.5±8.5%; **Figure A**), one patient underwent repeat ablation for AF recurrence and was ultimately classified as AIC after subsequent SR maintenance. Overall, 10 patients were classified as AIC and 6 as non-AIC. Among AIC patients, LVEDD decreased from 52.6±6.2 mm to 46.6±4.4 mm, and LVEF improved from 33.2±8.3% to 60.0±6.5%. LTBP4 levels were higher in AIC than non-AIC patients (P=0.0467) (**Figure D**). Exploratory Firth logistic regression yielded an odds ratio of 1.70 per 1-SD increase (95% CI 0.64–8.21). Receiver operating characteristic analysis yielded an AUC of 0.77 (95% CI 0.47–1.00) (**Figure F**). The optimal cutoff (4.14, log10 [RFU]) derived from the Youden index corresponded to 90% sensitivity and 67% specificity.

Discussion

Using high-throughput proteomics, we identified LTBP4 and CHST15 as elevated in AF patients with LV systolic dysfunction. In the validation cohort, LTBP4 levels were higher in patients with AIC, highlighting its potential as a biomarker for reversible myocardial dysfunction.

AIC remains a clinically defined entity without universally accepted diagnostic criteria and is increasingly regarded as a potentially reversible heart failure syndrome associated with sustained arrhythmia.[5] Our classification based on LV functional recovery after CA represents an operational definition aligned with this contemporary framework. Structural remodeling parameters, including LV dimensions, have been associated with recovery in AIC.[6] In parallel, proteomic studies in AF and cardiomyopathy have linked circulating proteins to structural remodeling, whereas biomarkers reflecting reversibility remain uncertain.[7] Our findings suggest that LTBP4 may serve as a biomarker of reversible myocardial dysfunction in AF-related LV impairment.

LTBP4 regulates extracellular matrix integrity and latent TGF-β signaling, pathways involved in inflammation-to-fibrosis transitions and myocardial remodeling.[8] Prior studies have linked circulating LTBP4 alterations to ventricular remodeling in cardiovascular disease, providing biological plausibility for the elevated levels observed in AF-related LV dysfunction in our study.[9, 10] The observed increase may reflect an early reparative response to myocardial stress consistent with reversible myocardial dysfunction. Further multicenter validation, including targeted quantitative assay confirmation (e.g., ELISA), is needed to support clinical translation.

Limitations

Because different SomaScan assay versions were used, quantitative comparison between cohorts was not performed, and cross-platform variability cannot be excluded. The validation cohort was small, resulting in exploratory estimates, and clinical confounders including AF burden, atrial remodeling, and cardiomyopathy duration were not comprehensively assessed. Classification at 3 months may not fully capture delayed reverse remodeling or early transient improvement. Nevertheless, the direction of association identified in the discovery cohort was reproduced in validation. These findings should be considered hypothesis-generating.

Conclusions

LTBP4 may help identify patients with reversible LV dysfunction after rhythm control, although prospective validation is required before clinical application.

Data Availability Statement

Data are available from the corresponding author on reasonable request.

Conflicts of interest

A.U., S.K., K.H., and I.W. are employees of NEC Solution Innovators, Ltd. S.Y. conducts joint research with the company. All other authors declare no conflicts of interest. This work was supported by JSPS KAKENHI (JP21K16014).

References

1. Gonzalez-Ferrero T, Bergonti M, Lopez-Canoa JN, Chierchia GB, de Asmundis C, Conte G, et al. Atrial fibrillation ablation in patients with arrhythmia-induced cardiomyopathy: a prospective multicentre study. *ESC Heart Fail.* 2023;10:3055-3066. doi:10.1002/ehf2.14488
2. Gold L, Ayers D, Bertino J, Bock C, Bock A, Schneider D, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. *PLoS One.* 2010;5:e15004. doi:10.1371/journal.pone.0015004
3. Williams SA, Ostroff R, Hinterberg MA, Zhang Z, Farkas R, Becker J, et al. A proteomic surrogate for cardiovascular outcomes that is sensitive to multiple mechanisms of change in risk. *Sci Transl Med.* 2022;14:eabj9625. doi:10.1126/scitranslmed.abj9625
4. Yamashita S, Tokuda M, Matsuo S, Hachiya H, Nozato T, Oseto A, et al. Comparison of atrial arrhythmia recurrence after persistent atrial fibrillation ablation between patients with or without tachycardia-induced cardiomyopathy. *J Cardiovasc Electrophysiol.* 2019;30:2310-2318. doi:10.1111/jce.14204
5. Serban T, Badertscher P, du Fay de Lavallaz J, Providencia R, Migliore F, Mugnai G, et al. Definition and management of arrhythmia-induced cardiomyopathy: findings from the European Heart Rhythm Association survey. *Europace.* 2024; 26, euae112 doi:10.1093/europace/euae112
6. Schach C, Körtl T, Zeman F, Luttenberger B, Mühleck F, Baum P, et al. Clinical characterization of arrhythmia-induced cardiomyopathy in patients with tachyarrhythmia and idiopathic heart failure. *JACC Clin Electrophysiol.* 2024;10(5):870-881. doi:10.1016/j.jacep.2024.102332.
7. Lumish HS, Harano N, Liang LW, Hasegawa K, Maurer MS, Tower-Rader A, et al. Prediction of new-onset atrial fibrillation in patients with hypertrophic cardiomyopathy using plasma proteomics profiling. *Europace.* 2024;26(11):euae267. doi:10.1093/europace/euae267.
8. Rosenkranz S. TGF-beta1 and angiotensin networking in cardiac remodeling. *Cardiovasc Res.* 2004;63:423-432. doi:10.1016/j.cardiores.2004.04.030
9. Boileau A, Lalem T, Vausort M, Zhang L, Devaux Y, Cardiolinc network. A 3-gene panel improves the prediction of left ventricular dysfunction after acute myocardial infarction. *Int J Cardiol.* 2018;254:28-35. doi:10.1016/j.ijcard.2017.08.056
10. Su CT, Urban Z. LTBP4 in Health and Disease. *Genes (Basel).* 2021;12:795. doi:10.3390/genes12060795

Figure Legends

Figure. Proteomic identification and validation of biomarkers distinguishing AIC from non-AIC

(A) Baseline characteristics of the discovery and validation cohorts.

(B, C) Plasma levels of LTBP4 and CHST15 in the discovery CHF cohort. Both proteins were significantly elevated in AF patients with reduced LVEF compared with those with preserved LVEF.

(D, E) Validation in the RFCA cohort. Plasma LTBP4 levels were significantly higher in patients diagnosed with AIC (n = 10) than in those with non-AIC (n = 6), whereas CHST15 showed a non-significant trend toward elevation.

(F) ROC curve for LTBP4 discriminating AIC from non-AIC in the validation cohort. The area under the curve (AUC) was 0.767 (95% CI 0.470–1.000).

Protein levels are expressed as log10-transformed relative fluorescence units (RFU). P values were calculated using logistic regression in the discovery cohort and the Wilcoxon rank-sum test in the validation cohort.

Abbreviations: AF, atrial fibrillation; AIC, arrhythmia-induced cardiomyopathy; CHST15, carbohydrate sulfotransferase 15; LTBP4, latent transforming growth factor-β binding protein 4; LVEF, left ventricular ejection fraction; ROC, receiver operating characteristic.

A

	Discovery cohort						Validation cohort		
	AF			SR					
	Reduced LVEF (N = 56)	Preserved LVEF (N = 148)	P value	Reduced LVEF (N = 109)	Preserved LVEF (N = 374)	P value	AIC (N = 10)	Non-AIC (N = 6)	P value
Age (year), mean (SD)	70 ± 12	72 ± 11	0.224	68 ± 12	68 ± 14	0.883	66 ± 8	66 ± 8	0.92
Male, n (%)	45 (80.4)	107 (72.3)	0.318	76 (69.7)	232 (62.0)	0.175	6 (60.0)	6 (100.0)	0.24
SBP (mmHg), mean (SD)	118 ± 18	124 ± 16	0.010	121 ± 19	128 ± 17	0.001	109 ± 15	113 ± 24	0.68
DBP (mmHg), mean (SD)	67 ± 14	73 ± 13	0.014	73 ± 13	73 ± 13	0.899	78 ± 18	70 ± 11	0.39
Heart rate (/min), mean (SD)	72 ± 13	68 ± 13	0.172	65 ± 10	65 ± 13	0.991	92 ± 14	75 ± 20	0.061
BNP (pg/mL), median [IQR]	131 [77-324]	67 [26-141]	<0.001	74 [33-161]	29 [13- 64]	<0.001	120 [52- 217]	101 [55- 290]	0.67
LVEDD (mm), mean (SD)	57.4 ± 9.0	47.2 ± 6.1	<0.001	56.7 ± 9.0	46.7 ± 6.1	<0.001	52.6 ± 6.2	57.0 ± 5.8	0.18
LVEF, mean (SD)	36.9 ± 9.1	63.8 ± 6.9	<0.001	37.8 ± 9.3	64.2 ± 7.3	<0.001	33.2 ± 8.3	36.6 ± 9.2	0.46