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Association of Circulating Autophagy Proteins ATG5 and Beclin 1 with Acute Myocardial Infarction in a Case-Control Study

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Keywords
Autophagy · ATG5 · Beclin 1 · Biomarker · Acute myocardial infarction · Risk prediction

Abstract
Introduction: Acute myocardial infarction (AMI) is a main contributor of sudden cardiac death worldwide. The discovery of new biomarkers that can improve AMI risk prediction meets a major clinical need for the identification of high-risk patients and the tailoring of medical treatment. Previously, we reported that autophagy a highly conserved catabolic mechanism for intracellular degradation of cellular components is involved in atherosclerotic plaque phenotype and cardiac pathological remodeling. The crucial role of autophagy in the normal and diseased heart has been well described, and its activation functions as a pro-survival process in response to myocardial ischemia. However, autophagy is dysregulated in ischemia/reperfusion injury, thus promoting necrotic or apoptotic cardiac cell death. Very few studies have focused on the plasma levels of autophagy markers in cardiovascular disease patients, even though they could be companion biomarkers of AMI injury. The aims of the present study were to evaluate (1) whether variations in plasma levels of two key autophagy regulators autophagy-related gene 5 (ATG5) and Beclin 1 (the mammalian yeast ortholog Atg6/Vps30) are associated with AMI and (2) their potential for predicting AMI risk. ***Methods:*** The case-control study population included AMI patients (n = 100) and control subjects (n = 99) at high cardiovascular risk but without known coronary disease. Plasma levels of ATG5 and Beclin 1 were measured in the whole population study by enzyme-linked immunosorbent assay. ***Results:*** Multivariate analyses adjusted on common cardiovascular factors and medical treatments, and receiver operating characteristic curves demonstrated that ATG5 and Beclin 1 levels were inversely associated with AMI and provided original biomarkers for AMI risk prediction. ***Conclusion:*** Plasma levels of autophagy regulators ATG5 and Beclin 1 represent relevant candidate biomarkers associated with AMI.

Introduction

Acute myocardial infarction (AMI) remains a major cause of death and disability worldwide. Unfortunately, the number of people with cardiovascular risk (CVR) factors is increasing due to the world's aging population and the rise in

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obesity and diabetes [1]. Despite considerable improvements in acute care and secondary prevention after AMI, there is always a need to discover novel biomarkers improving risk prediction models and therapeutic decision-making. Autophagy is a conserved pathway for the degradation and recycling of long-lived proteins and cytoplasmic organelles through the lysosome. In the general form of the process, cytoplasmic cargo targeted for destruction is sequestered inside double-membrane vesicles called autophagosomes and is delivered to the lysosome by fusion for breakdown [2]. Autophagy is critical for maintaining cellular homeostasis, and dysregulated autophagy has been associated with the pathogenesis of diseases including cardiovascular conditions [3–6]. Once activated, autophagy proceeds through four sequential steps, each step requiring specific regulatory proteins and complexes [7]. Cardiac autophagy has been extensively described in cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells, and endothelial cells [8, 9]. Our recent works reported that autophagy is involved in atherosclerotic plaque phenotype and development [6], and acted as an adaptive response to antagonize exchange protein directly activated by cAMP 1 (Epac1)-promoted cardiomyocyte hypertrophy [10]. Although autophagy has been observed in the failing myocardium and during ischemia and reperfusion (I/R) of both murine models and patients [11–15], the beneficial or detrimental effect of autophagy is not fully understood and requires further investigations. Several studies showed that ischemic injury activates autophagy in cardiomyocytes enabling them to cope with nutritional stress and improve cell survival [11, 14, 16]. On the other hand, autos is a novel form of cell death which is associated with reduced autophagic flux and blockade of autophagosome clearance occurred during the late reperfusion phase in I/R mouse model [17]. Notably, a meta-analysis including 40 studies (1,066,408 patients) showed that the autophagy inducer metformin commonly used in patients with diabetes could significantly reduce all-cause mortality in myocardial infarction (MI) and heart failure patients [18]. Minimizing myocardial tissue death is the ultimate goal of interventions in patients with AMI, and the study of autophagy marker levels is therefore highly relevant and could help clinicians to adapt the therapeutic strategy. However, despite the existence of several pharmacological agents and regimens that modulate autophagy, few clinical studies (e.g., interventional protocol with trehalose, hydroxychloroquine, or exercise) have investigated changes in circulating autophagy proteins as reliable markers for predicting/controlling disease progression and for monitoring clinical status. Currently, a small panel of autophagy proteins has been measured in human body fluids thanks to available immunoassay kits [19–22]. For example, circulating levels of the autophagy protein ATG5 (first discovered in yeast, is a protein involved in the early stages of autophagosome formation) and Parkin (involved in the ubiquitination of mitochondrial substrates during mitophagy) were found as diagnostic tools for early monitoring of patients with cognitive decline, for identifying the active phase of multiple sclerosis or related to the severity of hypoxic injury during perinatal hypoxic-ischemic encephalopathy [19–21]. Interestingly, it was shown that healthy centenarians have increased circulating Beclin 1 protein (a BH3-only protein core component of the class III PI3K complex required for autophagosome formation) levels in comparison with a population of young healthy subjects or patients with MI [22]. The level of serum circulating Beclin 1 is also related to the degree of airflow obstruction in chronic obstructive pulmonary disease patients [23]. A recent study showed that autophagy (e.g., Beclin 1, ATG5, and ATG7) and mitophagy (e.g., Parkin, optineurin) proteins are upregulated in human serum of calcific aortic valve stenosis and correlated with the pro-inflammatory cytokine interleukin-1β as well as with the severity of the disease [24]. Thus, our hypothesis for this work was to investigate in human plasma samples whether variations in levels of autophagy markers could be accurately measured and potentially associated with AMI. To address these objectives, we focused on the two essential pro-autophagic factors ATG5 and Beclin 1. We measured the plasma concentrations of ATG5 and Beclin 1 proteins in a case-control study including patients who experienced AMI and high-risk asymptomatic individuals according to a Systematic Coronary Risk Evaluation (SCORE) model [25].

Methods

Study Population

The case-control study population included AMI patients (n = 100) and control subjects (n = 99) at high CVR but without known coronary from a prospective cohort (NCT02405468) [26]. Cases and control subjects were recruited in the Cardiology, Arterial Hypertension and Therapeutic Department of Toulouse University Hospital Center, France. Cases (n = 100, men and women) were patients who were diagnosed with an AMI according to the "Third Universal Definition of MI" [27] and were included 3 days after the acute ischemic phase (hereinafter referred to as AMI patients). Demographic, clinical, and biological characteristics were recorded, and a blood sample was drawn at the time of inclusion. Coronary data were available for all patients. Left ventricular ejection fraction (LVEF) was available at inclusion. During the same period, subjects (n = 99, men and women) at high CVR (hereinafter referred to as controls) but without any previous vascular or coronary event, particularly AMI, were recruited in the Center for the Prevention of Cardiovascular Disease in the Cardiology Department of Toulouse University Hospital Center, France. Exclusion criteria for both

groups included the following: infectious disease within 1 week before the inclusion, immunocompromised patients, antibiotic treatment within 1 month before inclusion, chronic viral infection, chronic inflammatory intestinal bowel disease, renal failure (estimated glomerular filtration rate <50 mL/min per 1.73 m^2), and pregnancy. Written informed consent was obtained from each individual included in the study. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and Toulouse Hospital guidelines. The study protocol was previously reviewed and approved by the regional board of health authorities (Agence Régionale de Santé Occitanie-Midi-Pyrénées, Toulouse, France) and the institution's Ethics Committee on research on humans (Comité de Protection des Personnes du Sud-Ouest, Toulouse, France), approval number 2013-750.

Plasma Levels of Autophagy Marker Measurement

For each patient, an EDTA blood tube (4 mL per patient) was collected in fasting condition and transported at 4°C. The sample was then centrifuged, aliquoted, and stored at −80°C. Plasma concentrations of ATG5 and Beclin 1 were determined by using commercially available enzyme-linked immunosorbent assay kits (human ATG5 ELISA kit [HUEB1679]; Assay Genie, Dublin, Ireland; human BECN1 [Beclin 1] ELISA kit [E-EL-H0564]; Elabscience, Houston, TX, USA).

Statistical Analysis

Data are presented as means and standard deviations for quantitative variables and percentages for categorical variables. The distribution of qualitative variables between cases and controls was compared using the χ^2 test. When basic assumptions were not satisfied, data were subjected to Fisher's exact test. The comparison of the mean values of quantitative variables was performed by using the Student's *t* test. We used the Shapiro-Wilk's and Levene's tests to test the normality of distribution of residuals and the homogeneity of variances, respectively. When basic assumptions of Student's *t* test were not satisfied, we performed a logarithmic transformation of the variables or a Wilcoxon-Mann-Whitney test. The autophagy markers ATG5 and Beclin 1 were compared between cases and controls using the Mann and Whitney test. Relationships between the levels of autophagy markers and metabolic parameters or CVR factors were tested with a nonparametric measure of rank correlation (Spearman rank correlation). Autophagy regulators were tested in a multivariate analysis by adjusting for variables of the basic model. A basic model was created with the following variables (common CVR factors): gender, current smoker, hypertension, dyslipidemia, diabetes, obesity, age, and heredity. Medical treatments were added in the basic model. Logistic regression analyses were performed with polynomial models (quadratic and cubic) to examine for possible nonlinear relationships between continuous variables and case-control status. Because of a clearly nonlinear relationship with ATG5 and Beclin 1, logistic regression analyses were carried out using a categorization of the variable into quartiles. To establish the ability to differentiate cases and controls for autophagy markers, the receiver operating characteristic (ROC) curve analysis was used and the calculation of the standard error of the area under the curve (AUC) was done applying the Delong's method. All tests were two-tailed at the level of significance of 0.05. All analyses were carried out using SAS software, version 9.4 (SAS Institute, Cary, NC, USA) and STATA statistical software, release 14.1 (Stata Corporation, College Station, TX, USA).

Results

Population Characteristics

The case-control study was conducted on 199 individuals (men and women). The baseline characteristics of the AMI and control subjects are reported in Table 1. The high prevalence of hypertension, dyslipidemia, or obesity, as well as the significant differences in the medical treatments found in the control group, was explained by their recruitment in the Department of Prevention of Cardiovascular Disease (Toulouse University Hospital Center, Toulouse, France).

Measurement of ATG5 and Beclin 1 in Plasma Samples

Plasma levels of the autophagy biomarkers ATG5 and Beclin 1 were quantified in the studied subjects using an enzyme-linked immunosorbent assay. As shown in Figure 1, the circulating concentrations of ATG5 and Beclin 1 were statistically lower in AMI patients compared with control subjects (Fig. 1).

Correlation between Autophagy Biomarkers with Metabolic Parameters and CVR Factors

Spearman rank correlation coefficients between ATG5 and Beclin 1 plasma levels, and metabolic parameters or CVR markers were calculated. In the whole study population, a significant positive correlation of ATG5 levels with total cholesterol ($r = 0.134$, $p = 0.04$) and low-density lipoprotein cholesterol ($r = 0.157$, $p = 0.02$) was found. In AMI cases, a significant positive correlation between Beclin 1 ($r = 0.35$, $p = 0.0004$) and ATG5 ($r = 0.21$, $p = 0.03$) levels and triglycerides was found. Interestingly, Beclin 1 is also significantly correlated with the LVEF ($r = 0.24$, $p = 0.01$), thus supporting the involvement of autophagy in myocardial injury.

Association of ATG5 and Beclin 1 with AMI

The results presented in Table 2 show the crude estimates and unadjusted/adjusted associations of the autophagy markers with AMI. The unadjusted logistic regression analysis revealed that low levels of ATG5 ($p = 0.022$) and Beclin 1 ($p = 0.0032$) were significantly associated with AMI. After adjustment for potential confounders including the common CVR factors, gender, age, obesity, dyslipidemia, hypertension, heredity, smoking, and the medical treatments, the multivariate analysis showed that the association with AMI remained statistically significant for ATG5 ($p = 0.031$).

Table 1. Baseline characteristics of the study population

	AMI patients (n = 100)	Control subjects (n = 99)	p value
Gender, %			
Male	82	58	<0.001
Female	18	41	
Age, years	57.46 (8.84)	60.25 (7.61)	0.035
CVR factors			
BMI, kg/m^2	26.22 (4.13)	28.56 (4.78)	<0.001
Obesity, n (%)	15 (15.15)	36 (37.50)	<0.001
Dyslipidemia, n (%)	57 (57.58)	78 (81.25)	<0.001
Diabetes, n (%)	19 (19.19)	24 (25.00)	0.3
Hypertension, n (%)	42 (42.42)	87 (90.62)	<0.001
Current smoking, n (%)	41 (41.41)	16 (16.67)	<0.001
Heredity, n (%)	29 (29.29)	35 (36.46)	0.3
LVEF, %	51.90 (8.30)	60.09 (8.94)	<0.001
AMI category, %			
STEMI[a]	60		
NSTEMI[b]	40		
PCI revascularization[c,*], %	90		
PCI revascularization success, %	83		
Culprit artery, %			
Left anterior descending coronary artery	36		
Right coronary artery	36		
Left circumflex coronary artery	25		
Unidentified culprit lesion	3		
Involved vessels, %			
1-vessel disease	55		
2-vessel disease	28		
3-vessel disease	17		
Blood glucose, mg/dL	116.82 (41.49)	112.28 (58.72)	0.031
Triglycerides, mg/dL	146.63 (87.13)	137.67 (71.65)	0.6
Total cholesterol, mg/dL	195.03 (45.35)	198.45 (44.86)	0.4
LDL cholesterol, mg/dL[d]	119.56 (38.64)	117.86 (40.23)	0.8
HDL cholesterol, mg/dL[e]	47.77 (11.70)	52.77 (15.72)	0.054
Apo B, mg/dL	98.28 (26.48)	100.86 (22.26)	0.4
Medical treatment at admission, n (%)			
β-blocker agents	17 (17.17)	26 (27.08)	0.1
ACE inhibitors[f]	15 (15.15)	24 (25)	0.086
Statins	24 (24.24)	49 (51.04)	<0.001
Calcium channel blockers	11 (11.11)	36 (37.5)	<0.001
Antiplatelet agents	17 (17.17)	26 (27.08)	0.1
Antidiabetic treatment	10 (10.1)	21 (21.88)	0.025
Diuretic treatment	2 (2.02)	35 (36.46)	<0.001

Data are shown as mean with standard deviation or %. [a]STEMI, ST-segment elevation MI; [b]NSTEMI, non-ST-segment elevation MI; [c]PCI, percutaneous coronary intervention. *In 10% of cases, either the patient was beyond the time limit for PCI or the lesion identified did not warrant PCI (lesion <50% stenosis); [d]LDL, low-density lipoprotein; [e]HDL, high-density lipoprotein; [f]ACE, angiotensin-converting enzyme.

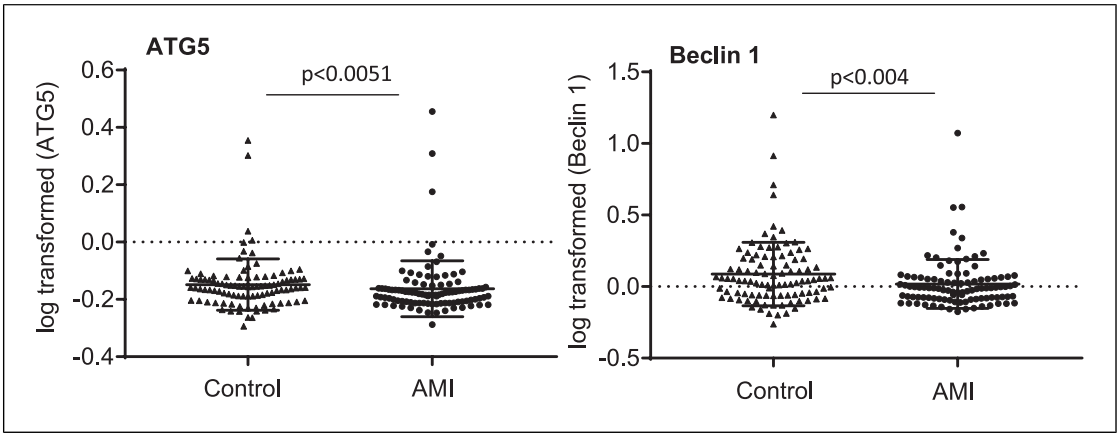

Fig. 1. Plasma levels of ATG5 and Beclin 1 proteins in the study population measured by enzyme-linked immunosorbent assay. The scatter plots represent the values (log-transformed data) with standard deviation of control subjects (control) and AMI patients (AMI).

Table 2. Logistic regression analysis for ATG5 and Beclin 1 association with AMI

Non-adjusted logistic regression (AMI case vs. control)					Adjusted logistic regression (AMI case vs. control)				
	Pr > Khi-2	point estimate	95% Wald confidence limits			Pr > Khi-2	point estimate	95% Wald confidence limits	
ATG5									
Q2 versus Q1	0.24	1.650	0.718	3.791	Q2 versus Q1	0.74	1.202	0.403	3.584
Q3 versus Q1	0.14	0.550	0.250	1.211	Q3 versus Q1	0.39	0.621	0.206	1.872
Q4 versus Q1	0.022	0.386	0.171	0.872	Q4 versus Q1	0.031	0.267	0.08	0.885
Beclin 1									
Q2 versus Q1	0.484	1.339	0.592	3.029	Q2 versus Q1	0.25	1.925	0.640	5.803
Q3 versus Q1	0.226	0.610	0.274	1.357	Q3 versus Q1	0.054	0.325	0.104	1.019
Q4 versus Q1	0.0032	0.287	0.125	0.658	Q4 versus Q1	0.078	0.345	0.105	1.128

Q1 = 0.62; Q2 = 0.677; Q3 = 0.735. Q1 = 0.841; Q2 = 1.023; Q3 = 1.283. Adjusted for gender, age, smoking, hypertension, dyslipidemia, diabetes, obesity, heredity, and medical treatments.

Analysis of ROC Curves and AMI Risk Prediction for ATG5 and Beclin 1

We then assessed whether ATG5 and Beclin 1 may serve as useful biomarkers for AMI risk prediction. ROC curve analyses were performed, and data in Figure 2 show the performance (AUC) of ATG5 (AUC 0.614, 95% confidence interval [CI] [0.534–0.694]) and Beclin 1 (AUC 0.618, 95% CI [0.539–0.696]). Interestingly, the combination of ATG5 and Beclin 1 resulted in a much higher AUC value of 0.656 (95% CI [0.579–0.732]) increasing the predictive power for AMI.

Discussion

The discovery of reliable and sensitive biomarkers in acute coronary events remains a critical clinical need to improve diagnosis, prognosis, and selection of appropriate treatment. In the present study, we investigated for the first time the circulating levels of two main autophagy regulators ATG5 and Beclin 1 in a population of AMI patients compared to high-risk control subjects. Our results showed a significantly decreased level of both proteins in AMI patients, which could be explained by a reduced autophagy flux and/or blockade

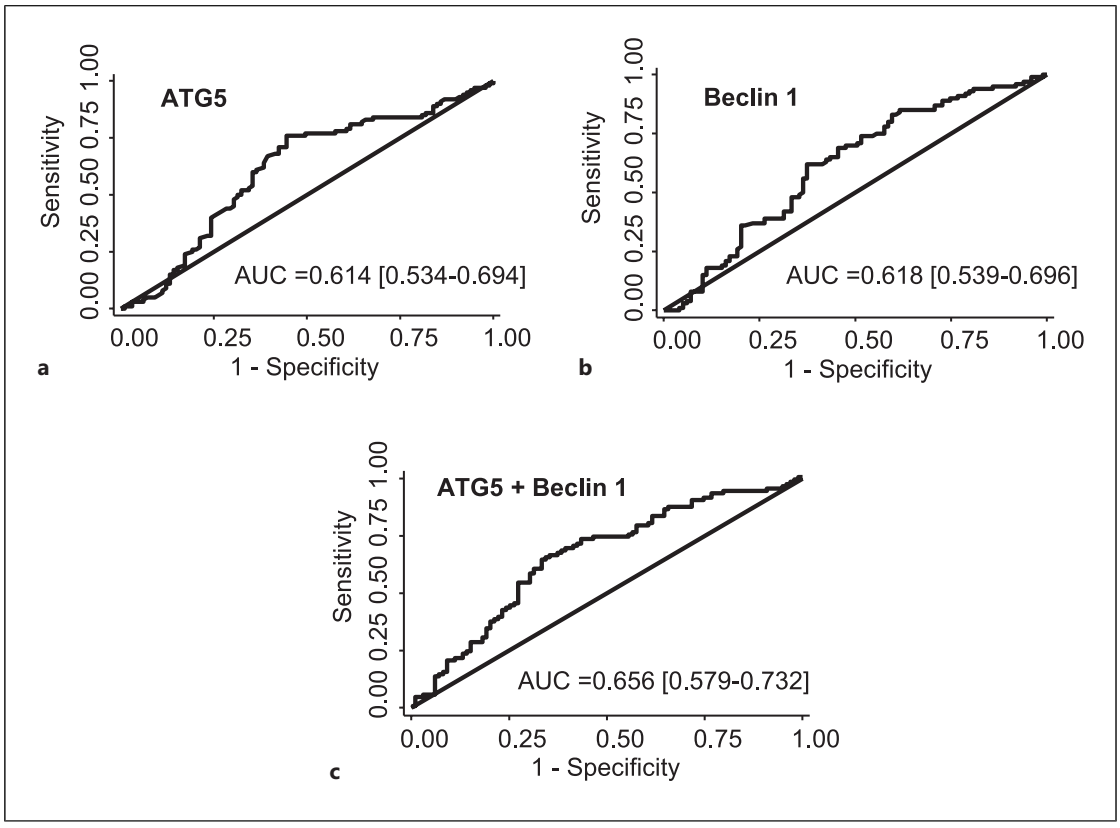

Fig. 2. ROC curve analysis of ATG5 (**a**), Beclin 1 (**b**), and ATG5 + Beclin 1 (**c**). AUC, area under the curve (95% CI).

of autophagosome clearance reporting on dysregulated autophagy in cardiac diseases. First, myocardial ischemia stimulates autophagy in cardiomyocytes enabling them to handle with hypoxic conditions and to improve cell survival [11, 14, 16]. On the other hand, during the early phase of reperfusion autophagy is further activated through upregulation of oxidative stress, and then due to simultaneously increased level of the autophagy inhibitor Rubicon, the autophagy flux is inactivated below basal levels in a time-dependent manner [17]. Therefore, on the basis of our results we could hypothesize that the decreased level of circulating ATG5 and Beclin 1 measured in AMI patients might reflect the inactivation of autophagy flux during the late phase of reperfusion. Further studies will be necessary to determine whether differential expression of ATG5 and Beclin 1 was a result of modulated intracellular production and/or different release mechanisms. The logistic regression analysis revealed that low levels of ATG5 and Beclin 1 were significantly associated with AMI. After adjustment for potential confounders including CVR factors and the medical treatments, the association with AMI remained statistically significant for ATG5. In addition, we identified significant positive correlations between ATG5 and Beclin 1 and lipid parameters including total cholesterol, low-density lipoprotein cholesterol, and triglycerides, which corroborated results showing increased regulatory markers of autophagy including Beclin 1, LC3B, and LAMP2 in heart tissues of hypercholesterolemic pigs [28] and high-fat feeding mice [29]. These results are particularly relevant since defective autophagy is associated with pro-atherogenic inflammasome activation triggered by hypercholesterolemia and metabolic syndrome [30]. It would be interesting in a further study to measure the association of inflammatory biomarkers including high-sensitivity C-reactive protein, interleukin-6, and interleukin-1β with ATG5 and Beclin 1.

The significant correlation found between Beclin 1 and LVEF, the principal determinant of heart failure in MI patients [31], supports recent data showing the

involvement of autophagy (e.g., increased expression of Beclin 1 protein) in left ventricular reverse remodeling following mechanical unloading [32]. Interestingly, the ROC curve analysis revealed that ATG5 and Beclin 1 could be novel candidate markers for predicting AMI. Furthermore, the combination of ATG5 and Beclin 1 managed to deliver an increased performance power with the AUC of 0.656 for predicting AMI.

Besides the novelty of comparing the level of circulating autophagy markers in AMI patients and control subjects, an original feature of our study is the inclusion of high-risk asymptomatic subjects (according to a SCORE model) seen in a prevention center specialized in the treatment of individuals with CVR factors. However, the selection bias between the two populations was strictly controlled by rigorous adjustment with CVR factors and medical treatments in the multivariate analyses. Thus, the association of autophagy regulators with the risk of AMI, independently of CVR factors and medical treatments, supports the significance of circulating autophagy proteins as novel candidate biomarkers.

Some limitations in our study have to be taken into account. First, although the sample size had sufficient statistical power to detect significant differences for all analyses, it would be useful to include a second, larger validation cohort to investigate the additional value of ATG5 and Beclin 1 when incorporated into a cardiovascular event risk prediction model. Second, it would have been valuable to measure the baseline expression of ATG5 and Beclin 1 before the MI event; however, such information required a longitudinal cohort study at high CVR with at least 10-year follow-up. Third, the lack of gender and age matching between AMI patients and control subjects could be a limit of the study. However, we did not find any difference in men and women, and there were no significant correlations between age and the concentrations of ATG5 and Beclin 1 in AMI patients and controls.

Conclusion

Our study displayed original data showing that circulating autophagy proteins ATG5 and Beclin 1 could be candidate biomarkers independently associated with AMI. In the perspective of our work, the discovery and monitoring of a panel of circulating autophagy biomarkers for translating into clinical practice represent a great opportunity to understand how levels of autophagy correlate with cardiac outcomes in individuals affected by cardiovascular diseases. Moreover, considering the active role of autophagy in myocardial I/R, our study encourages further research into targeting autophagy for therapeutic interventions.

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Statement of Ethics

The study (NCT02405468) was conducted in accordance with the Declaration of Helsinki, and reviewed and approved by Agence Régionale de Santé Occitanie-Midi-Pyrénées (Toulouse, France) and the institution's Ethics Committee on research on humans: Comité de Protection des Personnes du Sud-Ouest, Toulouse, France), approval number 2013-750. Written informed consent has been obtained from all patients/participants for the study and to publish this paper.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

The study was conceptualized by C.V. and M.E. M.-H.G and J.-B.R. conducted the study investigation. C.V. contributed to the writing (original draft) of the manuscript. W.M., J.-B.R., and C.V. contributed to the writing (review and editing) of the manuscript. We attest that all authors have reviewed and approved the article prior to its submission.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

1 Timmis A, Townsend N, Gale C, Grobbee R, Maniadakis N, Flather M, et al. European society of Cardiology: cardiovascular disease statistics 2017. Eur Heart J. 2018;39(7):508–79. doi: 10.1093/eurheartj/ehx628.

2 Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. Science. 2000;290(5497):1717–21. doi: 10.1126/science.290.5497.1717.

3 Vindis C. Autophagy: an emerging therapeutic target in vascular diseases. Br J Pharmacol. 2015;172(9):2167–78. doi: 10.1111/bph.13052.

4 De Meyer GR, Grootaert MOJ, Michiels CF, Kurdi A, Schrijvers DM, Martinet W. Autophagy in vascular disease. Circ Res. 2015;116(3):468–79. doi: 10.1161/CIRCRESAHA.116.303804.

5 Gustafsson AB, Gottlieb RA. Autophagy in ischemic heart disease. Circ Res. 2009;104(2):150–8. doi: 10.1161/CIRCRESAHA.108.187427.

6 Nahapetyan H, Moulis M, Grousset E, Faccini J, Grazide MH, Mucher E, et al. Altered mitochondrial quality control in Atg7-deficient VSMCs promotes enhanced apoptosis and is linked to unstable atherosclerotic plaque phenotype. Cell Death Dis. 2019;10(2):119. doi: 10.1038/s41419-019-1400-0.

7 Moulis M, Vindis C. Autophagy in metabolic age-related human diseases. Cells. 2018;7(10):149. doi: 10.3390/cells7100149.

8 Mialet-Perez J, Vindis C. Autophagy in health and disease: focus on the cardiovascular system. Essays Biochem. 2017;61(6):721–32. doi: 10.1042/EBC20170022.

9 Lavandero S, Chiong M, Rothermel BA, Hill JA. Autophagy in cardiovascular biology. J Clin Invest. 2015;125(1):55–64. doi: 10.1172/JCI73943.

10 Laurent AC, Bisserier M, Lucas A, Tortosa F, Roumieux M, De Régibus A, et al. Exchange protein directly activated by cAMP 1 promotes autophagy during cardiomyocyte hypertrophy. Cardiovasc Res. 2015;105(1):55–64. doi: 10.1093/cvr/cvu242.

11 Ma X, Liu H, Foyil SR, Godar RJ, Weinheimer CJ, Hill JA, et al. Impaired autophagosome clearance contributes to cardiomyocyte death in ischemia/reperfusion injury. Circulation. 2012;125(25):3170–81. doi: 10.1161/CIRCULATIONAHA.111.041814.

12 Ranek MJ, Kokkonen-Simon KM, Chen A, Dunkerly-Eyring BL, Vera MP, Oeing CU, et al. PKG1-modified TSC2 regulates mTORC1 activity to counter adverse cardiac stress. Nature. 2019;566(7743):264–9. doi: 10.1038/s41586-019-0895-y.

13 Saito T, Asai K, Sato S, Hayashi M, Adachi A, Sasaki Y, et al. Autophagic vacuoles in cardiomyocytes of dilated cardiomyopathy with initially decompensated heart failure predict improved prognosis. Autophagy. 2016;12(3):579–87. doi: 10.1080/15548627.2016.1145326.

14 Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, et al. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ Res. 2007;100(6):914–22. doi: 10.1161/01.RES.0000261924.76669.36.

15 Kassiotis C, Ballal K, Wellnitz K, Vela D, Gong M, Salazar R, et al. Markers of autophagy are downregulated in failing human heart after mechanical unloading. Circulation. 2009;120(11 Suppl):S191–7. doi: 10.1161/CIRCULATIONAHA.108.842252.

16 Kanamori H, Takemura G, Goto K, Maruyama R, Ono K, Nagao K, et al. Autophagy limits acute myocardial infarction induced by permanent coronary artery occlusion. Am J Physiol Heart Circ Physiol. 2011;300(6):H2261–71. doi: 10.1152/ajpheart.01056.2010.

17 Nah J, Zhai P, Huang CY, Fernández ÁF, Mareedu S, Levine B, et al. Upregulation of Rubicon promotes autosis during myocardial ischemia/reperfusion injury. J Clin Invest. 2020;130(6):2978–91. doi: 10.1172/JCI132366.

18 Han Y, Xie H, Liu Y, Gao P, Yang X, Shen Z. Effect of metformin on all-cause and cardiovascular mortality in patients with coronary artery diseases: a systematic review and an updated meta-analysis. Cardiovasc Diabetol. 2019;18(1):96. doi: 10.1186/s12933-019-0900-7.

19 Castellazzi M, Patergnani S, Donadio M, Giorgi C, Bonora M, Bosi C, et al. Autophagy and mitophagy biomarkers are reduced in sera of patients with Alzheimer's disease and mild cognitive impairment. Sci Rep. 2019;9(1):20009. doi: 10.1038/s41598-019-56614-5.

20 Tarocco A, Morciano G, Perrone M, Cafolla C, Ferrè C, Vacca T, et al. Increase of Parkin and ATG5 plasmatic levels following perinatal hypoxic-ischemic encephalopathy. Sci Rep. 2022;12(1):7795. doi: 10.1038/s41598-022-11870-w.

21 Patergnani S, Castellazzi M, Bonora M, Marchi S, Casetta I, Pugliatti M, et al. Autophagy and mitophagy elements are increased in body fluids of multiple sclerosis-affected individuals. J Neurol Neurosurg Psychiatry. 2018;89(4):439–41. doi: 10.1136/jnnp-2017-316234.

22 Emanuele E, Minoretti P, Sanchis-Gomar F, Pareja-Galeano H, Yilmaz Y, Garatachea N, et al. Can enhanced autophagy be associated with human longevity? Serum levels of the autophagy biomarker beclin-1 are increased in healthy centenarians. Rejuvenation Res. 2014;17(6):518–24. doi: 10.1089/rej.2014.1607.

23 Schlemmer F, Boyer L, Soumagne T, Ridoux A, Chouaid C, Maitre B, et al. Beclin1 circulating levels and accelerated aging markers in COPD. Cell Death Dis. 2018;9(2):156. doi: 10.1038/s41419-017-0178-1.

24 Morciano G, Patergnani S, Pedriali G, Cimaglia P, Mikus E, Calvi S, et al. Impairment of mitophagy and autophagy accompanies calcific aortic valve stenosis favouring cell death and the severity of disease. Cardiovasc Res. 2022;118(11):2548–59. doi: 10.1093/cvr/cvab267.

25 Knuuti J, Wijns W, Saraste A, Capodanno D, Barbato E, Funck-Brentano C, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J. 2020;41(3):407–77. doi: 10.1093/eurheartj/ehz425.

26 Elbaz M, Faccini J, Laperche C, Grousset E, Roncalli J, Ruidavets JB, et al. Identification of a miRNA based-signature associated with acute coronary syndrome: evidence from the FLORINF study. J Clin Med. 2020;9(6):1674. doi: 10.3390/jcm9061674.

27 Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD, et al. Third universal definition of myocardial infarction. Eur Heart J. 2012;33(20):2551–67. doi: 10.1093/eurheartj/ehs184.

28 Sabe AA, Elmadhun NY, Sadek AA, Chu LM, Bianchi C, Sellke FW. Differential effects of atorvastatin on autophagy in ischemic and nonischemic myocardium in Ossabaw swine with metabolic syndrome. J Thorac Cardiovasc Surg. 2014;148(6):3172–8. doi: 10.1016/j.jtcvs.2014.07.104.

29 Hsu HC, Chen CY, Lee BC, Chen MF. High-fat diet induces cardiomyocyte apoptosis via the inhibition of autophagy. Eur J Nutr. 2016;55(7):2245–54. doi: 10.1007/s00394-015-1034-7.

30 Razani B, Feng C, Coleman T, Emanuel R, Wen H, Hwang S, et al. Autophagy links inflammasomes to atherosclerotic progression. Cell Metab. 2012;15(4):534–44. doi: 10.1016/j.cmet.2012.02.011.

31 McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur J Heart Fail. 2022;24(1):4–131. doi: 10.1002/ejhf.2333.

32 Martin TG, Juarros MA, Cleveland JC Jr, Bristow MR, Ambardekar AV, Buttrick PM, et al. Assessment of autophagy markers suggests increased activity following LVAD therapy. JACC Basic Transl Sci. 2023;8(9):1043–56. doi: 10.1016/j.jacbts.2023.05.015.