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Serum amine-based metabolites and their association with outcomes in primary prevention implantable cardioverter-defibrillator patients

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Aims Heart failure patients are at increased risk of ventricular arrhythmias and all-cause mortality. However, existing clinical and serum markers only modestly predict these adverse events. We sought to use metabolic profiling to identify novel biomarkers in two independent prospective cohorts of patients with implantable cardioverter-defibrillators (ICDs) for primary prevention of sudden cardiac death (SCD).

Methods and results Baseline serum was quantitatively profiled for 42 known biologically relevant amine-based metabolites among 402 patients from the Prospective Observational Study of Implantable Cardioverter-Defibrillators (PROSE-ICD) Study (derivation group) and 240 patients from the Genetic Risk Assessment of Defibrillator Events (GRADE) Study (validation group) for ventricular arrhythmia-induced ICD shocks and all-cause mortality. Three amines, *N*-methyl-L-histidine, symmetric dimethylarginine (SDMA), and L-kynurenine, were derived and validated to be associated with all-cause mortality. The hazard ratios of mortality in PROSE-ICD and GRADE were 1.48 (95% confidence interval 1.14–1.92) and 1.67 (1.22–2.27) for *N*-methyl-L-histidine, 1.49 (1.17–1.91) and 1.77 (1.27–2.45) for SDMA, 1.31 (1.06–1.63) and 1.73 (1.32–2.27) for L-kynurenine, respectively. L-Histidine, SDMA, and L-kynurenine were associated with ventricular arrhythmia-induced ICD shocks in PROSE-ICD, but they did not reach statistical significance in the GRADE cohort.

Conclusion Utilizing metabolic profiling in two independent prospective cohorts of patients undergoing ICD implantation for primary prevention of SCD, we identified several novel amine markers that were associated with appropriate shock and mortality. These findings shed insight into the potential biologic pathways leading to adverse events in ICD patients. Further studies are needed to confirm the prognostic value of these findings.

Keywords Metabolomics • Amine • Implantable cardioverter-defibrillator • Ventricular arrhythmia • Mortality

Introduction

Individuals with systolic heart failure are at risk of ventricular arrhythmias and all-cause mortality. However, known clinical variables and serum-based biomarkers have demonstrated only modest prognostic power and incompletely predict the risk of adverse events in high-risk heart failure patients.[1] The dearth of effective new treatments in heart failure further highlights the importance of developing new insights into the underlying mechanisms and pathophysiology of heart failure, a prerequisite for guiding improved risk prediction, disease prevention, and more effective therapeutic strategies.

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What's new?

- Serum-based metabolic profiling was performed in two independent prospective cohorts of systolic heart failure patients with primary prevention ICDs. This was performed in order to derive and validate a panel of amine-based compounds that could predict ICD shocks for ventricular arrhythmias or all-cause mortality.
- We identified and validated *N*-methyl-L-histidine, SDMA, and L-kynurenine as three compounds associated with all-cause mortality. These findings suggest a role of the nitric oxide and other vascular relaxation pathways in modulating mortality risk among patients with systolic heart failure.
- L-Histidine, SDMA, and L-kynurenine were three novel compounds found to be associated with ICD shocks for ventricular arrhythmias, but they did not reach statistical significance in the validation cohort.

The metabolic environment of the injured cardiomyocytes may initiate and/or perpetuate fatal arrhythmias by advancing the existing injury or by acting as triggers. Metabolic profiling technology allows high-throughput quantitative assessment of thousands of small-molecule by-products of cellular metabolism found in the serum, thus reflecting the closest 'snapshot' of cellular processes both in normal physiology and disease.[2] It has been used to identify novel biomarkers and to improve understanding of the biological mechanism in several disease processes including coronary artery disease and diabetes.[3,4] A growing body of studies have also applied metabolic profiling to document metabolic alterations in heart failure patients,[5,6] but their diagnostic value in identifying future ventricular arrhythmias or mortality is largely unknown. Finding ways to fill this knowledge gap is particularly relevant to individuals at risk for sudden cardiac death (SCD) who have undergone primary prevention implantable cardioverter-defibrillators (ICDs) given the variability in their outcomes after device implantation.

Amine-based metabolites including amino acids and biogenic amines are a particularly important class of compounds and most widely studied because of their involvement in many metabolic processes including heart disease.[7] In an effort to better understand the role of amine-based metabolites in risk prediction for ventricular arrhythmias and all-cause mortality, we performed metabolic profiling of baseline sera from two independent prospective cohorts of patients with ischaemic and non-ischaemic systolic heart failure who underwent ICD implantation for primary prevention of SCD. Biologically relevant compounds were derived in one [the Prospective Observational Study of Implantable Cardioverter-Defibrillators (PROSE-ICD) Study[8]] and validated in the other [the Genetic Risk Assessment of Defibrillator Events (GRADE) Study[9]] with the aim of identifying novel biomarkers that might serve as new predictors of ventricular arrhythmia and all-cause mortality in this patient population.

Materials and methods

Study design and population

The PROSE-ICD is a multicentre prospective study of patients with systolic heart failure undergoing implantation of a primary prevention ICD conducted at four clinical centres in the United States from 2003 to 2013. Details of the study design have been described previously.[8] Briefly, patients 18–80 years of age referred for primary prevention ICD implantation were enrolled if they met any of the following criteria: (i) ischaemic cardiomyopathy (myocardial infarction >40 days prior to implant) with an ejection fraction of <30% and stable New York Heart Association (NYHA) Class I–III heart failure; (ii) ischaemic or non-ischaemic cardiomyopathy with an ejection fraction <35% and NYHA Class II or III heart failure; or (iii) ejection fraction <35% with NYHA Class II–IV heart failure undergoing guideline-indicated implantation of a cardiac resynchronization therapy device with an ICD. Among the 1189 participants enrolled in the PROSE-ICD Study, metabolic profiling of amines was performed in 402 individuals who had serum available for metabolic profiling.

The GRADE study is a multicentre prospective study of systolic heart failure patients with an ICD placed for primary or secondary prevention between 2002 and 2010 and followed through 2012.[9] Briefly, patients 18 years of age and older with ischaemic or non-ischaemic cardiomyopathy were enrolled if they had significant left ventricular systolic dysfunction (defined as a left ventricular ejection fraction <30%) and increased left ventricular size (defined as left ventricular end-diastolic dimension of >55 mm). Among the 1808 participants, the current analysis was based on 240 participants who were implanted for primary prevention and had serum available for metabolic profiling. Both studies complied with the Declaration of Helsinki and all centres obtained approval from their respective institutional review boards as well as signed informed consent from the patients.

Clinical data collection

In both PROSE-ICD and GRADE, participants underwent a comprehensive medical history and cardiovascular examination along with a digitally recorded resting 12-lead electrocardiogram (ECG), an echocardiogram or radionuclide ventriculography (if one was not previously available), and fasting blood collection at enrolment. The medical history included data on NYHA class, atrial fibrillation, smoking, comorbidities, and medication use. Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation. Chronic kidney disease was defined as eGFR < 60 mL/min/1.73 m^2.

Metabolic profiling

The amine platform covers amino acids and biogenic amines employing an Accq-tag derivatization strategy. An ACQUITY UPLC system with autosampler (Waters, Etten-Leur, The Netherlands) was coupled online with a Xevo Tandem quadrupole mass spectrometer (Waters) operated using QuanLynx data acquisition software (version 4.1; Waters). Fasting blood samples were collected at enrolment and analysed by UPLC-MS/MS using an Accq-Tag Ultra column (Waters). Each serum sample (5 μL) was spiked with an internal standard solution followed by deproteination with MeOH. The supernatant was transferred to a new Eppendorf tube and dried under N_2. The residue was reconstituted in borate buffer (pH 8.5) with AQC reagent. After reaction, the vials were transferred to an autosampler tray and cooled to 10°C until the injection. One microlitre of the reaction mixture was injected into the UPLC-MS/MS system.

Acquired data were evaluated using TargetLynx software (Waters), by integration of assigned MRM peaks and normalization using proper internal standards. For analysis of amino acids, their $^{13}C^{15}N$-labelled analogues were used. For other amines, the closest-eluting internal standard was employed. Blank samples were used to correct for background and in-house developed algorithms were applied using the

pooled QC samples to compensate for shifts in the sensitivity of the mass spectrometer over the batch. All baseline serum samples were analysed centrally using the same method.

Follow-up and outcomes

In PROSE-ICD, patients were evaluated every 6 months after ICD implantation either in person or by phone and soon after any ICD shock recognized by the patient. For the current analysis, participants were followed for events through 1 July 2013. An appropriate ICD shock was defined as one delivered for rapid ventricular tachyarrhythmias. Arrhythmic events were adjudicated by two clinical cardiac electrophysiologists blinded to patient demographic information. Disagreements were reconciled by a third electrophysiologist. Deaths were ascertained by phone interviews with the next of kin and by searches of the National Death Index.

In GRADE, patients were evaluated yearly after ICD implantation either in person or by phone, and ICD telemetry was examined. Clinical data and ICD telemetry following any ICD shock were evaluated when available. For the current analysis, participants were followed for events through 1 July 2011. An appropriate ICD shock was defined as an ICD shock for rapid ventricular tachyarrhythmias. Arrhythmic events were adjudicated by two cardiologists, and a third in cases of disagreement. Deaths were ascertained by phone interviews with the next of kin, medical records, and searches of the National Death Index.

Statistical analysis

Participants from PROSE-ICD and GRADE were analysed separately. Metabolites with missing values (i.e. metabolite levels below the lower limits of detection) were imputed with a value of the lower limits of detection divided by 2. Metabolites with >25% missing values were excluded from the analysis. Owing to the skewed distribution and different units of different metabolites, all metabolites were first log-transformed to approximate a normal distribution, and then standardized to have a mean of 0 and standard deviation (SD) of 1.

Cox proportional hazards regression model was used to assess the association between each individual metabolite and study endpoints. For all analyses, we used two models with increasing degrees of adjustment for confounding. The first model adjusted for age, sex, race, and enrolment centre. The second model further adjusted for smoking status, body mass index, ejection fraction, NYHA class, atrial fibrillation, diabetes, hypertension, and CKD (adjustment for kidney disease was only done in PROSE-ICD as the information was not available in GRADE). In sensitivity analyses further adjusting for ECG markers (QRS, QTc) and medications [aspirin, angiotensin converting enzyme inhibitor (ACE-I)/angiotensin receptor blocker (ARB), beta-blocker, diuretics, and aldosterone antagonist], the results were virtually unchanged (data not shown). Nominal *P*-values from the Cox regression models were reported since the nature of this analysis was exploratory and two independent cohorts were used for derivation and validation. We also used the Benjamini–Hochberg procedure with a false discovery rate of 0.05 to account for multiple comparisons, and identified the same three amine markers for mortality as statistically significant. All analyses were performed using STATA version 12 (StataCorp LP, College Station, TX, USA).

Results

In this analysis, the average age (SD) of participants at baseline was 60.1 ± 12.8 years in PROSE-ICD and 62.5 ± 11.8 years in GRADE (*Tables 1 and 2*). Men and African-Americans represented 73.6 and 35.6% of the PROSE-ICD population, and 77.1 and 16.7% in GRADE, respectively.

In PROSE-ICD, 55 of 402 participants experienced an appropriate ICD shock (incidence rate 3.4 per 100 person-years), and 120 participants died (incidence rate 5.5 per 100 person-years), during a median follow-up of 5.5 years. Patients who experienced an appropriate ICD shock were more likely to be current or former smokers and less likely to be hypertensive (*Table 1*), whereas patients who died were older, male, Caucasian, current or former smokers, had NYHA Class III heart failure, atrial fibrillation, and CKD (*Table 2*). In GRADE, 52 of 240 participants experienced an appropriate ICD shock (incidence rate 7.3 per 100 person-years), and 39 participants died (incidence rate 4.8 per 100 person-years) during a median follow-up of 3.7 years. Patients who experienced an appropriate ICD shock were younger, more likely to be male, and to have a lower ejection fraction (*Table 1*), whereas patients who died were older and had lower body mass index (*Table 2*).

In PROSE-ICD, L-histidine [hazard ratio (HR) 0.72, 95% confidence interval (CI) 0.52–0.98], symmetric dimethylarginine (SDMA; HR 1.79, 95% CI 1.19–2.69), and L-kynurenine (HR 1.54, 95% CI 1.04–2.29) were associated with the risk of appropriate ICD shock after adjusting for age, sex, race, enrolment centre, smoking status, body mass index, ejection fraction, NYHA class, atrial fibrillation, diabetes, hypertension, and CKD (*Figure 1*). In GRADE, these three compounds followed a similar trend in their associations with an appropriate shock but none achieved statistical significance. The corresponding HRs were 0.87 (0.60–1.25) for L-histidine, 1.28 (0.95–1.71) for SDMA, and 1.17 (0.83–1.63) for L-kynurenine. In addition, L-4-hydroxyproline and L-glutamine were found to be associated with appropriate shock in GRADE but not in the PROSE-ICD cohort.

In multivariate Cox models for mortality, three amines (*N*-methyl-L-histidine, SDMA, and L-kynurenine) were positively associated with the risk of all-cause mortality in both PROSE-ICD and GRADE (*Figure 2*). The HRs of mortality in PROSE-ICD and GRADE were 1.48 (1.14–1.92) and 1.67 (1.22–2.27) for *N*-methyl-L-histidine, 1.49 (1.17–1.91) and 1.77 (1.27–2.45) for SDMA, and 1.31 (1.06–1.63) and 1.73 (1.32–2.27) for L-kynurenine, respectively.

Discussion

Using metabolic profiling, we identified three amines (*N*-methyl-L-histidine, SDMA, and L-kynurenine) that were associated with all-cause mortality in two independent prospective cohorts of patients undergoing ICD implantation for primary prevention of SCD. The associations remained true after adjustment for demographic and clinical risk factors. In addition, L-histidine, SDMA, and L-kynurenine showed associations with the risk of appropriate ICD shock in PROSE-ICD but not in GRADE. Nevertheless, these findings suggest their potential to be novel markers of ventricular arrhythmia.

The metabolic environment, which the cardiomyocytes are continuously exposed, represents a collection of the final downstream products of a number from biologic processes including gene transcription, enzyme activity, nutrition, drugs, and hormones. Metabolic profiling allows the systematic assessment of thousands of small-molecule metabolites found in the serum and has been used in the search for novel biomarkers for cardiovascular disease.[2] A

Table 1 Baseline characteristics of participants, by appropriate ICD shock

Characteristic	PROSE-ICD				GRADE			
	Total (n = 402)	No appropriate ICD shock (n = 347)	Appropriate ICD shock (n = 55)	P-Value	Total (n = 240)	No appropriate ICD shock (n = 188)	Appropriate ICD shock (n = 52)	P-Value
Age (year)	60.1 ± 12.8	60.2 ± 12.8	59.6 ± 12.7	0.75	62.5 ± 11.8	63.5 ± 11.6	58.8 ± 11.9	0.01
Sex				0.41				0.01
Male	296 (73.6)	253 (72.9)	43 (78.2)		185 (77.1)	138 (73.4)	47 (90.4)	
Female	106 (26.4)	94 (27.1)	12 (21.8)		55 (22.9)	50 (26.6)	5 (9.6)	
Race				0.26				0.06
White	248 (61.7)	210 (60.5)	38 (69.1)		194 (80.8)	158 (84.0)	36 (69.2)	
Black	143 (35.6)	126 (36.3)	17 (30.9)		40 (16.7)	26 (13.8)	14 (26.9)	
Other	11 (2.7)	11 (3.2)	0 (0.0)		6 (2.5)	4 (2.1)	2 (3.8)	
Smoking				0.05				0.96
Never	137 (34.1)	126 (36.3)	11 (20.0)		87 (36.3)	68 (36.2)	19 (36.5)	
Former	191 (47.5)	158 (45.5)	33 (60.0)		153 (63.7)[a]	120 (63.8)[a]	33 (63.5)[a]	
Current	74 (18.4)	63 (18.2)	11 (20.0)					
Body mass index (kg/m^2)	29.4 ± 6.5	29.1 ± 6.5	30.9 ± 6.3	0.06	28.7 ± 5.5	28.8 ± 5.6	28.2 ± 5.2	0.56
Ejection fraction (%)	21.6 ± 7.5	21.6 ± 7.5	21.4 ± 7.3	0.88	20.4 ± 6.6	20.9 ± 6.3	18.7 ± 7.1	0.03
NHYA class				0.88				0.55
Class I	59 (14.7)	52 (15.0)	7 (12.7)		45 (18.8)	32 (17.0)	13 (25.0)	
Class II	162 (40.3)	141 (40.6)	21 (38.2)		125 (52.1)	101 (53.7)	24 (46.2)	
Class III	180 (44.8)	153 (44.1)	27 (49.1)		69 (28.8)	54 (28.7)	15 (28.8)	
Class IV	1 (0.2)	1 (0.3)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	
Ischaemic cardiomyopathy	216 (53.7)	184 (53.0)	32 (58.2)	0.48	168 (70.0)	131 (69.7)	37 (71.2)	0.84
QRS (ms)	121.4 ± 32.0	121.2 ± 32.4	122.2 ± 30.1	0.84	136.6 ± 37.9	137.2 ± 38.3	134.3 ± 36.5	0.66
QTc (ms)	459.1 ± 43.4	458.2 ± 44.1	465.1 ± 38.2	0.28	470.9 ± 52.2	472.9 ± 53.8	463.6 ± 45.5	0.27
Atrial fibrillation	103 (25.6)	89 (25.6)	14 (25.5)	0.98	38 (15.8)	26 (13.8)	12 (23.1)	0.16
Diabetes	128 (31.8)	110 (31.7)	18 (32.7)	0.88	74 (30.8)	55 (29.3)	19 (36.5)	0.54
Hypertension	242 (60.2)	220 (63.4)	22 (40.0)	0.001	160 (66.7)	125 (66.5)	35 (67.3)	0.87
Chronic kidney disease	111 (27.6)	99 (28.5)	12 (21.8)	0.44	NA	NA	NA	NA
Medications								
Aspirin	264 (65.7)	229 (66.0)	35 (63.6)	0.73	NA	NA	NA	NA
ACE-I/ARB	291 (72.4)	252 (72.6)	39 (70.9)	0.79	184 (76.7)	143 (76.1)	41 (78.8)	0.68
Beta-blocker	357 (88.8)	312 (89.9)	45 (81.8)	0.08	213 (88.8)	168 (89.4)	45 (86.5)	0.55
Thiazide/loop diuretics	275 (68.4)	237 (68.3)	38 (69.1)	0.91	164 (68.3)	126 (67.0)	38 (73.1)	0.46
Aldosterone antagonist	99 (24.6)	91 (26.2)	8 (14.5)	0.06	64 (26.7)	51 (27.1)	13 (25.0)	0.71

Values are number (%) or mean (SD).
[a]Values denote former or current smokers.

few recent studies have applied metabolic profiling techniques to patients with heart failure and have documented metabolic alterations that correlate with heart failure severity.[5,6] These studies were limited by small sample sizes and by the cross-sectional designs which could not link the observed metabolic changes to future ventricular arrhythmia or mortality events. Our analysis of two independent prospective cohorts of primary prevention ICD recipients aims to fill this knowledge gap by applying metabolic profiling to high-risk heart failure patients. In doing so, we identified several amine metabolites that could serve as novel markers of ventricular arrhythmia and mortality in this patient population.

The role of *N*-methyl-L-histidine in heart disease is unknown beyond a recent observation showing that its levels are elevated among those with heart failure.[7] Dimethylarginines, asymmetric dimethylarginine (ADMA), and SDMA, on the other hand, have been studied extensively. These compounds are endogenously occurring analogues of L-arginine and are generated by the post-translational methylation of arginine residues. Most previous research has been focused on ADMA since it is the predominant endogenous inhibitor of nitric oxide (NO) synthase and has been shown to be a risk factor for cardiovascular and all-cause mortality.[10] In a study of 106 primary prevention ICD patients from Germany, ADMA was also found to be associated with the risk of

Table 2 Baseline characteristics of participants, by all-cause mortality

Characteristic	PROSE-ICD				GRADE			
	Total (n = 402)	Alive (n = 282)	Dead (n = 120)	P-Value	Total (n = 240)	Alive (n = 201)	Dead (n = 39)	P-Value
Age (year)	60.1 ± 12.8	57.8 ± 11.9	65.5 ± 13.2	<0.001	62.5 ± 11.8	61.6 ± 11.3	67.3 ± 13.2	0.006
Sex				0.004				0.22
Male	296 (73.6)	196 (69.5)	100 (83.3)		185 (77.1)	152 (75.6)	33 (84.6)	
Female	106 (26.4)	86 (30.5)	20 (16.7)		55 (22.9)	49 (24.4)	6 (15.4)	
Race				0.007				0.50
White	248 (61.7)	160 (56.7)	88 (73.3)		194 (80.8)	165 (82.1)	29 (74.4)	
Black	143 (35.6)	113 (40.1)	30 (25.0)		40 (16.7)	31 (15.4)	9 (23.1)	
Other	11 (2.7)	9 (3.2)	2 (1.7)		6 (2.5)	5 (2.5)	1 (2.6)	
Smoking				0.27				0.68
Never	137 (34.1)	103 (36.5)	34 (28.3)		87 (36.3)	74 (36.8)	13 (33.3)	
Former	191 (47.5)	130 (46.1)	61 (50.8)		153 (63.7)[a]	127 (63.2)[a]	26 (66.7)[a]	
Current	74 (18.4)	49 (17.4)	25 (20.8)					
Body mass index (kg/m^2)	29.4 ± 6.5	29.7 ± 6.7	28.5 ± 6.0	0.08	28.7 ± 5.5	29.1 ± 5.3	26.5 ± 6.3	0.009
Ejection fraction (%)	21.6 ± 7.5	21.9 ± 7.6	20.6 ± 7.1	0.11	20.4 ± 6.6	20.5 ± 6.5	19.6 ± 7.0	0.47
NHYA class				0.004				0.51
Class I	59 (14.7)	48 (17.0)	11 (9.2)		45 (18.8)	39 (19.4)	6 (15.4)	
Class II	162 (40.3)	123 (43.6)	39 (32.5)		125 (52.1)	107 (53.2)	18 (46.2)	
Class III	180 (44.8)	110 (39.0)	70 (58.3)		69 (28.8)	54 (26.9)	15 (38.5)	
Class IV	1 (0.2)	1 (0.4)	0 (0.0)		0 (0.0)	0 (0.0)	0 (0.0)	
Ischaemic cardiomyopathy	216 (53.7)	142 (50.4)	74 (61.7)	0.04	168 (70.0)	138 (68.7)	30 (76.9)	0.30
QRS (ms)	121.4 ± 32.0	118.5 ± 30.9	127.9 ± 33.9	0.007	136.6 ± 37.9	135.8 ± 37.5	141.0 ± 40.1	0.48
QTc (ms)	459.1 ± 43.4	455.1 ± 41.1	468.6 ± 47.0	0.004	470.9 ± 52.2	471.0 ± 51.3	470.2 ± 57.3	0.93
Atrial fibrillation	103 (25.6)	59 (20.9)	44 (36.7)	0.001	38 (15.8)	29 (14.4)	9 (23.1)	0.16
Diabetes	128 (31.8)	82 (29.1)	46 (38.3)	0.07	74 (30.8)	62 (30.8)	12 (30.8)	0.91
Hypertension	242 (60.2)	165 (58.5)	77 (64.2)	0.29	160 (66.7)	135 (67.2)	25 (64.1)	0.83
Chronic kidney disease	111 (27.6)	55 (19.5)	56 (46.7)	<0.001	NA	NA	NA	NA
Medications								
Aspirin	264 (65.7)	185 (65.6)	79 (65.8)	0.96	NA	NA	NA	NA
ACE-I/ARB	291 (72.4)	203 (72.0)	88 (73.3)	0.78	184 (76.7)	154 (76.6)	30 (76.9)	0.97
Beta-blocker	357 (88.8)	254 (90.1)	103 (85.8)	0.22	213 (88.8)	180 (89.6)	33 (84.6)	0.45
Thiazide/loop diuretics	275 (68.4)	188 (66.7)	87 (72.5)	0.25	164 (68.3)	133 (66.2)	31 (79.5)	0.19
Aldosterone antagonist	99 (24.6)	67 (23.8)	32 (26.7)	0.54	64 (26.7)	57 (28.4)	7 (17.9)	0.32

Values are number (%) or mean (SD).
[a]Values denote former or current smokers.

appropriate ICD therapy.[11] Unlike ADMA, its structural isomer SDMA, has been less well investigated and is thought to be biologically inert as it does not directly inhibit NO synthesis.[12] However, recent studies have reported associations of SDMA with cardiovascular outcomes and mortality in various study populations including patients undergoing coronary angiography,[10] and with coronary heart disease.[13] Moreover, in several studies in which ADMA and SDMA were both examined, SDMA showed a similar or even stronger association with cardiovascular and mortality endpoints compared with ADMA.[10,13] Our results from two independent cohorts of heart failure patients with primary prevention ICDs also showed that SDMA, but not ADMA, was positively associated with all-cause mortality. In addition, we found a statistically significant association between SDMA and the risk of appropriate ICD shock in the PROSE-ICD cohort and a non-significant trend towards higher risk in the GRADE cohort. These findings suggest that besides being a risk marker for mortality, SDMA might also be associated with the development of ventricular arrhythmias.

Several mechanisms may explain the link between SDMA and cardiovascular endpoints. Symmetric dimethylarginine is a marker of kidney function as it is exclusively eliminated by renal secretion (as opposed to ADMA which is mainly hydrolysed enzymatically by dimethylarginine dimethylamino-hydrolase). However, consistent with previous studies, SDMA remained associated with mortality and ICD shock in our study after adjustment for kidney disease, suggesting that these associations were not fully explained by kidney function and alternative mechanisms may exist. Although not a direct NO synthase inhibitor, SDMA indirectly reduces NO synthesis

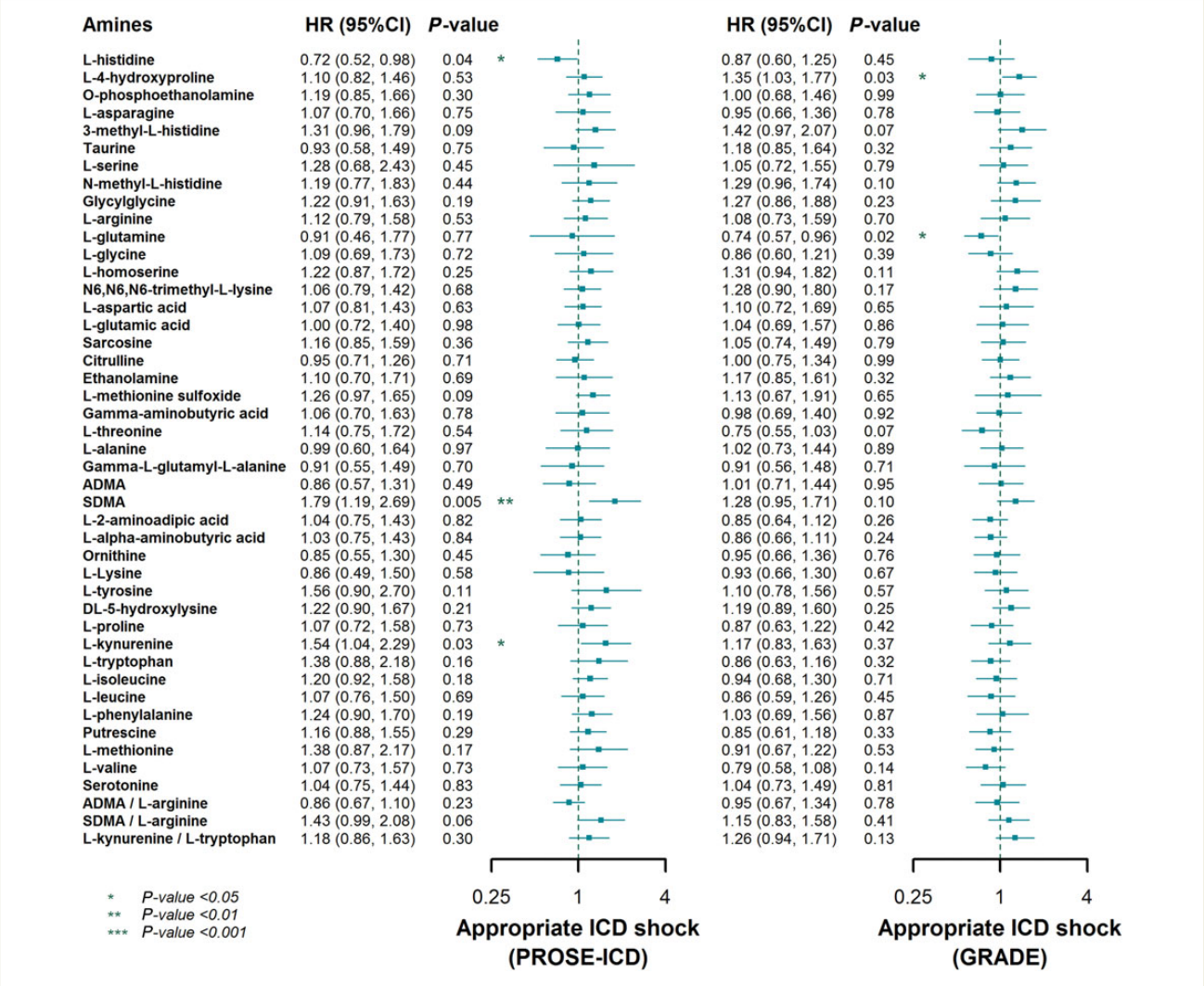

Figure 1 Multivariate-adjusted HRs (95% CI) for appropriate shock associated with each metabolite in the PROSE-ICD (left panel) and GRADE (right panel) studies. Models were adjusted for age, sex, race, enrolment centre, smoking status, body mass index, ejection fraction, NYHA class, atrial fibrillation, diabetes, hypertension, and CKD (adjustment for kidney disease was only done in PROSE-ICD as the information was not available in GRADE).

by inhibiting cellular uptake of the NO precursor L-arginine.[12] Symmetric dimethylarginine also stimulates the generation of reactive oxygen species in monocytes by acting on Ca^{2+} entry to the cell and promotes vascular inflammation.[12] Studies have shown that SDMA is associated with inflammatory markers including C-reactive protein (CRP), interleukin-6, and tumour necrosis factor-alpha.[14]

In addition to SDMA, our study also found positive associations of L-kynurenine with all-cause mortality and appropriate ICD shock. Kynurenine is a metabolite of the essential amino acid tryptophan.[15] It has been shown to be involved in vessel relaxation in experimental model of systemic inflammation and is associated with oxidative stress, inflammation, and the prevalence of cardiovascular disease in patients with renal disease.[15] L-Tryptophan is catalysed into kynurenine by two-dioxygenases, indoleamine 2,3-dioxygenase (IDO), and tryptophan 2,3-dioxygenase.[15] Tryptophan 2,3-dioxygenase resides primarily in the liver, whereas IDO is present in various cells including macrophages and neurons.[16] Indoleamine 2,3-dioxygenase is an important immune modulator suppressing the activation of T lymphocytes and is up-regulated by cytokines and inflammatory molecules particularly interferon gamma.[16] Several lines of evidence have shown that IDO activity, measured by the kynurenine/tryptophan ratio, is associated with risk factors for atherosclerosis (such as LDL cholesterol, body mass index, and CRP)[17] and mortality.[18] Additionally, activation of the kynurenine pathway has recently been shown to increase the risk of death after out-of-hospital cardiac arrest.[19] In our study, the association with mortality was stronger for the kynurenine/tryptophan ratio when compared with kynurenine alone; however, the kynurenine/tryptophan ratio was not associated with appropriate ICD shock. Further experimental and clinical studies are needed to better understand the role of kynurenine in heart failure patients.

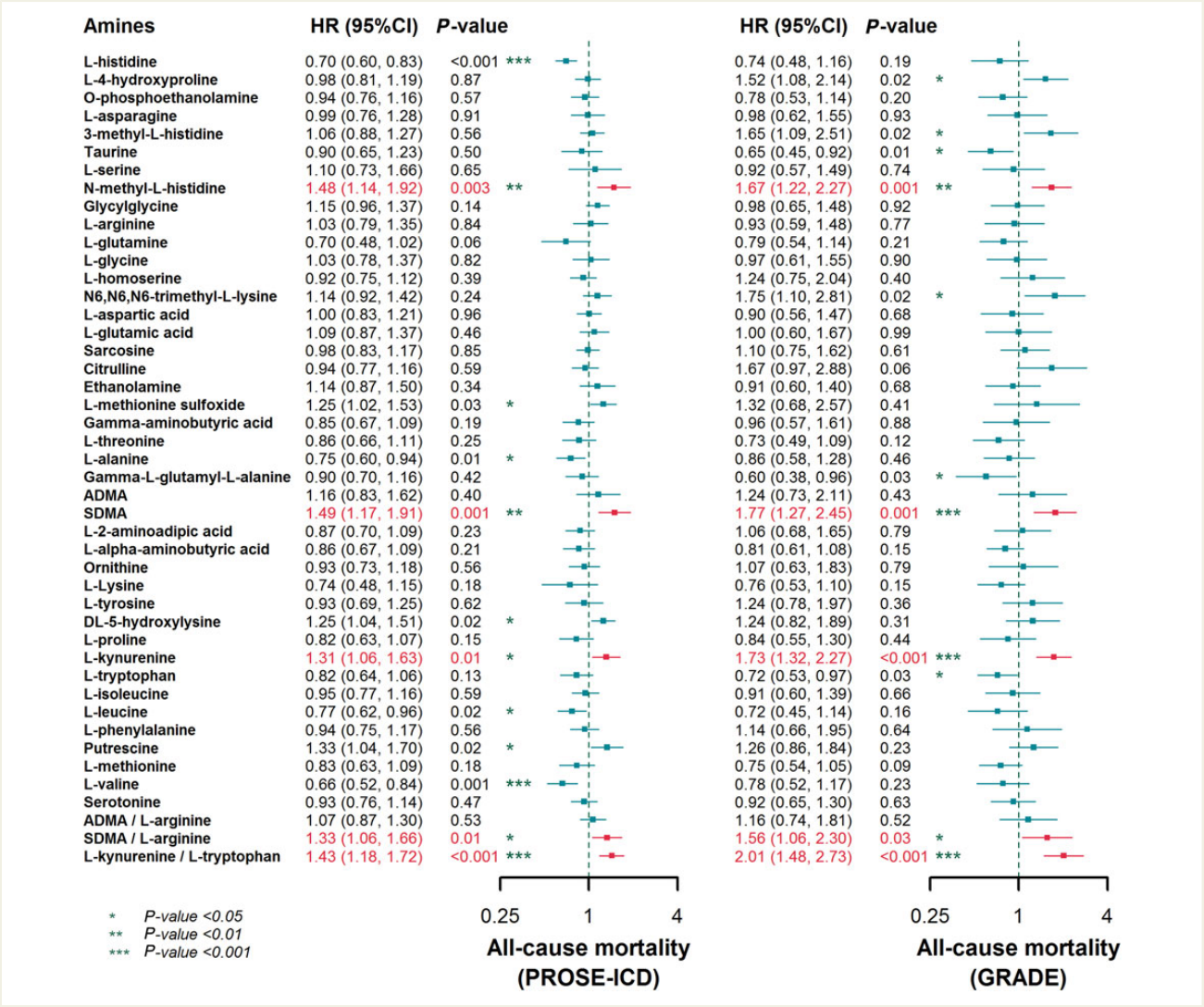

Figure 2 Multivariate-adjusted HRs (95% CI) for all-cause mortality associated with each metabolite in the PROSE-ICD (left panel) and GRADE (right panel) studies. Models were adjusted for age, sex, race, enrolment centre, smoking status, body mass index, ejection fraction, NYHA class, atrial fibrillation, diabetes, hypertension, and CKD (adjustment for kidney disease was only done in PROSE-ICD as the information was not available in GRADE). Amines that were significantly associated with mortality in both cohorts were highlighted in red.

Several limitations and strengths need to be considered when interpreting our results. Owing to the observational design of our study, we could only identify associations, but not establish causal links between amine-based metabolites and outcomes. Although our study included hundreds of patients with primary prevention ICDs, it still may be underpowered to detect associations with appropriate shock as only a few patients had the event. The mode of death could not be firmly established in many patients due to the lack of reliable records when patients died out of hospital. As a consequence, we could not examine differences in the cause-specific mortality or whether different amines may have different impact on cardiac vs. non-cardiac mode of death. In addition, our findings may not be applicable in all populations at risk of sudden death including those with preserved left ventricular function. The major strengths of this proposal include the availability of two independent cohorts of ICD patients, extensive and stringent phenotyping, uniformly collected and stored blood samples for analysis, and state-of-the-art tools for metabolic profiling.

Conclusions

Utilizing two independent prospective cohorts of patients undergoing ICD implantation for primary prevention of SCD, we identified several novel amine markers that were associated with appropriate shock and mortality using metabolic profiling. These findings may provide novel insights into the biologic pathways leading to adverse events in ICD patients, which will in turn aid the development of newer therapeutic measures for reducing SCD and mortality risk. Our study was exploratory and further experimental and clinical research is needed to validate our findings in other study populations, to elucidate the underlying mechanism of the observed associations, and to examine the added prognostic value of these amine-based

metabolites in risk prediction beyond established serum and ECG markers.

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References

1. Fishman GI, Chugh SS, Dimarco JP, Albert CM, Anderson ME, Bonow RO *et al.* Sudden cardiac death prediction and prevention: report from a National Heart, Lung, and Blood Institute and Heart Rhythm Society Workshop. *Circulation* 2010; **122**:2335–48.
2. Griffin JL, Atherton H, Shockcor J, Atzori L. Metabolomics as a tool for cardiac research. *Nat Rev Cardiol* 2011;**8**:630–43.
3. Shah SH, Bain JR, Muehlbauer MJ, Stevens RD, Crosslin DR, Haynes C *et al.* Association of a peripheral blood metabolic profile with coronary artery disease and risk of subsequent cardiovascular events. *Circ Cardiovasc Gene* 2010;**3**:207–U33.
4. Wang TJ, Larson MG, Vasan RS, Cheng S, Rhee EP, McCabe E *et al.* Metabolite profiles and the risk of developing diabetes. *Nat Med* 2011;**17**:448–U83.
5. Wang J, Li ZF, Chen JX, Zhao HH, Luo LT, Chen C *et al.* Metabolomic identification of diagnostic plasma biomarkers in humans with chronic heart failure. *Mol Biosyst* 2013;**9**:2618–26.
6. Cheng ML, Wang CH, Shiao MS, Liu MH, Huang YY, Huang CY *et al.* Metabolic disturbances identified in plasma are associated with outcomes in patients with heart failure: diagnostic and prognostic value of metabolomics. *J Am Coll Cardiol* 2015;**65**:1509–20.
7. Hakuno D, Hamba Y, Toya T, Adachi T. Plasma amino acid profiling identifies specific amino acid associations with cardiovascular function in patients with systolic heart failure. *PLoS ONE* 2015;**10**:e0117325.
8. Cheng A, Dalal D, Butcher B, Norgard S, Zhang Y, Dickfeld T *et al.* Prospective observational study of implantable cardioverter-defibrillators in primary prevention of sudden cardiac death: study design and cohort description. *J Am Heart Assoc* 2013;**2**:e000083.
9. Refaat MM, Lubitz SA, Makino S, Islam Z, Frangiskakis JM, Mehdi H *et al.* Genetic variation in the alternative splicing regulator RBM20 is associated with dilated cardiomyopathy. *Heart Rhythm* 2012;**9**:390–6.
10. Meinitzer A, Kielstein JT, Pilz S, Drechsler C, Ritz E, Boehm BO *et al.* Symmetrical and asymmetrical dimethylarginine as predictors for mortality in patients referred for coronary angiography: The Ludwigshafen Risk and Cardiovascular Health Study. *Clin Chem* 2011;**57**:112–21.
11. Lehmann HI, Goette A, Martens-Lobenhoffer J, Hammwohner M, Rohl FW, Bukowska A *et al.* Asymmetric dimethylarginine predicts appropriate implantable cardioverter-defibrillator intervention in patients with left ventricular dysfunction. *Europace* 2011;**13**:1428–35.
12. Schwedhelm E, Boger RH. The role of asymmetric and symmetric dimethylarginines in renal disease. *Nat Rev Nephrol* 2011;**7**:275–85.
13. Siegerink B, Maas R, Vossen CY, Schwedhelm E, Koenig W, Boger R *et al.* Asymmetric and symmetric dimethylarginine and risk of secondary cardiovascular disease events and mortality in patients with stable coronary heart disease: the KAROLA follow-up study. *Clin Res Cardiol* 2013;**102**:193–202.
14. Schepers E, Barreto DV, Liabeuf S, Glorieux G, Eloot S, Barreto FC *et al.* Symmetric dimethylarginine as a proinflammatory agent in chronic kidney disease. *Clin J Am Soc Nephrol* 2011;**6**:2374–83.
15. Pawlak K, Domaniewski T, Mysliwiec M, Pawlak D. The kynurenines are associated with oxidative stress, inflammation and the prevalence of cardiovascular disease in patients with end-stage renal disease. *Atherosclerosis* 2009;**204**:309–14.
16. Chen Y, Guillemin GJ. Kynurenine pathway metabolites in humans: disease and healthy states. *Int J Tryptophan Res* 2009;**2**:1–19.
17. Pertovaara M, Raitala A, Juonala M, Lehtimaki T, Huhtala H, Oja SS *et al.* Indoleamine 2,3-dioxygenase enzyme activity correlates with risk factors for atherosclerosis: the Cardiovascular Risk in Young Finns Study. *Clin Exp Immunol* 2007;**148**:106–11.
18. Pertovaara M, Raitala A, Lehtimaki T, Karhunen PJ, Oja SS, Jylha M *et al.* Indoleamine 2,3-dioxygenase activity in nonagenarians is markedly increased and predicts mortality. *Mech Ageing Dev* 2006;**127**:497–9.
19. Ristagno G, Latini R, Vaahersalo J, Masson S, Kurola J, Varpula T *et al.* Early activation of the kynurenine pathway predicts early death and long-term outcome in patients resuscitated from out-of-hospital cardiac arrest. *J Am Heart Assoc* 2014;**3**:e001094.