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Guanidino compound ratios are associated with stroke etiology, internal carotid artery stenosis and CHA_2DS_2-VASc score in three cross-sectional studies

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ABSTRACT

Introduction: Guanidino compounds, including L-homoarginine (L-hArg), symmetric dimethylarginine (SDMA), asymmetric dimethylarginine (ADMA) and L-arginine (L-Arg) are associated with mortality, fatal strokes, stroke incidence, and atherosclerosis.
Objectives: We aimed to study the association of guanidino compounds (L-hArg/ADMA and L-hArg/SDMA) with stroke etiology, internal carotid artery (ICA) stenosis and CHA_2DS_2-VASc score in patients with cerebrovascular disease.
Methods: We analyzed L-hArg, SDMA, ADMA, L-Arg, and compound molar ratios, i.e. L-hArg/ADMA and L-hArg/SDMA, in 272 patients with cerebrovascular disease in a cross-sectional discovery cohort and two cross-sectional validation cohorts of acute stroke patients from Germany (n = 137) and UK (n = 394). The guanidino compound levels were compared with clinical, imaging, and ultrasound parameters.
Results: Low L-hArg/ADMA and L-hArg/SDMA molar ratios predicted territorial infarcts (OR 1.74; 95% CI 1.34–2.26 and OR 1.64; 95% CI 1.26–2.15, respectively) and were associated with stroke subtypes due to large vessel disease or cardio-embolism (OR 1.52; 95% CI 1.12–2.06 and OR 2.01; 95% CI 1.35–3.00, respectively) in meta-analysis of the discovery and validation cohort data. In line with these results, a low L-hArg/ADMA and L-hArg/SDMA molar ratio was found in patients with ICA stenosis (OR 0.73; 95% CI 0.55–0.97 and OR 0.69; 95% CI 0.50–0.94, respectively) in the discovery and validation cohort. Furthermore, guanidino compound ratios (i.e. L-hArg/ADMA and L-hArg/SDMA) were strongly correlated with CHA_2DS_2-VASC score ($p < .001$) in all three cohorts.
Discussion: The results from these three cross-sectional studies reveal that guanidino compound ratios (i.e. L-hArg/ADMA and L-hArg/SDMA) can discriminate stroke etiologies, predict ICA stenosis and estimate risk prediction in patients with cerebrovascular disease.

1. Introduction

L-Arginine (L-Arg), L-homoarginine (L-hArg), symmetric dimethylarginine (SDMA), and asymmetric dimethylarginine (ADMA) are guanidino compounds involved in nitric oxide (NO) metabolism and vascular function. NO is produced from L-Arg by NO-synthase (NOS) and is a crucial modulator of vascular function, i.e. elicit vasodilatation, decrease atherosclerosis, reduce platelet aggregation, and modulate inflammatory processes [1]. Numerous clinical studies underline the strong correlation between L-Arg and its derivatives, such as ADMA, SDMA, and L-hArg with stroke and cardiovascular events [2,3]. Although multi-factorial mechanisms are responsible for atherogenesis, experimental in vitro and animal models as well as clinical studies have shown the importance of NO metabolism in these processes [4].

In clinical studies, ADMA levels were associated with progression of carotid intima media thickness (IMT) [5]. However, whilst some studies revealed a positive association of SDMA with carotid IMT [6,7] others did not validate this correlation [5,8]. Both, SDMA and L-hArg are

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associated with vascular remodeling, but in contrast to SDMA, L-hArg showed an inverse correlation with measures of aortic atherosclerosis [9,10], and low L-hArg levels were associated with progressive carotid atherosclerosis assessed by IMT [11]. Given that ADMA and SDMA are risk factors for cardiovascular disease, it is presumed that they promote atherosclerotic disease [12]. But the association of guanidino compounds between each other is less well understood. Recently, a low L-hArg/ADMA molar ratio was reported to significantly correlate with aortic IMT in a single cross-sectional study and therefore suggested as composite biomarker in the cardiovascular system [13,14]. Furthermore, low L-hArg/ADMA and L-hArg/SDMA molar ratios were associated with cardiovascular mortality and events in patients with lower extremity arterial disease [15]. At present, it is unclear if L-hArg/ADMA and L-hArg/SDMA molar ratios predict parameters of atherosclerosis (i.e. carotid IMT, carotid artery stenosis and occlusion) in stroke patients.

With respect to stroke subtypes, low L-hArg levels have been associated with territorial infarcts in patients with acute ischemic stroke [16]. Different stroke etiologies underlie ischemic stroke events (e.g. small and large vessel disease, cardio-embolism), which require different stroke treatments. But associations of guanidino compounds with stroke subtypes, stroke etiologies and their underlying diseases (i.e. carotid artery stenosis, atrial fibrillation) have not been evaluated yet.

In three independent cross-sectional studies we tested the hypothesis that guanidino compounds and their ratios are associated with stroke subtype, stroke etiology and risk prediction scores (i.e. CHA_2DS_2-VASc score). The concentrations of circulating L-Arg, L-hArg, ADMA, and SDMA were determined and associated with clinical parameters in a discovery cohort of 272 patients with stroke risk recruited at a German neurological outpatient clinic (UKE cohort) and two validation cohorts of 137 acute stroke patients at a German neurological clinic (Harburg Stroke Study) and 394 hospitalized acute stroke patients recruited in Leeds, United Kingdom (Leeds Stroke Study).

2. Materials and methods

2.1. Study design

For the UKE discovery cohort 274 patients were recruited from the neurovascular outpatient clinic at the University Medical Center Hamburg-Eppendorf from October 2015 until September 2016 (Supplementary Fig. 1). The inclusion criteria were as follows: age > 18 years, diagnosis of prior stroke or patients at stroke risk, patient provided informed consent. Previous history of myocardial infarction, stroke, hypertension, diabetes mellitus, hypercholesterolemia, atrial fibrillation, and coronary artery disease, was determined from case notes, as were current use of antihypertensive, hypoglycemic and lipid-lowering medication use. Two patients were excluded due to missing data for ACI stenosis and therefore 272 patients were included in this study. 226 subjects (83%) had experienced prior stroke. Blood samples were taken directly after inclusion, serum and plasma samples were obtained by centrifugation and subsequently subjected to biochemical analyses. Written informed consent was obtained from all participants. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The study protocol has been priorly approved by the Ethics Committee of the Hamburg Board of Physicians (PV4715). The two other study cohorts, i.e. Harburg and Leeds Stroke Study, have been described elsewhere [16,17].

2.2. Biochemical analyses

Cholesterol, LDL, HDL, serum creatinine, glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), creatine kinase, and C-reactive protein (hsCRP) were determined with routine laboratory assays after inclusion of participants. Estimated glomerular filtration rate (eGFR) was calculated with the MDRD formula. The laboratory assessors were blinded to clinical data. Biochemical analyses of the Harburg and Leed Stroke cohorts have been described elsewhere [7,16].

Additional plasma samples were stored at −20 °C for the UKE discovery cohort, at −40 °C for the Leeds Stroke Study, and −80 °C for the Harburg Stroke Study. L-hArg, L-Arg, ADMA, and SDMA measurements were performed batch-wise using a previously described liquid chromatography–tandem mass spectrometric (LC–MS/MS) assay [18,19]. Even though sample analyses were performed with the same analytical platform, patient specimens were harvested at different time points and storage conditions were not identical. Briefly, 25 µL aliquots of plasma were spiked with internal standards, i.e. stable isotope-labelled L-hArg, L-Arg, and ADMA (stable isotope-labelled ADMA being used to quantify unlabelled ADMA and SDMA). By adding 100 µL methanol proteins were precipitated. After filtrating the samples through a 0.22 µm hydrophilic membrane (Multiscreen HTS™, Millipore, Molsheim, France), compounds were derivatized to their butylester derivatives with butanolic 1 N HCl, and analyzed by LC-MS/MS (Varian 1200 MS, Agilent Technologies, Santa Clara, USA).

2.3. Stroke subtype classification

According to the Oxfordshire Community Stroke Project (OCSP) classification, ischemic stroke was subclassified as lacunar, partial anterior, total anterior, or posterior circulation infarction based on cranial computed tomographic (CT) or magnetic resonance (MR) scans, as previously described [16]. Lacunar infarction represents stroke of probable small-vessel origin; partial anterior and total anterior circulation infarctions represent stroke of probable large-vessel origin; and posterior circulation infarction represents stroke of mixed vascular pathology. According to TOAST (Trial of Org 10,172 in Acute Stroke Treatment) criteria, all patients with ischemic stroke were further classified into different stroke subtypes: small-vessel atherosclerosis, large-vessel atherosclerosis, cardioembolic, other determined etiology and undetermined etiology. Electrocardiography, echocardiography, imaging of extracranial and intracranial arteries, computed tomography, magnetic resonance imaging, and laboratory assessments were determined from case notes and were performed after admission for acute stroke. Classification of stroke subtype was based on the detailed review of patient's clinical symptoms and results of diagnostic tests.

2.4. Carotid artery ultrasound

Carotid arteries were evaluated with high-resolution B-mode and Duplex ultrasound using a GE Logiq7 system with a 7.5-MHz linear-array transducer (GE Healthcare, Solingen, Germany) as previously described [20,21]. Ultrasound assessment of the severity of carotid stenosis was evaluated by using combined Doppler acoustic standard criteria. IMT was evaluated based on the Mannheim IMT Consensus [20]. The IMT was measured at the far wall as the distance from the leading edge of the first echogenic line to the leading edge of the second echogenic line with 10 mm proximal to the reference point at its thickest point in a region free of plaques. The greatest IMT measured in the right and left common carotid artery was used to define individual IMT. All carotid ultrasound studies were performed by two investigators, and offline analysis of all frozen ultrasound images were performed by the same experienced reader, who was blinded to clinical data.

2.5. Statistics

The sample size for the discovery cohort was estimated to be 240 on the basis of previous studies with *P* value of 0.001, mean values of 1.48 and 1.93 µmol/L and standard deviation of 1 µmol/L L-hArg [16]. Continuous variables are given as mean ± standard deviation (SD) if normally distributed, otherwise as median [25th–75th percentile], and categorical variables are given as number (percentage) of participants. Relationships between guanidino compounds and continuous variables were assessed by Spearman correlation analyses (correlation coefficient ρ) or linear regression analyses (beta coefficient and 95% confidence interval, CI). Statistical comparisons of two groups were made by Student's *t*-test (two-tailed) or by logistic regression analyses and odds ratios (OR) and corresponding 95% CI are given. We calculated beta coefficients and ORs for different models: unadjusted (model 1), adjusted for age and sex (model 2), and additionally adjusted for the cardiovascular risk factors hypertension, diabetes, hypercholesterolemia, eGFR, and smoking status (model 3). Model 3 was calculated by the backward elimination method based on the likelihood-ratio statistics. For the meta-analysis all models additionally included the study cohort as adjustment. A p value < .05 was considered as statistically significant. Due to the explorative nature of the study we did not adjust for multiple testing. Statistical analysis was performed with IBM SPSS Statistics (version 22, IBM Corp., Armonk, NY) and GraphPad Prism (version 5 for Windows, La Jolla, USA).

2.6. Data availability

De-identified participant data, related documents such as study protocol and statistical analysis will be shared by request from any qualified investigator for 3 years after the date of publication.

3. Results

3.1. Baseline characteristics

The baseline characteristics of the UKE discovery cohort and the two validation cohorts (Harburg Stroke Study and Leeds Stroke Study) are summarized in Supplementary Tables 1, 2 and 3 respectively. In the UKE stroke cohort, L-hArg positively correlated with renal function (estimated glomerular filtration rate) and negatively with age (Supplementary Table 4). In contrast, ADMA and SDMA levels were negatively correlated with renal function and positively with age. Previous studies suggested a strong association of L-hArg/ADMA levels with vascular risk factors [13], but we did not observe a correlation with body mass index, blood pressure, or total cholesterol (Supplementary Table 4).

3.2. Guanidino compounds and stroke subtype

We explored the association of guanidino compounds with stroke subtypes and etiology assessed by brain imaging, ultrasound and cardiological assessment. In the UKE discovery cohort, the L-hArg/ADMA and L-hArg/SDMA molar ratios were able to differentiate lacunar from territorial infarcts in unadjusted and adjusted models of logistic regression analyses (model 3: OR 1.95; 95% CI 1.26–3.02 and OR 1.74; 95% CI 1.15–2.62, respectively) (Supplementary Table 5). In the Harburg Stroke Study, the OR (95% CI) was also significantly increased in unadjusted (model 1) and adjusted models (model 2 and 3). Similar results were observed in a combined analysis of both studies in crude and adjusted models for L-hArg/ADMA and L-hArg/SDMA (model 3: OR 1.74; 95% CI 1.34–2.26 and OR 1.64; 95% CI 1.26–2.15, respectively). Guanidino compounds were also associated with stroke etiology according to the TOAST classification. In the UKE discovery cohort, lower L-hArg/ADMA and L-hArg/SDMA were observed in stroke patients due to large vessel disease or cardio-embolism compared with small vessel disease in unadjusted and fully adjusted models (model 3: OR 1.58; 95% CI 1.04–2.41 and OR 1.87; 95% CI 1.13–3.12, respectively) (Supplementary Table 6). A similar association was found in the Harburg Stroke Study in unadjusted model 1, but not after adjustment. The combined analysis of discovery and validation cohort revealed a significant association with stroke etiology in crude and adjusted models for L-hArg/ADMA and L-hArg/SDMA (model 3: OR 1.52; 95% CI

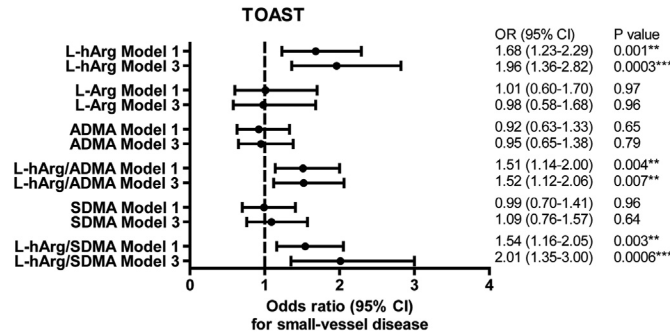

Fig. 1. Associations of guanidino compounds with TOAST classification in meta-analysis of UKE cohort and Harburg Stroke Study. Logistic regression analyses with odds ratios (OR) with 95% confidence intervals (CI) per SD increase of guanidino compounds for small-vessel disease compared to cardioembolic or large-vessel disease (model 1: adjusted for the study cohort; model 3: additionally adjusted for age, sex, hypertension, diabetes, hypercholesterolemia, estimated glomerular filtration rate, and smoking; model 3 was calculated by backward elimination). ADMA indicates asymmetric dimethylarginine; CI, confidence interval; L-Arg, L-arginine; L-hArg, L-homoarginine; OR, odds ratio; SDMA, symmetric dimethylarginine; TOAST, Trial of Org 10172 in Acute Stroke Treatment.

1.12–2.06 and OR 2.01; 95% CI 1.35–3.00, respectively) (Fig. 1).

3.3. Guanidino compounds and atherosclerosis

In the UKE discovery cohort, L-hArg/SDMA was negatively correlated in linear regression analyses with IMT in the common carotid artery (model 1: beta coefficient − 0.06; 95% CI -0.12–0.00), but did not remain significant after adjustment for age, sex and vascular risk factors (Supplementary Table 7). L-hArg/ADMA did not correlate with IMT in the common carotid artery. Correspondingly, L-hArg/SDMA was lower in patients with ICA stenosis compared with patients without ICA stenosis in the UKE discovery cohort (3.09 ± 1.78 vs 3.61 ± 2.18, p < .05). Similar results were observed in logistic regression analysis, which did not reach statistical significance due to a low number of patients with ICA stenosis (model 1: OR 0.75; 95% CI 0.55–1.02) (Supplementary Table 8). In the Harburg Stroke Study and combined analysis of both studies, L-hArg/SDMA was significantly lower in patients with ICA stenosis or occlusion compared with patients without any ICA stenosis in unadjusted and adjusted models (Fig. 2,

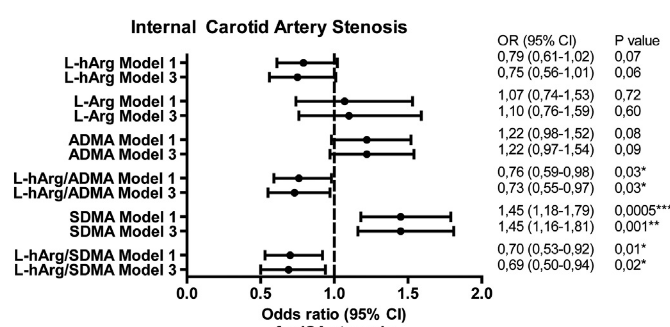

Fig. 2. Associations of guanidino compounds with ICA stenosis/occlusion in meta-analysis of UKE cohort and Harburg Stroke Study. Logistic regression analyses with odds ratios (OR) with 95% confidence intervals (CI) per SD increase of guanidino compounds for stenosis or occlusion of the internal carotid artery (model 1: adjusted for the study cohort; model 3: additionally adjusted for age, sex, hypertension, diabetes, hypercholesterolemia, estimated glomerular filtration rate, and smoking; model 3 was calculated by backward elimination). ADMA indicates asymmetric dimethylarginine; CI, confidence interval; L-Arg, L-arginine; L-hArg, L-homoarginine; OR, odds ratio; SDMA, symmetric dimethylarginine; ICA, internal carotid artery.

Supplementary Table 8) (model 3: OR 0.69; 95% CI 0.50–0.94).

3.4. Guanidino compounds and atrial fibrillation

In the UKE discovery cohort, low L-hArg/ADMA and L-hArg/SDMA ratios were found in patients with large vessel or cardio-embolic etiologies. In addition to large vessel disease, we also studied correlations of L-hArg/ADMA and L-hArg/SDMA with cardio-embolic stroke. L-hArg/ADMA was lower in patients of the Leeds Stroke Study with atrial fibrillation (AF) compared to patients without AF (1.86 ± 0.99 vs 2.35 ± 1.19, $p < .001$), whereas no difference was found in patients of the UKE cohort and Harburg Stroke Study (3.01 ± 1.69 vs 3.62 ± 1.87 and 3.13 ± 1.46 vs 3.35 ± 1.72; p = n.s. for both) In all three cohorts, L-hArg/SDMA was significantly lower in patients with AF compared with patients without AF (UKE cohort: 2.70 ± 1.57 vs 3.52 ± 2.12, $p < .05$; Harburg Stroke Study: 3.38 ± 1.57 vs 4.28 ± 2.6, $p < .05$; Leeds Stroke Study: 1.84 ± 1.04 vs 2.56 ± 1.50, $p < .001$). A logistic regression analysis of all three cohorts revealed a significantly lower L-hArg/ADMA and L-hArg/SDMA ratio in patients with AF in a crude logistic regression model (model 1: OR 0.70; 95% CI 0.53–0.92 and OR 0.60; 95% CI 0.45–0.80, respectively), but this association did not remain significant after adjustment for age, sex, and vascular risk factors (Fig. 3, Supplementary Table 9).

3.5. Guanidino compounds and risk of stroke recurrence

We analyzed whether compound molar ratios, i.e. L-hArg/ADMA and L-hArg/SDMA, were associated with CHA_2DS_2-VASc score in the UKE cohort, Harburg and Leeds Stroke Studies, which is a well-established risk prediction score used to estimate the annual stroke risk. Spearman correlation analysis revealed significant associations of L-hArg, ADMA, L-hArg/ADMA, SDMA and L-hArg/SDMA with CHA_2DS_2-VASc score in all three stroke cohorts (Table 1). In the Harburg Stroke Study, and Leeds Stroke Study we found the strongest and most significant correlation with CHA_2DS_2-VASc score for L-hArg/SDMA. These data indicate that especially a low L-hArg/SDMA ratio associates with a higher risk of stroke recurrence in patients with established cerebrovascular disease.

4. Discussion

Our results from three cross-sectional studies of cerebrovascular disease indicate that low guanidino compound ratios (i.e. L-hArg/ADMA and L-hArg/SDMA molar ratios) are associated with 1) territorial infarcts, 2) cardio-embolic or large vessel stroke, 3) internal carotid artery stenosis/occlusion, 4) atrial fibrillation and 5) CHA_2DS_2-VASC-score.

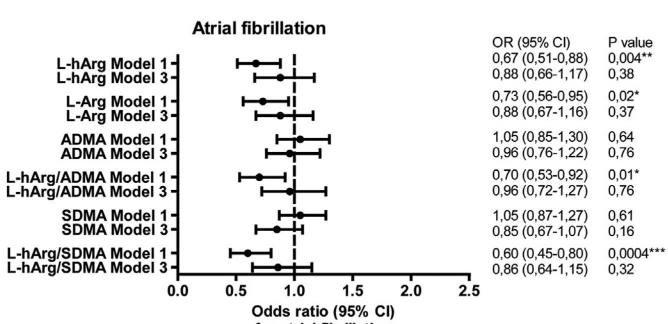

Fig. 3. Associations of guanidino compounds with atrial fibrillation in meta-analysis of UKE cohort, Harburg Stroke Study and Leeds Stroke Study. Logistic regression analyses with odds ratios (OR) with 95% confidence intervals (CI) per SD increase of guanidino compounds for atrial fibrillation (model 1: adjusted for the study cohort; model 3: additionally adjusted for age, sex, hypertension, diabetes, hypercholesterolemia, estimated glomerular filtration rate, and smoking; model 3 was calculated by backward elimination). ADMA indicates asymmetric dimethylarginine; CI, confidence interval; L-Arg, L-arginine; L-hArg, L-homoarginine; OR, odds ratio; SDMA, symmetric dimethylarginine.

4.1. ICA stenosis and atrial fibrillation

Predictive blood-based biomarkers, which could support the diagnostic process to determine stroke subtype and etiology, are currently unavailable. Guanidino compounds and ratios thereof have been previously associated with endothelial (dys)function and atherosclerotic burden in different vascular segments as well as vascular disease [22–26]. Our data now add that a low L-hArg/ADMA and L-hArg/SDMA molar ratio is associated with common carotid artery atherosclerosis and ICA stenosis/occlusion, which underlie ischemic strokes due to larger vessel disease. Compared with L-hArg/ADMA, L-hArg/SDMA was more consistently and stronger associated with ICA stenosis/occlusion and carotid IMT. L-hArg/ADMA and L-hArg/SDMA are both associated with atherosclerotic disease. But in line with our findings, other studies have also revealed associations of SDMA with parameters of atherosclerosis (i.e. IMT, aortic wall thickness), but not with ADMA [6,10]. In addition to large vessel disease, a low L-hArg/ADMA and L-hArg/SDMA molar ratio predicted the presence of atrial fibrillation in all three cross-sectional studies. Correspondingly, a recent study revealed increased ADMA and SDMA plasma concentrations in patients with permanent atrial fibrillation [27]. Interestingly, missense variants of alanine-glyoxylate aminotransferase 2 (AGXT2) – the only enzyme capable of metabolizing all three guanidino compounds at least to some extent, i.e. hArg, ADMA and SDMA – were associated with chronic atrial fibrillation in coronary angiography patients [28]. This finding is in line with our association of L-hArg/ADMA and L-hArg/SDMA with atrial fibrillation and might indicate an involvement of NO bioavailability in the pathogenesis of atrial fibrillation. Taken together, a low L-hArg/ADMA and L-hArg/SDMA is a marker for large vessel disease and possibly atrial fibrillation, which are main causes of ischemic strokes.

4.2. Stroke subtype

In patients with acute ischemic stroke, low L-hArg is a risk factor for territorial infarcts [16]. Even though similar associations of ADMA or SDMA with stroke subtypes are not available, we previously reported a weak association of AGXT2 gene variants with territorial infarcts [29]. The main causes of territorial infarcts are large vessel disease and cardio-embolism. Therefore, it is not surprising that reduced L-hArg/ADMA and L-hArg/SDMA ratios were found in patients with stroke presumably due to large vessel disease or cardio-embolism. No difference was found for ADMA and SDMA only, therefore the effect for L-hArg/ADMA and L-hArg/SDMA was primarily driven by L-hArg. Despite different pathophysiological mechanisms of atrial fibrillation and large vessel disease, L-hArg/ADMA and L-hArg/SDMA were able to predict both. In line with these results, the AGXT2 gene variant rs16899974 was associated with large vessel atherosclerosis, but not with small vessel disease stroke subtype [28].

4.3. CHA_2DS_2-VASc score

In addition to stroke subtype and etiology, precise estimation of stroke recurrence risk is essential for individualized stroke management. Risk prediction is commonly assessed with the CHA_2DS_2-VASc score in patients with atrial fibrillation. Recent studies extend the importance of the CHA_2DS_2-VASc score beyond patients with atrial fibrillation predicting vascular function, thromboembolism and cardiovascular mortality in patients without atrial fibrillation [30–35]. Our data reveal that low L-hArg/ADMA and L-hArg/SDMA are associated with an increased CHA_2DS_2-VASc Score possibly indicating an increased risk for vascular events and death. SDMA plasma

Table 1
Spearman correlation of arginine derivatives with CHA_2DS_2-VASc-Score.

	UKE cohort		Leeds stroke cohort		Harburg stroke cohort	
	Spearman-Rho	P value	Spearman-Rho	P value	Spearman-Rho	P value
L-hArg	−0.13	0.03*	−0.34	6×10^{-12}***	−0.27	0.001**
L-Arg	−0.08	0.18	−0.26	1×10^{-7}***	−0.11	0.21
ADMA	0.17	0.004**	0.09	0.06	0.16	0.07
L-hArg/ADMA	−0.22	3×10^{-4}***	−0.35	2×10^{-12}***	−0.28	0.001**
SDMA	0.32	7×10^{-8}***	0.28	1×10^{-8}***	0.33	8×10^{-5}***
L-hArg/SDMA	−0.27	5×10^{-6}***	−0.41	8×10^{-17}***	−0.37	1×10^{-5}***

ADMA indicates asymmetric dimethylarginine; L-Arg, L-arginine; L-hArg, L-homoarginine; SDMA, symmetric dimethylarginine. *$p < .05$, **$p < .01$, ***$p < .001$.

concentrations increased with CHA_2DS_2-VASc Score in anticoagulated patients with AF in the ARISTOTLE substudy [27]. Given that increased CHA_2DS_2-VASc Score predict vascular events and death, it is not surprising that low L-hArg/SDMA ratios were associated with increased cardiovascular events in patients with lower extremity arterial disease [15]. Therefore, a low L-hArg/SDMA ratio might help to differentiate patients at high risk and proximal embolic stroke. In the light of current research, we suggest that a low L-hArg/SDMA ratio in patients with embolic strokes of undetermined source (ESUS) might therefore be helpful to further differentiate patients with underlying proximal embolic sources and high risk for vascular events.

4.4. Biochemical associations of guanidino compounds

The studied guanidino compounds L-hArg, ADMA and SDMA are all involved in NO metabolism. ADMA inhibits NO synthase and therefore reduces NO availability [36]. Similarly, SDMA competes with L-Arg at the cationic amino acid transporter to enter the cell and subsequent substrate reduction indirectly reduces NO synthesis [37]. Consequently, impaired NO signaling was supposed to underlie the association of ADMA and SDMA with cardiovascular disease. In contrast, L-hArg is a substrate for NO synthase [38]. Furthermore, L-hArg is a competitive inhibitor of arginase, which could indirectly increase substrate availability of the NO synthase [39]. Therefore, the current notion is that L-hArg would facilitate NO availability [2]. Generally, ADMA and SDMA might act antagonistically to L-hArg in the vascular system (Graphical Abstract). Others have also suggested that L-hArg/AMDA and possibly L-hArg/SDMA might be more useful than using a single guanidino compound alone [14].

4.5. Strengths and limitations

The strength of our study is the analysis of three independent cross-sectional studies. Furthermore, one study of patients at cerebrovascular risk and two studies of patients with acute ischemic stroke were included, which broadens the clinical relevance. Even though sample analyses were performed with the same analytical platform, specimen of participants were harvested at different time points and storage conditions were not identical. The limitation of our study is the cross-sectional design, therefore no statement about long-term follow-up and no causal conclusion can be made.

5. Conclusion

In summary, low L-hArg/ADMA and L-hArg/SDMA ratios were associated with territorial infarcts, internal carotid artery stenosis and an increased stroke risk prediction (i.e. CHA_2DS_2-VASc score). Our findings suggest that these novel guanidino compound ratios, especially L-hArg/SDMA, are useful as biomarkers for stroke patients to facilitate diagnosis and prognosis. We conclude that prospective clinical trials are required to establish their significance for patients with cerebrovascular disease and experimental studies are needed to further elucidate the (patho)physiological role of L-hArg/ADMA and L-hArg/SDMA in animal models.

Ethical approval

Ethical approval for this study was obtained from the ethics committee of the medical chamber in Hamburg (PV4715) and Leeds Teaching Hospitals Research Ethics Committee. Written informed consent was obtained from all subjects before the study.

Competing interests

The author(s) declare the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: KC, RG, SL, NL, DA, AN, SH, RB, TM, ES and PJG report no disclosures. DA received funding from the LMU Munich's Institutional Strategy LMUexcellent within the framework of the German Excellence Initiative. GT received fees as a consultant or lecture fees from Acandis, Bayer Vital, Bristol-Myers Squibb/Pfizer, Boehringer Ingelheim, Daichii Sankyo, GlaxoSmithKline, and Stryker, as well as funding from the German Research Foundation and the German Ministry of Science and Research. CG received personal fees and others from Bayer Healthcare and Boehringer Ingelheim, personal fees from Acticor Biotech, Sanofi Aventis Amgene, and Prediction Bioscience, grants from German Research Council, German Ministery of Science and Education, and European Community. CC received lecture fees from Bristol-Myers Squibb/Pfizer and research grants from the Else Kröner Fresenius Foundation and German Research Foundation.

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Authors' contributions

KC, RG, ES and CC recruited patients, acquired, analyzed and interpreted data. ES, PJG and CC conceived, designed and supervised the research. CC wrote the first draft of the manuscript. SL, NL, DA, AN, SH, RB, TM, GT and CG acquired, analyzed and interpreted data. All authors reviewed and edited the manuscript and approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jns.2018.12.037.

References

[1] S. Godo, H. Shimokawa, Endothelial functions, Arterioscler. Thromb. Vasc. Biol. 37 (9) (2017) e108–e114.
[2] D. Atzler, E. Schwedhelm, C.U. Choe, L-homoarginine and cardiovascular disease, Curr. Opin. Clin. Nutr. Metab. Care 18 (1) (2015) 83–88.
[3] E. Schwedhelm, R.H. Boger, The role of asymmetric and symmetric dimethylarginines in renal disease, Nat. Rev. Nephrol. 7 (5) (2011) 275–285.
[4] D. Tousoulis, R.H. Boger, C. Antoniades, G. Siasos, E. Stefanadi, C. Stefanadis, Mechanisms of disease: L-arginine in coronary atherosclerosis—a clinical perspective, Nat. Clin. Pract. Cardiovasc. Med. 4 (5) (2007) 274–283.
[5] P.W. Nanayakkara, T. Teerlink, C.D. Stehouwer, D. Allajar, A. Spijkerman, C. Schalkwijk, P.M. ter Wee, C. van Guldener, Plasma asymmetric dimethylarginine (ADMA) concentration is independently associated with carotid intima-media thickness and plasma soluble vascular cell adhesion molecule-1 (sVCAM-1) concentration in patients with mild-to-moderate renal failure, Kidney Int. 68 (5) (2005) 2230–2236.
[6] M. Bahls, N. Friedrich, D. Atzler, S.B. Felix, M.A. Nauck, R.H. Boger, H. Volzke, E. Schwedhelm, M. Dorr, L-Arginine and SDMA serum concentrations are associated with subclinical atherosclerosis in the Study of Health in Pomerania (SHIP), PLoS One 10 (6) (2015) e0131293.
[7] N. Lüneburg, R.A. von Holten, R.F. Topper, E. Schwedhelm, R. Maas, R.H. Böger, Symmetric dimethylarginine is a marker of detrimental outcome in the acute phase after ischaemic stroke: role of renal function, Clin. Sci. (Lond.) 122 (3) (2012) 105–111.
[8] I. Kanazawa, S. Yano, Y. Notsu, T. Yamaguchi, T. Nabika, T. Sugimoto, Asymmetric dimethylarginine as a risk factor for cardiovascular disease in Japanese patients with type 2 diabetes mellitus, Clin. Endocrinol. 74 (4) (2011) 467–472.
[9] D. Atzler, M.O. Gore, C.R. Ayers, C.U. Choe, R.H. Boger, J.A. de Lemos, D.K. McGuire, E. Schwedhelm, Homoarginine and cardiovascular outcome in the population-based Dallas heart study, Arterioscler. Thromb. Vasc. Biol. 34 (11) (2014) 2501–2507.
[10] M.O. Gore, N. Luneburg, E. Schwedhelm, C.R. Ayers, M. Anderssohn, A. Khera, D. Atzler, J.A. de Lemos, P.J. Grant, D.K. McGuire, R.H. Boger, Symmetrical dimethylarginine predicts mortality in the general population: observations from the Dallas heart study, Arterioscler. Thromb. Vasc. Biol. 33 (11) (2013) 2682–2688.
[11] C.M.C. Mels, A.E. Schutte, H.W. Huisman, W. Smith, R. Kruger, J.M. van Rooyen, E. Schwedhelm, D. Atzler, R.H. Boger, N.T. Malan, L. Malan, Asymmetric dimethylarginine and symmetric dimethylarginine prospectively relates to carotid wall thickening in black men: the SABPA study, Amino Acids 49 (11) (2017) 1843–1853.
[12] S. Schlesinger, S.R. Sonntag, W. Lieb, R. Maas, Asymmetric and symmetric dimethylarginine as risk markers for total mortality and cardiovascular outcomes: a systematic review and meta-analysis of prospective studies, PLoS One 11 (11) (2016) e0165811.
[13] A. Haghikia, G.R. Yanchev, A.A. Kayacelebi, E. Hanff, N. Bledau, C. Widera, K. Sonnenschein, A. Haghikia, K. Weissenborn, J. Bauersachs, U. Bavendiek, D. Tsikas, The role of L-arginine/L-homoarginine/nitric oxide pathway for aortic distensibility and intima-media thickness in stroke patients, Amino Acids 49 (6) (2017) 1111–1121.
[14] D. Tsikas, A.A. Kayacelebi, Do homoarginine and asymmetric dimethylarginine act antagonistically in the cardiovascular system? Circ. J. 78 (8) (2014) 2094–2095.
[15] P. Jud, F. Hafner, N. Verheyen, A. Meinitzer, T. Gary, M. Brodmann, G. Seinost, G. Hackl, Homoarginine/ADMA ratio and homoarginine/SDMA ratio as independent predictors of cardiovascular mortality and cardiovascular events in lower extremity arterial disease, Sci. Rep. 8 (1) (2018) 14197.
[16] C.U. Choe, D. Atzler, P.S. Wild, A.M. Carter, R.H. Boger, F. Ojeda, O. Simova, M. Stockebrand, K. Lackner, C. Nabuurs, B. Marescau, T. Streichert, C. Muller, N. Luneburg, P.P. De Deyn, R.A. Benndorf, S. Baldus, C. Gerloff, S. Blankenberg, A. Heerschap, P.J. Grant, T. Magnus, T. Zeller, D. Isbrandt, E. Schwedhelm, Homoarginine levels are regulated by L-arginine:glycine amidinotransferase and affect stroke outcome: results from human and murine studies, Circulation 128 (13) (2013) 1451–1461.
[17] F. Schulze, A.M. Carter, E. Schwedhelm, R. Ajjan, R. Maas, R.A. von Holten, D. Atzler, P.J. Grant, R.H. Boger, Symmetric dimethylarginine predicts all-cause mortality following ischemic stroke, Atherosclerosis 208 (2) (2010) 518–523.
[18] D. Atzler, M. Mieth, R. Maas, R.H. Böger, E. Schwedhelm, Stable isotope dilution assay for liquid chromatography-tandem mass spectrometric determination of L-homoarginine in human plasma, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 879 (23) (2011) 2294–2298.
[19] E. Schwedhelm, R. Maas, J. Tan-Andresen, F. Schulze, U. Riederer, R.H. Böger, High-throughput liquid chromatographic-tandem mass spectrometric determination of arginine and dimethylated arginine derivatives in human and mouse plasma, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 851 (1–2) (2007) 211–219.
[20] P.J. Touboul, M.G. Hennerici, S. Meairs, H. Adams, P. Amarenco, N. Bornstein, L. Csiba, M. Desvarieux, S. Ebrahim, R. Hernandez Hernandez, M. Jaff, S. Kownator, T. Naqvi, P. Prati, T. Rundek, M. Sitzer, U. Schminke, J.C. Tardif, A. Taylor, E. Vicaut, K.S. Woo, Mannheim carotid intima-media thickness and plaque consensus (2004-2006-2011). An update on behalf of the advisory board of the 3rd, 4th and 5th watching the risk symposia, at the 13th, 15th and 20th European Stroke Conferences, Mannheim, Germany, 2004, Brussels, Belgium, 2006, and Hamburg, Germany, 2011, Cerebrovasc. Dis. 34 (4) (2012) 290–296.
[21] M. Rosenkranz, A. Russjan, E. Goebell, S. Havemeister, G. Thomalla, B. Cheng, C. Beck, A. Krutzelmann, J. Fiehler, C. Gerloff, Carotid plaque surface irregularity predicts cerebral embolism during carotid artery stenting, Cerebrovasc. Dis. 32 (2) (2011) 163–169.
[22] M. Juonala, J.S. Viikari, G. Alfthan, J. Marniemi, M. Kahonen, L. Taittonen, T. Laitinen, O.T. Raitakari, Brachial artery flow-mediated dilation and asymmetrical dimethylarginine in the cardiovascular risk in young Finns study, Circulation 116 (12) (2007) 1367–1373.
[23] W. März, A. Meinitzer, C. Drechsler, S. Pilz, V. Krane, M.E. Kleber, J. Fischer, B.R. Winkelmann, B.O. Bohm, E. Ritz, C. Wanner, Homoarginine, cardiovascular risk, and mortality, Circulation 122 (10) (2010) 967–975.
[24] R. Schnabel, S. Blankenberg, E. Lubos, K.J. Lackner, H.J. Rupprecht, C. Espinola-Klein, N. Jachmann, F. Post, D. Peetz, C. Bickel, F. Cambien, L. Tiret, T. Munzel, Asymmetric dimethylarginine and the risk of cardiovascular events and death in patients with coronary artery disease: results from the AtheroGene Study, Circ. Res. 97 (5) (2005) e53–e59.
[25] P. Valtonen, T. Laitinen, T. Lyyra-Laitinen, O.T. Raitakari, M. Juonala, J.S. Viikari, N. Heiskanen, E. Vanninen, K. Punnonen, S. Heinonen, Serum L-homoarginine concentration is elevated during normal pregnancy and is related to flow-mediated vasodilatation, Circ. J. 72 (11) (2008) 1879–1884.
[26] L. Vogl, J. Pohlhammer, A. Meinitzer, B. Rantner, M. Stadler, S. Peric, A. Hammerer-Lercher, P. Klein-Weigel, G. Fraedrich, F. Kronenberg, B. Kollerits, Serum concentrations of L-arginine and L-homoarginine in male patients with intermittent claudication: a cross-sectional and prospective investigation in the CAVASIC Study, Atherosclerosis 239 (2) (2015) 607–614.
[27] J.D. Horowitz, R. De Caterina, T. Heresztyn, J.H. Alexander, U. Andersson, R.D. Lopes, P.G. Steg, E.M. Hylek, P. Mohan, M. Hanna, P. Jansky, C.B. Granger, L. Wallentin, A. Investigators, Asymmetric and symmetric dimethylarginine predict outcomes in patients with atrial fibrillation: an ARISTOTLE Substudy, J. Am. Coll. Cardiol. 72 (7) (2018) 721–733.
[28] I. Seppala, M.E. Kleber, S. Bevan, L.P. Lyytikainen, N. Oksala, J.A. Hernesniemi, K.M. Makela, P.M. Rothwell, C. Sudlow, M. Dichgans, N. Mononen, E. Vlachopoulou, J. Sinisalo, G.E. Delgado, R. Laaksonen, T. Koskinen, H. Scharnagl, M. Kahonen, H.S. Markus, W. Marz, T. Lehtimaki, Associations of functional alanine-glyoxylate aminotransferase 2 gene variants with atrial fibrillation and ischemic stroke, Sci. Rep. 6 (2016) 23207.
[29] N. Luneburg, W. Lieb, T. Zeller, M.H. Chen, R. Maas, A.M. Carter, V. Xanthakis, N.L. Glazer, E. Schwedhelm, S. Seshadri, M.A. Ikram, W.T. Longstreth Jr., M. Fornage, I.R. Konig, C. Loley, F.M. Ojeda, A. Schillert, T.J. Wang, H. Sticht, A. Kittel, J. Konig, E.J. Benjamin, L.M. Sullivan, I. Bernges, M. Anderssohn, A. Ziegler, C. Gieger, T. Illig, C. Meisinger, H.E. Wichmann, P.S. Wild, H. Schunkert, B.M. Psaty, K.L. Wiggins, S.R. Heckbert, N. Smith, K. Lackner, K.L. Lunetta, S. Blankenberg, J. Erdmann, T. Munzel, P.J. Grant, R.S. Vasan, R.H. Boger, Genome-wide association study of L-arginine and dimethylarginines reveals novel metabolic pathway for symmetric dimethylarginine, Circ. Cardiovasc. Genet. 7 (6) (2014) 864–872.
[30] L. Melgaard, A. Gorst-Rasmussen, D.A. Lane, L.H. Rasmussen, T.B. Larsen, G.Y. Lip, Assessment of the CHA2DS2-VASc score in predicting ischemic stroke, thromboembolism, and death in patients with heart failure with and without atrial fibrillation, JAMA 314 (10) (2015) 1030–1038.
[31] L.B. Mitchell, D.A. Southern, D. Galbraith, W.A. Ghali, M. Knudtson, S.B. Wilton, the APPROACH investigators, for, Prediction of stroke or TIA in patients without atrial fibrillation using CHADS2 and CHA2DS2-VASc scores, Heart 100 (19) (2014) 1524–1530.
[32] G. Ntaios, G.Y. Lip, D. Lambrou, V. Papavasileiou, E. Manios, H. Milionis, K. Spengos, K. Makaritsis, K. Vemmos, Leukoaraiosis and stroke recurrence risk in patients with and without atrial fibrillation, Neurology 84 (12) (2015) 1213–1219.
[33] G. Ntaios, G.Y. Lip, K. Makaritsis, V. Papavasileiou, A. Vemmou, E. Koroboki, P. Savvari, E. Manios, H. Milionis, K. Vemmos, CHADS(2), CHA(2)S(2)DS(2)-VASc, and long-term stroke outcome in patients without atrial fibrillation, Neurology 80 (11) (2013) 1009–1017.
[34] G. Ntaios, K. Vemmos, G.Y. Lip, E. Koroboki, E. Manios, A. Vemmou, A. Rodriguez-Campello, E. Cuadrado-Godia, E. Giralt-Steinhauer, V. Arnao, V. Caso, M. Paciaroni, E. Diez-Tejedor, B. Fuentes, J. Perez Lucas, A. Arauz, S.F. Ameriso, M.A. Hawkes, L. Pertierra, M. Gomez-Schneider, F. Bandini, B. Chavarria Cano, A.M. Iglesias Mohedano, A. Garcia Pastor, A. Gil-Nunez, J. Putaala, T. Tatlisumak, M.A. Barboza, G. Athanasakis, K. Makaritsis, V. Papavasileiou, Risk stratification for recurrence and mortality in embolic stroke of undetermined source, Stroke 47 (9) (2016) 2278–2285.
[35] K.M. Henriksson, B. Farahmand, S. Asberg, N. Edvardsson, A. Terent, Comparison of cardiovascular risk factors and survival in patients with ischemic or hemorrhagic stroke, Int. J. Stroke 7 (4) (2012) 276–281.
[36] R.H. Boger, Asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthase, explains the "L-arginine paradox" and acts as a novel cardiovascular risk factor, J. Nutr. 134 (10 Suppl) (2004) 2842S–2847S (discussion 2853S).
[37] E.I. Closs, F.Z. Basha, A. Habermeier, U. Forstermann, Interference of L-arginine analogues with L-arginine transport mediated by the y+ carrier hCAT-2B, Nitric Oxide 1 (1) (1997) 65–73.
[38] A. Hrabak, T. Bajor, A. Temesi, Comparison of substrate and inhibitor specificity of arginase and nitric oxide (NO) synthase for arginine analogues and related compounds in murine and rat macrophages, Biochem. Biophys. Res. Commun. 198 (1) (1994) 206–212.
[39] C.W. Lin, W.H. Fishman, L-Homoarginine. An organ-specific, uncompetitive inhibitor of human liver and bone alkaline phosphohydrolases, J. Biol. Chem. 247 (10) (1972) 3082–3087.