Genetic and Environmental Determinants of Dimethylarginines and Association With Cardiovascular Disease in Patients With Type 2 Diabetes

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OBJECTIVE

To investigate determinants of asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA), including single nucleotide polymorphisms (SNPs), in the *DDAH1*, *DDAH2*, and *AGXT2* genes and their associations with prevalent and incident cardiovascular disease (CVD) in older adults with type 2 diabetes mellitus.

RESEARCH DESIGN AND METHODS

Prevalent CVD was assessed in men and women aged 60–75 years with type 2 diabetes as part of the Edinburgh Type 2 Diabetes Study (ET2DS), and the participants were prospectively followed up for 4 years for incident CVD. Dimethylarginines were measured in 783 of these subjects, and genotyping for tag SNPs in the *DDAH1*, *DDAH2*, and *AGXT2* genes was performed in 935 subjects.

RESULTS

Plasma ADMA levels were significantly associated with SNPs in *DDAH1* (top SNP rs1554597; P = 9.0E-09), while SDMA levels were associated with SNPs in *AGXT2* (top SNP rs28305; P = 1.3E-04). Significant, independent determinants of plasma ADMA were sex, L-arginine, creatinine, fasting glucose, and rs1554597 (all P < 0.05; combined R^2 = 0.213). Determinants of SDMA were age, sex, creatinine, L-arginine, diabetes duration, prevalent CVD, and rs28305 (all P < 0.05; combined R^2 = 0.425). Neither dimethylarginine was associated with incident CVD. None of the investigated SNPs were associated with overall CVD, although subgroup analysis revealed a significant association of *AGXT2* rs28305 with intermittent claudication.

CONCLUSIONS

Our study in a well-characterized population with type 2 diabetes does not support reported associations or causal relationship between ADMA and features of diabetes or CVD.

Diabetes Care 2014;37:846–854 | DOI: 10.2337/dc13-0546

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Received 5 March 2013 and accepted 27 October 2013.

Asymmetric dimethylarginine (ADMA) is considered an independent risk factor for cardiovascular (CV) events and mortality in patients with a range of CV risk (1). Although ADMA plasma levels were reported to be elevated in patients with type 2 diabetes (2) and insulin resistance (3), there are discrepancies concerning the association of ADMA with glycemic control and its prognostic value regarding CV disease (CVD) in diabetes patients (4–8). Results from prospective studies reporting that high levels of ADMA predicted CV events in patients with diabetes mellitus (9,10) could not be confirmed in population-based prospective cohorts (7). A possible explanation for these conflicting results may be that activity of the enzyme responsible for degradation of ADMA, dimethylarginine dimethylaminohydrolase (DDAH), is influenced by diabetes and diabetes therapy (11,12). Variation in the *DDAH1* and/or *DDAH2* genes were associated with differences in ADMA levels in diabetes subjects (13) and with insulin sensitivity in nondiabetic subjects (14) and predicted major CV events in diabetes subjects (15).

Recent evidence indicates that symmetric dimethylarginine (SDMA) might also play a role in diabetes mellitus and CVD. In patients with type 2 diabetes mellitus, SDMA was inversely associated with glycemic control and with insulin resistance in nondiabetic Caucasians (5,16). Furthermore, prospective studies found that SDMA was an independent predictor for mortality in patients with CVD (17,18). While the majority of ADMA is metabolized by DDAH, SDMA is mainly cleared via the kidneys. However, both dimethylarginines can also be degraded by the enzyme alanine–glyoxylate aminotransferase (AGXT2) (19,20).

There appears to be a complex interaction between dimethylarginines, the genes involved in their metabolism, glycemic control, and CVD in patients with type 2 diabetes mellitus. Therefore, the aims of this study were to *1*) investigate determinants of dimethylarginine plasma levels in a representative sample of older men and women with type 2 diabetes mellitus; *2*) explore the influence of single nucleotide polymorphisms (SNPs) in the *DDAH1*, *DDAH2*, and *AGXT2* genes on plasma levels of dimethylarginines; and *3*) determine whether plasma dimethylarginines and/or dimethylarginine-related SNPs are associated with pre-existing or incident CVD in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Study Population

The Edinburgh Type 2 Diabetes Study (ET2DS) is a prospective, population-based cohort study of 1,066 men and women aged 60–75 years with established type 2 diabetes mellitus recruited between 2006 and 2007 in the Lothian region of Scotland, U.K. The design of the ET2DS and details of the physical examination undertaken at baseline and 1 year after recruitment have been described previously (21). The ET2DS population has been shown to be representative of patients aged 60–75 years with type 2 diabetes living in Lothian (22), and subjects returning after 1 year (n = 939) are similar to the original study population (23). Genotyping for the current analysis was performed in participants attending the 1-year examination using DNA extracted from whole blood samples taken at baseline (n = 935), and plasma L-arginine and dimethylarginines were measured in 783 of these subjects (data missing at random due to insufficient plasma in stored aliquots). All ET2DS participants were followed up after 4 years for CV events (in 2010–2011). Use of routine data sources (record linkage) and general practitioner (GP)/hospital notes as well as direct patient contact ensured that follow-up for CVD events included all ET2DS participants.

Determination of Dimethylarginines

Mass spectrometric determination of L-arginine, ADMA, and SDMA plasma samples was performed as described by Schwedhelm et al. using a validated high-throughput liquid chromatography–mass spectrometry/mass spectrometry assay (24).

Assessment of CVD Events

Prevalent CVD at baseline was recorded according to predefined criteria using a combination of subject report of a doctor diagnosis of disease, positive World Health Organization (WHO) chest pain questionnaire, and positive Edinburgh Claudication questionnaire and electrocardiogram (ECG) findings (Minnesota coded) together with hospital discharge codes from record linkage at the Information and Statistics Division of the National Health Service for Scotland, details of which have been described previously (22). Four years after recruitment, participants were followed up for new CV events using a combination of repeat self-completion (or GP) questionnaire, repeat Information and Statistics Division record linkage for hospital discharge and death certificate data (both ICD-10 coded), and review of clinical case notes as required. Criteria for fatal and nonfatal events were as follows: myocardial infarction (MI), *1*) ICD-10 code for new MI on hospital discharge/death record, dated after baseline, plus either subject report of a doctor diagnosis of MI, positive WHO chest pain questionnaire for MI, report of MI on GP questionnaire (all with date consistent with ICD-10-coded event), or ECG codes for MI that were not present at baseline or *2*) clinical criteria for MI met following scrutiny of hospital and/or GP notes; new angina (in subjects without a diagnosis of angina at baseline), *1*) ICD-10 code for angina as primary diagnosis on hospital discharge record, dated after baseline, *2*) at least two of (a) subject report of a doctor diagnosis (self-report) of angina or of starting angina medication since baseline, (b) ECG codes for ischemia that were not present at baseline, and (c) positive WHO chest pain questionnaire, or *3*) clinical diagnosis of angina on scrutiny of hospital notes; stroke, *1*) ICD-10 code for stroke as primary diagnosis on hospital discharge/death record, dated after baseline, or *2*) clinical criteria for stroke met on scrutiny of clinical notes in subjects with either self-report of stroke or with nonprimary ICD-10 hospital discharge/death code for stroke; transient ischemic attack (TIA), *1*) ICD-10 code for TIA as primary diagnosis on hospital discharge record or *2*) clinical criteria for TIA met on scrutiny of clinical notes in subjects with either self-report of stroke or with nonprimary ICD-10 hospital discharge code for stroke or TIA.

Genotyping

Genotyping was performed at the Genetics Core of the Welcome Trust Clinical Research Facility in Edinburgh. SNPs of the *DDAH1* gene, the *DDAH2* gene, and the *AGXT2* gene were determined using the TaqMan OpenArray Genotyping System (Applied Biosystems Inc., Carlsbad, CA). The sample success rate and SNP success rate were 96.5% and 100%, respectively. In total, 96.5% of the genotypes could be determined. Using the tagger algorithm implemented in Haploview 4 (25), tag SNPs across *DDAH1*, *DDAH2*, and *AGXT2* genes, including the known or expected promoter regions, were selected. The selection was based on the linkage disequilibrium patterns observed in Caucasian samples genotyped within the International HapMap Project. From each linkage disequilibrium block identified, 2–3 tag SNPs with an r^2 of at least 0.8 were chosen to cover the whole locus, including SNPs reported to be associated with diabetes (rs2474123 and rs2935) (13). Linkage equilibrium was assessed using the locus zoom software. Haplotypes were generated using the THESIAS software and analyzed for possible effects. For further analyses, only those haplotypes with an estimated effect were considered.

Data Analysis

Normality of L-arginine and dimethylarginines was assessed using the Kolmogorov–Smirnov test. L-arginine, SDMA, and ADMA were naturally log transformed for parametric tests. One L-arginine value of 252.7 μmol/L was excluded as an outlier. Differences between groups were calculated using the χ^2 test for categorical data and the *t* test, Mann–Whitney test, or Kruskal–Wallis test for continuous data. Nonparametric Spearman's correlation coefficients and multiple linear regression analysis with stepwise backward selection were used to assess determinants of dimethylarginines. To determine the associations of clinical variables and SNPs with prevalent and incident CVD, logistic regression analyses were performed. To correct for multiple testing in the SNP analyses, Bonferroni correction was used, which set the *P* value for statistical significance at $P \leq 0.0021$. A *P* value of <0.05 was considered statistically significant for all other analyses. Statistical analyses were performed using SPSS 17.0/19.0 for Windows (SPSS Inc., Chicago, IL).

RESULTS

Dimethylarginines and Clinical Characteristics

Clinical characteristics of participants attending the year 1 research clinic (the total study population used in the current analysis; *n* = 939) and of the study population with plasma dimethylarginine available (*n* = 783) together with their associations with dimethylarginines are summarized in Table 1.

Association of Plasma Dimethylarginines With CVD

Median (interquartile range [IQR]) plasma ADMA levels were statistically significantly higher in subjects with history of stroke (0.50 [0.40–0.57] vs. 0.45 [0.39–0.51] μmol/L; $P = 0.01$), but no further associations with CVD were apparent. Significantly higher median (IQR) plasma SDMA levels were found in subjects with MI (0.55 [0.46–0.66] vs. 0.48 [0.41–0.57] μmol/L; $P < 0.001$), angina (0.52 [0.43–0.64] vs. 0.48 [0.41–0.56] μmol/L; $P < 0.001$), stroke (0.57

Table 1—Clinical characteristics of the population at year 1 visit (unless otherwise indicated) and Spearman correlations with dimethylarginines

	Entire year 1 cohort (*n* = 939)		Dimethylarginine subset (*n* = 783)				
				Correlation with ADMA		Correlation with SDMA	
	Max *n*	Mean ± SD	Mean ± SD, median (IQR)	*R*	*P*	*R*	*P*
Age (years)	939	68.9 ± 4.2	68.8 ± 4.2	0.040	0.26	***0.234***	***<0.001***
Female (*n*, %)	939	451 (48.0)	373 (47.6)	0.065	0.07	−0.053	0.14
Diabetes duration (years)	931	9.0 ± 6.4	8.9 ± 6.3	0.030	0.40	0.168	<0.001
BMI at baseline (kg/m^2)	938	31.3 ± 5.7	31.4 ± 5.7	0.039	0.28	−0.069	0.052
Waist–hip ratio at baseline	935	0.97 ± 0.08	0.97 ± 0.07	−0.019	0.59	0.044	0.22
SBP (mmHg)	933	138.1 ± 18.5	138.4 ± 18.5	0.027	0.45	−0.007	0.84
DBP (mmHg)	933	74.1 ± 9.6	74.4 ± 9.5	−0.003	0.92	***−0.158***	***<0.001***
Fasting glucose (mmol/L)	924	6.87 ± 2.31	6.88 ± 2.30	***0.106***	***0.003***	−0.036	0.31
HbA_{1c} % (mmol/mol)	927	7.19 ± 1.07 (55 ± 11.7)	7.26 ± 1.06 (56 ± 11.6)	−0.014	0.70	−0.030	0.40
Total cholesterol (mmol/L)	669	4.14 ± 0.79	4.15 ± 0.81	0.021	0.63	−0.035	0.42
HDL (mmol/L)	669	1.27 ± 0.35	1.27 ± 0.34	0.006	0.89	−0.059	0.18
Triglyceride (mmol/L)	668	1.66 ± 0.94	1.65 ± 0.96	−0.049	0.27	−0.024	0.58
Creatinine at baseline (μmol/L)	931	89.1 ± 31.1	88.6 ± 29.1	***0.143***	***<0.001***	***0.541***	***<0.001***
L-arginine (μmol/L)	783		63.0 (52.4–75.6)	***0.296***	***<0.001***	***0.199***	***<0.001***
ADMA (μmol/L)	783		0.45 (0.39–0.51)	N/A		***0.397***	***<0.001***
SDMA (μmol/L)	782		0.49 (0.42–0.59)	***0.397***	***<0.001***	N/A	

Data are displayed as mean ± SD, median (IQR), or frequency (%). SBP, systolic blood pressure; DBP, diastolic blood pressure. Boldface and italicized data indicate significant correlations. N/A, not applicable.

[0.48–0.74] vs. 0.49 [0.42–0.58] μmol/L; $P < 0.001$), and intermittent claudication (0.56 [0.45–0.70] vs. 0.49 [0.42–0.58] μmol/L; $P = 0.004$), with a maximum difference of 0.08 μmol/L between subjects with and without stroke. Neither of the dimethylarginines was significantly associated with categories of diabetes treatment (diet-only treatment, oral agents only, or insulin therapy), although a trend toward slightly higher SDMA levels in subjects with insulin treatment was apparent (median [IQR] SDMA 0.52 [0.42–0.66] vs. 0.49 [0.42–0.58]; $P = 0.051$). Associations of ADMA and SDMA with prevalent CVD lost statistical significance after adjusting for age, sex, and renal function. In the prospective analysis, neither of the dimethylarginines was significantly associated with incident coronary artery disease or cerebrovascular disease (Table 2).

Association of Dimethylarginines With SNPs

Plasma ADMA was related to SNPs in the *DDAH1* gene but not to SNPs in the *DDAH2* or *AGXT2* genes, whereas plasma SDMA was related to SNPs in the *AGXT2* gene only (Table 3). SNPs that were significantly associated with dimethylarginines showed an increase in plasma levels of the respective dimethylarginine from the major to the minor allele, with a maximum increase in ADMA of 0.06 μmol/L for the *DDAH1* rs1554597 SNP and a maximum increase of SDMA of 0.17 μmol/L for the *AGXT2* rs28305 SNP.

Determinants of Plasma Dimethylarginines

In univariate regression analysis, SDMA was significantly associated with age, sex, creatinine, L-arginine, diastolic blood pressure, waist–hip ratio, diabetes duration, and prevalent CVD, and ADMA was significantly associated with age, sex, creatinine, fasting glucose, L-arginine, and triglycerides. SNPs were entered into the regression models individually according to *P* value and as common haplotypes restricted to SNPs under linkage equilibrium (*DDAH1*: rs1554597, rs11161614, rs1146381, and the TTG haplotype; *AGXT2*: rs28305, rs16899974, rs344156, and the GCG haplotype). Table 4 displays the final models for the multivariate analyses. Addition of further SNPs or analysis by haplotypes did not improve the overall models.

Association of SNPs With CVD

The distribution of genotypes did not differ significantly between subjects with or without prevalent CVD for the SNPs we investigated (Table 3). Only weak associations between SNPs in the *DDAH1* and *AGXT2* genes and CVD were apparent in the logistic regression analysis, and these did not reach statistical significance after correction for multiple testing. In subgroup analyses, carriers of the *AGXT2* rs28305 C allele more frequently had a history of intermittent claudication. In the analysis of this SNP according to genotype, the frequency of prevalent intermittent claudication was 4.6% in the GG genotype, 11.9% in the GC genotype, and 33.3% in the CC genotype ($P < 0.001$). In the allelic analysis, intermittent claudication was present in 12.5% of the carriers of the C allele as compared with 5.4% in carriers of the G allele ($P < 0.001$). The age-, sex-, and creatinine-adjusted odds ratio (95% CI) for carriers of the C allele was 2.50 (1.56–4.01) ($P < 0.001$) and the odds ratio for the GC genotype versus GG genotype was 2.80 (1.60–4.92) ($P < 0.001$) and the CC versus GG genotype was 12.86 (1.10–150.05) ($P = 0.04$).

CONCLUSIONS

In our cohort of older adults with type 2 diabetes, we observed a lack of association of dimethylarginines or SNPs in the genes of their metabolizing enzymes with prevalent or incident CVD after adjustment for relevant covariates. However, subgroup analysis revealed an interaction between plasma SDMA levels, the *AGXT2* rs28305 SNP, and intermittent claudication. Furthermore, we observed strong association of SNPs in the *DDAH1* gene with plasma levels of ADMA and of SNPs in the *AGXT2* gene with SDMA.

Associations With CVD

The lack of association of ADMA with CVD in our cohort is in contrast to a number of previous studies. Krzyzanowska et al. reported that elevated ADMA levels were associated with macrovascular disease in diabetic patients (26). Furthermore, ADMA was predictive of future CV events and all-cause mortality in two different cohorts of diabetes patients (9,10). However,

Table 2—Associations of dimethylarginines with CVD

		ADMA				SDMA			
		Unadjusted		Adjusted		Unadjusted		Adjusted	
	CVD (*n*)	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*	OR (95% CI)	*P*
Cross-sectional	Prevalent								
CVD	285	1.22 (0.62–2.40)	0.56	0.93 (0.46–1.90)	0.85	3.21 (1.86–5.52)	<0.001	1.53 (0.76–3.08)	0.23
Coronary artery disease	236	1.00 (0.49–2.03)	0.99	0.74 (0.35–1.55)	0.42	2.88 (1.65–5.04)	<0.001	1.20 (0.58–2.47)	0.63
Cerebrovascular disease	71	3.53 (1.07–11.67)	0.04	2.28 (0.67–7.74)	0.19	3.67 (1.59–8.47)	0.002	1.45 (0.48–4.39)	0.51
Prospective	Incident								
CVD	99	2.80 (0.85–9.23)	0.09	2.23 (0.66–7.48)	0.20	2.02 (0.85–4.81)	0.11	0.84 (0.28–2.53)	0.76
Coronary artery disease	64	2.50 (0.58–10.88)	0.22	2.14 (0.48–9.55)	0.32	1.33 (0.45–3.96)	0.61	0.78 (0.2–3.06)	0.72
Cerebrovascular disease	42	3.17 (0.54–18.78)	0.20	2.24 (0.35–14.30)	0.39	2.96 (0.86–10.25)	0.09	0.59 (0.11–3.01)	0.52

Data are displayed as odds ratios and 95% CIs unadjusted and adjusted for age, sex, creatinine, and, in case of prospective analysis, prevalent event. *n* is the number of individuals with prevalent disease (cross-sectional) or incident event (prospective). CVD includes MI, angina, stroke, TIA, or intermittent claudication (cross-sectional) or MI, TIA, stroke, or new onset of angina (prospective). Coronary artery disease includes MI and/or angina (cross-sectional and prospective). Cerebrovascular disease includes stroke and/or TIA (cross-sectional and prospective). OR, odds ratio.

Table 3—SNP in the *DDAH1* and *AGXT2* genes and their associations with ADMA/SDMA plasma levels and prevalent CVD

			Association with ADMA/SDMA (max *n* = 770)		Association with CVD (max *n* = 920)		Odds ratio (95% CI) for CVD			
SNP ID	MAF	Genotype	Median (IQR) ADMA (μmol/L)	*P*	No CVD, *n* (%)	CVD, *n* (%)	Genotypic	*P*	Major vs. minor allele	*P*
DDAH1										
rs1554597	0.388 (C)	TT	0.43 (0.37–0.49)	9.0E-09	221 (39.5)	113 (35.6)	1.00	—	1.16 (0.95–1.41)	0.15
		TC	0.46 (0.39–0.52)		258 (46.1)	148 (46.7	1.12 (0.83–1.52)	0.46		
		CC	0.49 (0.43–0.54)		81 (14.5)	56 (17.7)	1.35 (0.90–2.04)	0.15		
rs2268667	0.375 (A)	GG	0.43 (0.37–0.49)	5.4E-08	237 (40.8)	121 (37.1)	1.00	—	1.16 (0.95–1.41)	0.14
		GA	0.46 (0.39–0.52)		267 (46.0)	151 (46.3)	1.11 (0.82–1.49)	0.50		
		AA	0.49 (0.43–0.54)		77 (13.3)	54 (16.6)	1.37 (0.91–2.07)	0.13		
rs233118	0.388 (C)	TT	0.43 (0.37–0.49)	7.3E-08	227 (39.1)	113 (34.9)	1.00	—	1.18 (0.97–1.44)	0.10
		TC	0.46 (0.40–0.52)		272 (46.9)	154 (47.5)	1.14 (0.84–1.54)	0.40		
		CC	0.49 (0.43–0.54)		81 (14.0)	57 (17.6)	1.41 (0.94–2.12)	0.10		
rs18582	0.380 (G)	AA	0.43 (0.37–0.49)	9.1E-08	233 (39.8)	119 (36.4)	1.00	—	1.13 (0.93–1.38)	0.21
		AG	0.46 (0.39–0.52)		273 (46.6)	155 (47.4)	1.11 (0.83–1.50)	0.48		
		GG	0.49 (0.43–0.53)		80 (13.7)	53 (16.2)	1.30 (0.86–1.96)	0.22		
rs233093	0.373 (A)	GG	0.43 (0.37–0.49)	1.0E-07	226 (40.3)	118 (37.1)	1.00	—	1.13 (0.93–1.38)	0.23
		GA	0.46 (0.40–0.52)		263 (46.9)	151 (47.5)	1.10 (0.82–1.48)	0.53		
		AA	0.49 (0.43–0.54)		72 (12.8)	49 (15.4)	1.30 (0.85–2.00)	0.22		
rs233112	0.397 (C)	TT	0.43 (0.37–0.49)	5.4E-07	208 (37.3)	112 (34.6)	1.00	—	1.13 (0.92–1.37)	0.24
		CT	0.46 (0.39–0.51)		267 (47.9)	155 (47.8)	1.08 (0.80–1.36)	0.63		
		CC	0.49 (0.42–0.54)		82 (14.7)	57 (17.6)	1.29 (0.86–1.94)	0.22		
rs11161606	0.426 (C)	TT	0.44 (0.37–0.49)	1.6E-06	194 (34.2)	99 (30.7)	1.00	—	1.10 (0.90–1.34)	0.35
		CT	0.45 (0.39–0.51)		272 (48.0)	162 (50.3)	1.17 (0.86–1.59)	0.33		
		CC	0.49 (0.43–0.53)		101 (17.8)	61 (18.9)	1.18 (0.79–1.77)	0.41		
rs11161614	0.227 (G)	TT	0.43 (0.38–0.50)	3.7E-06	356 (60.0)	188 (56.8)	1.00	—	1.18 (0.94–1.48)	0.14
		TG	0.46 (0.40–0.52)		211 (35.8)	123 (37.2)	1.10 (0.83–1.47)	0.50		
		GG	0.50 (0.44–0.56)		22 (3.7)	20 (6.0)	1.72 (0.92–3.24)	0.09		
rs11161616	0.229 (T)	CC	0.44 (0.38–0.50)	6.9E-05	342 (59.4)	188 (58.0)	1.00	—	1.04 (0.83–1.30)	0.75
		CT	0.46 (0.40–0.52)		207 (35.9)	121 (37.3)	1.06 (0.80–1.42)	0.67		
		TT	0.47 (0.43–0.55)		27 (4.7)	15 (4.6)	1.01 (0.53–1.95)	0.98		
rs1146381	0.496 (A)	GG	0.43 (0.38–0.48)	1.5E-04	154 (26.7)	81 (24.5)	1.00	—	1.11 (0.92–1.34)	0.29
		GA	0.46 (0.39–0.51)		285 (49.4)	161 (48.6)	1.07 (0.77–1.50)	0.67		
		AA	0.47 (0.41–0.53)		138 (23.9)	89 (26.9)	1.23 (0.84–1.79)	0.29		
rs1874807	0.484 (G)	AA	0.47 (0.41–0.53)	1.5E-03	144 (25.2)	88 (27.4)	1.00	—	0.95 (0.79–1.16)	0.63
		AG	0.45 (0.38–0.51)		297 (51.9)	160 (49.8)	0.88 (0.64–1.22)	0.45		
		GG	0.44 (0.38–0.48)		131 (22.9)	73 (22.7)	0.91 (0.62–1.35)	0.64		
rs506733	0.359 (C)	TT	0.44 (0.38–0.51)	0.03	242 (42.2)	137 (41.5)	1.00	—	1.01 (0.83–1.24)	0.90
		TC	0.46 (0.40–0.51)		252 (44.0)	148 (44.8)	1.03 (0.78–1.39)	0.81		
		CC	0.46 (0.41–0.52)		79 (13.8)	45 (13.6)	1.01 (0.66–1.53)	0.98		
rs233128	0.297 (G)	CC	0.46 (0.40–0.52)	0.04	292 (49.8)	159 (48.0)	1.00	—	0.99 (0.81–1.22)	0.94
		CG	0.45 (0.38–0.50)		239 (40.8)	148 (44.7)	1.14 (0.86–1.51)	0.37		
		GG	0.43 (0.37–0.51)		55 (9.4)	24 (7.3)	0.80 (0.48–1.34)	0.40		

Continued on p. 851

Table 3—Continued

			Association with ADMA/SDMA (max *n* = 770)		Association with CVD (max *n* = 920)		Odds ratio (95% CI) for CVD			
SNP ID	MAF	Genotype	Median (IQR) ADMA (μmol/L)	*P*	No CVD, *n* (%)	CVD, *n* (%)	Genotypic	*P*	Major vs. minor allele	*P*
rs7555486	0.265 (A)	TT	0.46 (0.40–0.52))	0.07	319 (54.3)	186 (56.2)	1.00	—	0.89 (0.72–1.11)	0.31
		TA	0.44 (0.39–0.50)		216 (36.8)	124 (37.5)	0.99 (0.74–1.31)	0.92		
		AA	0.43 (0.37–0.50		52 (8.9)	21 (6.3)	0.69 (0.40–1.19)	0.18		
rs17384213	0.169 (A)	GG	0.45 (0.38–0.51)	0.07	404 (70.0)	217 (67.4)	1.00	—	1.15 (0.89–1.48)	0.29
		GA	0.47 (0.41–0.52)		159 (27.6)	93 (28.9)	1.09 (0.80–1.48)	0.58		
		AA	0.48 (0.40–0.51)		14 (2.4)	12 (3.7)	1.60 (0.73–3.51)	0.25		
rs2935	0.118 (A)	GG	0.45 (0.40–0.51)	0.11	428 (76.7)	252 (78.5)	1.00	—	0.88 (0.64–1.19)	0.39
		GA	0.44 (0.37–0.51)		123 (22.0)	68 (21.2)	0.94 (0.67–1.31)	0.71		
		AA	0.42 (0.33–0.47)		7 (1.3)	1 (0.3)	0.24 (0.03–1.98)	0.19		
rs11587512	0.277 (G)	TT	0.46 (0.40–0.52)	0.15	305 (52.9)	167 (52.0)	1.00	—	1.04 (0.84–1.29)	0.71
		TG	0.45 (0.39–0.50)		228 (39.5)	127 (39.6)	1.02 (0.76–1.36)	0.91		
		GG	0.44 (0.37–0.50)		44 (7.6)	27 (8.4)	1.12 (0.67–1.88)	0.66		
AGXT2			Median (IQR) SDMA (μmol/L)							
rs28305	0.109 (C)	GG	0.48 (0.41–0.57)	1.3E-04	475 (80.8)	248 (74.7)	1.00	—	1.35 (1.01–1.83)	0.05
		GC	0.54 (0.45–0.63)		111 (18.9)	83 (25.0)	1.43 (1.04–1.98)	0.03		
		CC	0.65		2 (0.3)	1 (0.3)	0.96 (0.09–10.61)	0.97		
rs16899974	0.235 (A)	CC	0.48 (0.41–0.56)	3.1E-03	338 (59.6)	189 (57.1)	1.00	—	0.97 (0.77–1.21)	0.77
		CA	0.51 (0.43–0.62)		189 (33.3)	131 (39.6)	1.24 (0.93–1.64)	0.14		
		AA	0.56 (0.45–0.72)		40 (7.1)	11 (3.3)	0.49 (0.25–0.98)	0.04		
rs37369	0.089 (T)	CC	0.48 (0.41–0.57)	5.5E-03	487 (83.4)	267 (80.7)	1.00	—	1.16 (0.83–1.61)	0.39
		CT	0.54 (0.45–0.63)		95 (16.3)	64 (19.3)	1.23 (0.87–1.74)	0.25		
		TT	0.51		2 (0.3)	0	—	—		
rs344156	0.330 (A)	GG	0.48 (0.41–0.57)	0.42	259 (44.3)	149 (45.0)	1.00	—	1.02 (0.83–1.24)	0.89
		GA	0.49 (0.42–0.60)		267 (45.7)	144 (43.5)	0.94 (0.70–1.25)	0.66		
		AA	0.52 (0.42–0.60)		59 (10.0)	38 (11.5)	1.12 (0.71–1.76)	0.63		
rs237252	0.329 (G)	AA	0.49 (0.41–0.58)	0.46	245 (44.5)	144 (44.9)	1.00)	—	1.01 (0.82–1.24)	0.94
		AG	0.50 (0.42–0.60)		250 (45.4)	142 (44.2)	0.97 (0.72–1.29)	0.82		
		GG	0.52 (0.42–0.60)		56 (10.2)	35 (10.9)	1.06 (0.67–1.70	0.80		
rs2291701	0.328 (T)	CC	0.49 (0.41–0.58)	0.61	257 (44.5)	146 (45.3)	1.00	—	1.00 (0.82–1.12)	0.97
		CT	0.49 (0.42–0.60)		261 (45.2)	140 (43.5)	0.94 (0.71–1.26)	0.70		
		TT	0.51 (0.42–0.60)		59 (10.2)	36 (11.2)	1.07 (0.68–1.70)	0.76		
rs13163727	0.352 (T)	CC	0.49 (0.42–0.59)	0.78	247 (42.3)	140 (42.9)	1.00	—	1.04 (0.85–1.27)	0.73
		CT	0.49 (0.42–0.59)		266 (45.5)	139 (42.6)	0.92 (0.69–1.23)	0.59		
		TT	0.51 (0.42–0.60)		71 (12.2)	47 (14.4)	1.17 (0.77–1.78)	0.47		

Prevalent CVD includes angina, MI, stroke, TIA, or intermittent claudication. Dimethylarginines are displayed as median (IQR); differences between genotypes were calculated using the Kruskal–Wallis test. Genotypic and allelic odds ratios were calculated for prevalent CVD. Bonferroni correction set significant *P* values at $P < 0.0021$. MAF, minor allele frequency.

Table 4—Multiple linear regression analysis: determinants of ADMA and SDMA cross-sectionally

	Unstandardized		Standardized	
	B	SE	β	*P*
ln ADMA, R^2 = 0.213				
ln L-arginine	0.272	0.021	0.367	<0.001
Sex (female vs. male)	0.075	0.013	0.174	<0.001
Creatinine	0.001	0.000	0.184	<0.001
rs1554597 (C vs. T)	0.049	0.013	0.111	<0.001
Glucose	0.008	0.003	0.083	0.004
ln SDMA, R^2 = 0.425				
Creatinine	0.005	0.000	0.571	<0.001
ln L-arginine	0.167	0.019	0.175	<0.001
Sex (female vs. male)	0.074	0.011	0.135	<0.001
Age (years)	0.008	0.001	0.119	0.001
Diabetes duration (years)	0.003	0.001	0.080	0.014
rs28305 (C vs. G)	0.067	0.017	0.075	<0.001
Prevalent CVD	0.024	0.011	0.043	0.035

Prevalent CVD includes angina, MI, stroke, TIA, or intermittent claudication. B, unstandardized regression coefficient.

data from two larger study collectives recently published showed ADMA levels to be associated with CV events and all-cause mortality only in nondiabetic patients (7,15). In our large cohort of diabetes patients, only weak associations of ADMA with clinical features of diabetes and no association with medication was apparent, making it less likely that glycemic control or medication directly affects ADMA and its associated risk. However, there is some evidence that early stages of diabetic nephropathy with renal vasodilatation and hyperfiltration could lower circulating ADMA, while in later stages of renal insufficiency, ADMA levels rise (8,27). The predictive value of ADMA could therefore be highly dependent on the selection of study subjects. In this context, another possible bias is that we investigated older, obese subjects, and results might be different in younger cohorts with lower BMI.

The association between SDMA, rs28305, and CVD in our study adds to increasing evidence that links SDMA with macrovascular disease. In previous studies, SDMA was an independent predictor of mortality or CVD events in patients with prevalent CVD (17,18,28). To date, no data are available on the associations of SDMA or SNPs in *AGXT2* and CVD in patients with diabetes. Therefore, it is interesting to note that in our cohort, SDMA was more strongly related to CVD than ADMA in unadjusted analyses. Furthermore, the independent association of *AGXT2* rs28305 with intermittent claudication strengthens the hypothesis that SDMA might be both a marker of increased risk and involved in the mechanisms leading to vascular disease, which are most likely nitric oxide synthase independent. There is some evidence linking SDMA to an increased release of reactive oxygen species as well as proinflammatory cytokines (29,30). Because the development of macroangiopathy in type 2 diabetes is associated with chronic subclinical inflammation (31), it would be important to investigate the molecular mechanisms that connect SDMA, inflammation, and macrovascular disease in diabetes patients.

Determinants of Dimethylarginines

Consistent with our results, Abhary et al. reported that several tag SNPs in the *DDAH1* gene were associated with ADMA levels in an elderly cohort of patients with type 2 diabetes (13). Moreover, they found a significant, but weaker, association of one tag SNP in the *DDAH2* gene (rs313183) and ADMA levels. We cannot rule out that the lack of association between *DDAH2* and dimethylarginine levels in our group results from the selection of the SNPs, which did not include rs31318. However, subgroup analysis by retinopathy status among the participants of the above-mentioned study revealed that the majority of the SNPs were associated with ADMA levels in patients without documented diabetic retinopathy. Therefore genetic differences or differences in the clinical manifestation of the disease between the cohorts could have contributed to the discrepancies between the observed associations. Nonetheless, despite the hypothesis that DDAH2 might be the enzyme responsible for degrading plasma ADMA owing to its high expression in vascular tissue (32), the clinical data indicate a greater contribution of DDAH1 in regulating circulating ADMA levels in patients with type 2 diabetes. This observation is in line with our previous genome-wide association study in a population-based cohort, in which *DDAH1* emerged as the only gene locus significantly associated with ADMA in multivariate analysis (33).

Another enzyme capable of degrading dimethylarginines in vivo is AGXT2 (19,20). In our study, SNPs in the *AGXT2* gene were not related to ADMA but only to SDMA plasma concentrations. *AGXT2* rs28305 was significantly associated with SDMA plasma concentrations after adjustment for clinical variables, including renal function. This is an interesting observation, as SDMA is mainly eliminated via renal glomerular excretion, and our results suggest that enzymatic degradation by AGXT2 contributes to SDMA clearance. Preliminary data from our group support this finding, as we observed an association of *AGXT2* and SDMA in genome-wide association studies (33). This observation remains consistent with the close relation between SDMA and renal function, as AGXT2 is predominantly expressed in the kidneys (20).

Discrepancies between previous studies regarding ADMA plasma levels in diabetic patients raise questions about determinants of dimethylarginines in diabetes and their associations with glycemic state and diabetes medication. In our cohort, there were nominally significant, but weak, positive correlations of ADMA with fasting glucose levels and of SDMA with BMI, but neither of the dimethylarginines were significantly related to HbA_{1c} or diabetes treatment. In line with our

results, previous studies found that ADMA was related to markers of renal function and L-arginine plasma concentrations but not to diabetes duration, glucose concentrations, HbA_{1c}, or insulin therapy in patients with type 2 diabetes (9,26). In a population-based study, including diabetic and nondiabetic subjects, ADMA levels were associated with age, BMI, and smoking status, but these clinical variables only explained approximately 3.5% of interindividual variation in ADMA (7). In contrast, the multivariable model in our study, including rs7554597 and the precursor molecule L-arginine, was able to explain 21.6% of the variation of ADMA. In another sample of the same population-based cohort, SDMA plasma levels were related to age, creatinine, homocysteine, BMI, and diastolic blood pressure, very similar to our results (34). However, while the multivariate model including these parameters was only able to account for 8% of variation in SDMA levels, the multivariate model we used explained 44.5% of the interindividual variation in circulating SDMA. Therefore, it is important to consider the regulatory pathways of dimethylarginines, including genetic variation in the metabolizing enzymes, when assessing the determinants of ADMA or SDMA in diabetic patients, whereas the contribution of glycemic control or antihyperglycemic agents seems to be only minor.

We conclude that dimethylarginines are only weakly related to glycemic state, antihyperglycemic medication, or CVD in older patients with type 2 diabetes mellitus. Furthermore, their predictive value for incident CVD appears to be limited. The strong associations between *DDAH1* SNPs and ADMA levels, as well as *AGXT2* SNPs and SDMA levels, demonstrate that genetic variations in the metabolizing genes should be taken into consideration when determining dimethylarginines.

Acknowledgments. The authors thank the participants, staff, investigators, and collaborators on the ET2DS. Furthermore, the authors thank the Wellcome Trust Clinical Research Facility for their collaboration (research clinic and genotyping) and Tanja Zeller and Christian Müller from the Cardiology Department of the University Medical Center Hamburg-Eppendorf for helping with the genetic analyses. The excellent technical assistance of Mariola Kastner and Anna Steenpass, both of the Department of Clinical Pharmacology and Toxicology, University Medical Center Hamburg-Eppendorf, is gratefully acknowledged.

Funding. This study was supported by the European Association for the Study of Diabetes, the U.K. Medical Research Council, and the European Commission (EU-FP7 grant number 278397, EuRhythDia).

Duality of Interest. Additional support was provided by Pfizer. E.S. and R.H.B. are named as inventors on patents relating to analytical assays for methylarginines and receive royalties from them. No other potential conflicts of interest relevant to this article were reported.

Author Contributions. M.A. and S.M. researched data and wrote the manuscript. N.L. and E.S. contributed to the data analysis and discussion. C.R., R.M.W., and M.W.J.S. researched data and reviewed and edited the manuscript. R.A. reviewed and edited the manuscript. P.J.G., R.H.B., and J.F.P. designed the study, contributed to the discussion, and reviewed and edited the manuscript. R.H.B. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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