Molecular Biology Reports (2018) 45:2411–2419
https://doi.org/10.1007/s11033-018-4407-1

ORIGINAL ARTICLE

AGXT2 and DDAH-1 genetic variants are highly correlated with serum ADMA and SDMA levels and with incidence of coronary artery disease in Egyptians

Mina Amir[1] · Sally I. Hassanein[1] · Mohamed F. Abdel Rahman[2] · Mohamed Z. Gad[1]

Received: 10 June 2018 / Accepted: 25 September 2018 / Published online: 3 October 2018

Abstract

Dimethylarginine aminodehydrolase (DDAH1) and alanine glyoxylate aminotransferase2 (AGXT2) are two enzymes that contribute in asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) metabolism. Hence they affect production and bioavailability of eNOS-derived nitric oxide (NO) and consequently healthy blood vessels. The major aims of the current study were to investigate the association of genetic variants of AGXT2 rs37369, AGXT2 rs16899974 and DDAH1 rs997251 SNPs with incidence of coronary artery disease (CAD) in Egyptians and to correlate these variants with the serum levels of ADMA and SDMA. The study included 150 subjects; 100 CAD patients and 50 healthy controls. Genotyping was performed by qPCR while the ADMA and SDMA concentrations were assayed by ELISA. Both serum ADMA and SDMA concentrations were significantly higher in CAD patients compared to controls (both $p < 0.0001$). Genotype distributions for all studied SNPs were significantly different between CAD patients and controls. Carriers of AGXT2 rs37369-T allele (CT + TT genotypes) and AGXT2 rs16899974-A allele (CA + AA genotypes) had 2.4- and 2.08-fold higher risk of having CAD than CC genotype in both SNPs ($p = 0.0050$ and 0.0192, respectively). DDAH1 rs997251 TC + CC genotypes were associated with 2.3-fold higher risk of CAD than TT genotype ($p = 0.0063$). Moreover, the AGXT2 rs37369 TT and AGXT2 rs16899974 AA genotypes were associated with the highest serum ADMA and SDMA while DDAH1 rs997251 CC genotype was associated with the highest ADMA. AGXT2 rs37369-T, AGXT2 rs16899974-A, and DDAH1 rs997251-C alleles represent independent risk factors for CAD in the Egyptians.

Keywords Asymmetric dimethylarginine · Coronary artery disease · Alanine glyoxylate aminotransferase 2 · Dimethylarginine dimethylaminohydrolase · Polymorphisms · Symmetric dimethylarginine · Nitric oxide

Introduction

Nitric oxide (NO) is generated from the metabolism of L-arginine by three isoforms of nitric oxide synthase namely endothelial nitric oxide synthase (eNOS), inducible nitric oxide synthase (iNOS) and neuronal nitric oxide synthase (nNOS). The eNOS plays a major role in regulating vasomotor tone [1]. NO plays a key role in the physiological regulation of the cardiovascular system, hence abnormalities in its production or bioavailability are associated with cardiovascular diseases like hypertension, atherosclerosis and angiogenesis-associated disorders [2]. Reduced NO production were observed as a result of elevated serum levels of methylarginines; endogenous competitive inhibitors of NOS [3].

Methylarginines are produced from the degradation of methylated proteins that are formed by the reaction of two

✉ Sally I. Hassanein
sally.ibrahim@guc.edu.eg

Mina Amir
Mina.amir@student.guc.edu.eg

Mohamed F. Abdel Rahman
mmfarouk@msa.eun.eg

Mohamed Z. Gad
Mohamed.gad@guc.edu.eg

[1] Clinical Biochemistry Unit, Faculty of Pharmacy and Biotechnology, German University in Cairo (GUC), Main Entrance El-Tagamoa El-Khames, New Cairo City, Cairo, Egypt

[2] Biochemistry Department, Faculty of Pharmacy, October University for Modern Sciences and Arts (MSA), Cairo, Egypt

enzymes; arginine methyltransferase type 1 (PRMT1) and type 2 (PRMT2) which transfer the methyl group from S-adenosylmethionine. PRMT1 catalyses the formation of N^G-monomethyl-L-arginine (L-NMMA) and asymmetric N^G,N^G-dimethyl-L-arginine (ADMA), while PRMT2 catalyzes the formation of symmetric N^G,N^G-dimethyl-L-arginine (SDMA) [4]. The L-NMMA and ADMA are competitive inhibitors of the NOSs [5]. SDMA, unlike ADMA, does not inhibit NO synthesis directly but rather is transported efficiently by human cationic amino acid transporter (hCAT-2B) and is exchanged against intracellular L-arginine, resulting in an L-arginine depletion of the cells. SDMA also inhibits renal tubular L-arginine uptake and consequently enhancing its excretion from the body [6].

Elevated circulating ADMA and SDMA levels were reported to be associated with cardiovascular [7] and renal diseases [8]. In a German study, ADMA has been identified as predictor of cardiovascular events and all-cause mortality in the studied population [9].

ADMA is metabolized by the enzyme N^G dimethylarginine dimethylaminohydrolase (DDAH-1) to citrulline and dimethylamine [10]. L-citrulline can be reincorporated into proteins while the dimethylamine is excreted in the urine. ADMA is also a substrate for alanine-glyoxylate aminotransferase 2 (AGXT2), which is expressed only in kidney and liver, leading to the formation of asymmetric dimethylguanidino valeric acid (ADGV) that is also excreted in the urine [11].

SDMA is not hydrolyzed by DDAH enzyme, but is a substrate for the AGXT2 enzyme leading to the formation of symmetric α-keto-δ-*N,N*-dimethylguanidino valeric acid (SDGV) that is excreted in the urine. SDMA can also be excreted intact in the urine [12]. Compared to DDAH, AGXT2 can metabolize both ADMA and SDMA.

Several single nucleotide polymorphisms (SNPs) have been identified in DDAH-1 and AGXT2 genes. Few SNPs in DDAH-1 have been associated with an increase in serum ADMA and hypertension [13, 14]. Also recent genomic studies on AGXT2 gene reported many SNPs influencing the enzyme activity and the association with several clinical disorders [15, 16].

rs37369 and rs16899974 are two SNPs that cause valine to isoleucine (Val140Ile) and valine to leucine (Val498Leu) substitutions, respectively in human AGXT2 gene region on chrosome 5p13. DDAH1 rs997251 is a transition substitution between cytosine and thymine in the intron of the gene on chromosome 1.

Previous studies from our lab investigated the link between incidence of acute myocardial infarction (AMI) in Egyptians and presence of variants of several genes relevant to cardiovascular homeostasis including eNOS and DDAH2 genes [17–23]. In the current study we aimed to assess the association of AGXT2 variants; rs37369, rs16899974 and DDAH1 variant rs997251 with the CAD incidence in Egyptians. Moreover, to compare the levels of ADMA and SDMA in the CAD patients with those of controls and to explore the relationship between these biomarkers and the selected SNPs.

Materials and methods

Subjects

The patient group comprised 100 CAD patients having a history of coronary artery bypass graft (CABG) and/or myocardial infarction. They were recruited from in- and out-patient settings of the National Heart Institute (NHI) and Kasr El Einy hospital, Cairo. All patients involved in the study had history of percutaneous intervention (stents) where either recurrence occurred or blockage in another coronary artery so CABG was performed. All patients recruited in the study with history of STEMI were put on fibrinolytics. After CABG, all patients were given the standard treatment including antihypertensives and asprin.

The control group comprised 50 healthy controls with no diagnostic signs of CVDs. Both controls and patients had a controlled blood pressure of below 140/90 mmHg as all patients were receiving antihypertensive medications. Local ethics committee approved the study protocols. Subjects' characteristics are listed in Table 1.

Sample collection

A sample of peripheral venous blood was taken from each subject at the time of enrolment in the study. Blood samples were centrifuged immediately after collection at 5000 RPM for 15 min at 25 °C. Plasma was stored at − 80 °C till further analysis.

DNA extraction

DNA was extracted from blood using Thermo Scientific Gene Jet Whole Blood Genomic DNA Purification Minikit. DNA concentration and purity were determined by

Table 1 Characteristics of CAD and controls study groups

	CAD (n = 100)	Controls (n = 50)	*p* value
Gender (M/F)	45/55	21/29	0.8616
Age (Year)	41.5 ± 1.5	43.9 ± 1.9	0.3789
BMI (Kg/m^2)	47.9 ± 1.1	45.6 ± 0.8	0.1162
Smoking (n)	57	5	< 0.0001

Data are expressed as mean ± SEM

measuring the absorbance at wave lengths 260 and 280 nm using the NanoDropR ND-1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA).

SNPs genotyping

SNPs in the AGXT2 (rs37369 and rs1689997 in chromosome 5p13,) and DDAH1 (rs997251 in chromosome 1p22) genes were selected using HapMap. The PCR reaction was performed in a 96-well plate. Each well received the following: 3 µL of genomic DNA, 5 µL of SNP reaction mixture (Applied Biosystems P/N 4371355) consisting of 2.5 µL of TaqMan Universal PCR Master mix, 0.125 µL of 40X working stock of SNP1 rs37369, SNP2 rs1689997, SNP3 rs997251 and 2.375 µL DNase-free water. The PCR programme started with an initial denaturation at 95 °C for 10 min for activating the AmpliTaq Gold enzyme followed by 40 cycles of denaturation at 92 °C for 15 s and an annealing/extension temperature at 60 °C for 1 min. Allelic discrimination assays were performed using two TaqMan MGB probes (FAM/VIC dye) that target the SNP sites.

ADMA and SDMA assay

Plasma levels of ADMA and SDMA were measured using ELISA kits (DLD, Germany).

Statistical analysis

Statistical analysis was performed using the statistical program GraphPad Prism 7. Data are represented as mean ± SEM. To compare differences between groups odds ratio (Fischer test), nonparametric student *t* test (Mann–Whitney) and nonparametric one-way ANOVA (Kruskal–Wallis) were used. In all statistical tests two-tailed $p \leq 0.05$ was considered statistically significant. Cubex online calculator was used to calculate linkage disequilibrium [24].

Results

Serum concentrations of ADMA and SDMA

The current study results showed that the average serum concentration of ADMA in CAD patients (1.34 ± 0.05 µmol/L) was significantly higher than controls (0.72 ± 0.06 µmol/L) ($p < 0.0001$). The average serum concentration of SDMA among CAD patients (1.72 ± 0.05 µmol/L) was also significantly higher than controls (1.11 ± 0.05 µmol/L) ($p < 0.0001$) (Fig. 1).

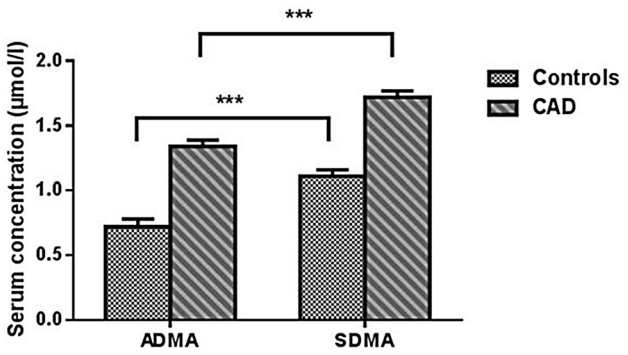

Fig. 1 Serum ADMA and SDMA concentrations in AMI patients and controls. Results are expressed as mean ± SEM. ***Significant difference from the control group at $p < 0.0001$

AGXT2 and DDAH1 genotyping

Genotype distribution of all 3 studied polymorphisms were in line with Hardy–Weinberg equilibrium ($p = 0.33$ for AGXT2 rs37369, $p = 0.06$ for AGXT2 rs16899974 and $p = 0.31$ for DDAH-1 rs997251). Moderate linkage disequilibrium was observed between the 2 studied AGXT2 SNPs ($D' = 0.825$ and $r^2 = 0.2274$).

Genotype distribution and allele frequencies for all studied SNPs were significantly different between CAD patients and controls (Table 2). Genotype distributions of AGXT2 rs37369 SNP were 54% CC, 37% CT and 9% TT in CAD patients and 74% CC, 22% TC and 4% TT in controls ($p = 0.0119$) (Fig. 2a) while its allele frequencies were 72.5% C and 27.5% T in CAD patients and 85% C and 15% T in control subjects ($p = 0.02$). The current study results also showed that individuals who do not have CC genotype (CT + TT) had 2.4-fold higher risk of having CAD than individuals who have CC genotype ($p = 0.005$). It also shows that T allele was associated with 2.1-fold higher risk for CAD than C allele ($p = 0.02$) (Table 2).

AGXT2 rs16899974 genotype distribution was 29% CC, 36% AC and 35% AA in CAD and 46% CC, 34% AC and 20% AA in controls ($p = 0.0183$) (Fig. 2b) while its allele frequencies in CAD were C 47% and A 53% while in controls they were C 63% and A 37% ($p = 0.0101$). Individuals who do not have CC genotype (CA + AA) showed 2.1-fold higher risk of CAD than individuals with CC genotype ($p = 0.0192$). AGXT2 rs16899974-A allele represented 1.9-fold higher risk for CAD than C allele ($p = 0.0101$) (Table 2).

Regarding DDAH1 rs997251, the genotype distribution was 32% TT, 48% TC and 20% CC in CAD and 52% TT, 36% TC and 12% CC in controls ($p = 0.0144$) (Fig. 2c) while the allele frequencies were 56% T and 44% C in CAD patients and 70% T and 30% C in controls ($p = 0.0239$). The study results showed that individuals who do not have TT genotype (TC + CC) had 2.3-fold higher risk of CAD than

Table 2 Genotype distribution, allele frequencies and odds ratio for all of the AGXT2 studied SNPs

SNP	CAD patients n = 100 (%)	Controls n = 50 (%)	Odds ratio	*p* value
AGXT2 rs37369				
Genotypes				
CT + TT	46	26	2.4	0.0050
CC	54	74		
Alleles				
T	27.5	15	2.1	0.0200
C	72.5	85		
AGXT2 rs16899974				
Genotypes				
CA + AA	71	54	2.1	0.0192
CC	29	46		
Alleles				
A	53	37	1.9	0.0101
C	47	63		
DDAH-1 rs997251				
Genotypes				
CT + CC	68	48	2.3	0.0063
TT	32	52		
Alleles				
C	44	30	1.8	0.0239
T	56	70		

individuals who have TT genotype ($p = 0.0063$). It also showed that C allele was associated with 1.8 times higher risk for CAD than T allele ($p = 0.0239$) (Table 2).

Correlation of AGXT2 and DDAH1 genotypes with serum ADMA and SDMA concentrations

Serum ADMA and SDMA concentrations were significantly different among various AGXT2 rs37369 genotypes in both CAD patients ($p < 0.0001$) and controls subjects ($p < 0.0001$) where TT genotype showed the highest concentrations of ADMA and SDMA followed by CT genotype while CC genotype was associated with the lowest serum concentrations of ADMA and SDMA (Fig. 3; Table 3).

Serum ADMA and SDMA concentrations were also significantly different among various AGXT2 rs16899974 genotypes in both CAD patients and control subjects ($p < 0.0001$) where highest serum ADMA and SDMA concentrations were in AA (Fig. 4; Table 3).

Serum ADMA concentrations were also significantly different among various DDAH-1 rs997251 where CC genotypes possessed the highest ADMA concentrations in both CAD patients and control subjects ($p < 0.0001$) (Fig. 5; Table 4).

Discussion

In the current study only patients with history of CABG were included as these are the most severe cases of coronary artery disease to investigate the link between these studied SNPs and CAD in Egyptians. As there is evidence that these studied SNPs are genetic determinants for the ADMA and SDMA levels [25]. Besides, there is also evidence that ADMA has been identified as predictor of cardiovascular events and all-cause mortality in the studied population [9]. So we aimed to have homogenous group of cardiovascular patients, we decided to select patients with chronic heart condition as patients with history of CABG and/or myocardial infarction (MI) to ensure the involvement of gene variants in the cardiovascular disease incidence.

In the current study, the mean serum levels of ADMA in controls was 0.72 ± 0.06 µmol/L. This value is identical to the mean serum ADMA concentration reported by

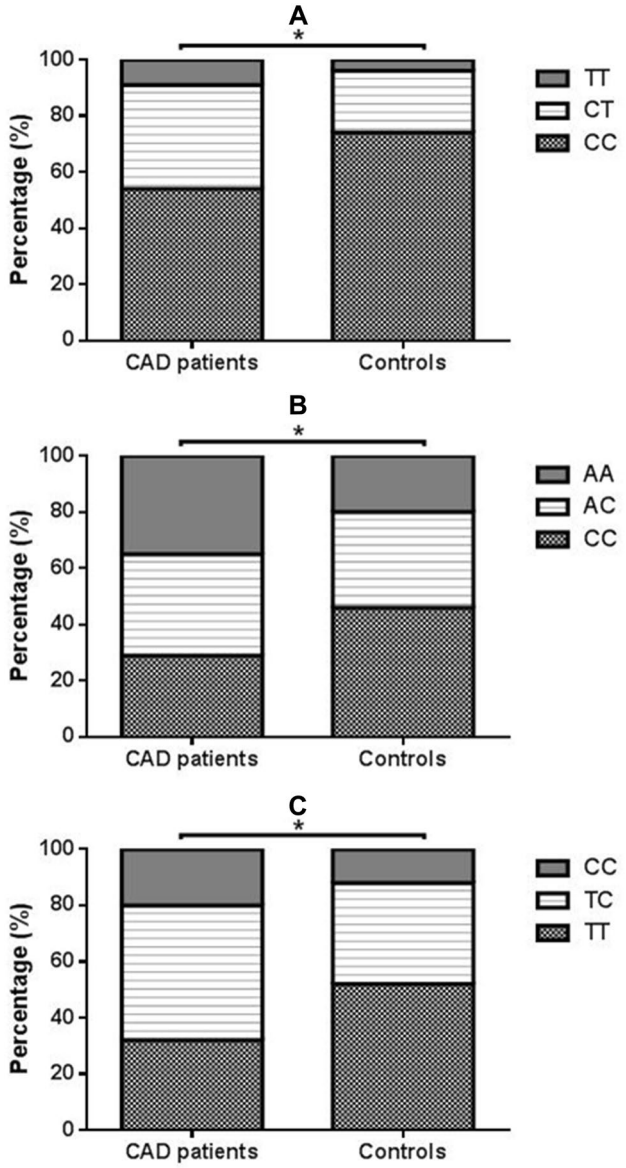

Fig. 2 Genotype distributions in **a** AGXT2 rs37369, **b** AGXT2 rs16899974 and **c** DDAH-1 rs997251. *Significant difference from the control group at $p < 0.05$

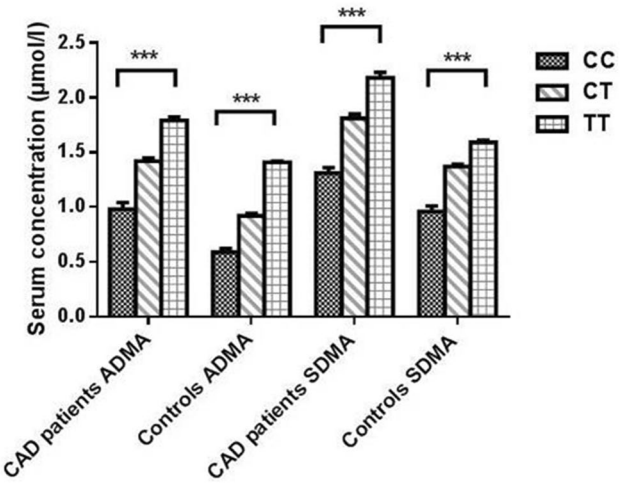

Fig. 3 Serum ADMA and SDMA concentrations in various AGXT2 rs37369 genotypes in CAD patients and controls subjects. Results are expressed as mean ± SEM. ***Significant difference from the control group at $p < 0.0001$

Martens-Lobenhoffer et al. [26] and very close to the mean serum level reported in a study performed on 500 healthy subjects to determine the reference interval of serum ADMA (0.69 µmol/L) [27]. Our level also falls within the reference interval for ADMA reported recently in Pomerania [28]. The mean serum SDMA concentration in the current study was 1.11 ± 0.05 µmol/L. This value is higher than the reported mean SDMA concentration in various studies [29, 30]. Similarly, it is higher than the recently reported Pomeranian reference intervals for SDMA [28]. SDMA variability might be attributed to different assay procedures [31]. SDMA is a sensitive marker for renal function [12]. Although controls are free from any renal disease but variability in their renal function cannot be excluded. A limitation in the current study is that no renal function biomarkers were measured.

Elevated serum levels of ADMA and SDMA reduce NO bioavailability leading to endothelial dysfunction and consequently to CAD [32]. ADMA inhibits NO synthesis directly by acting as a competitive inhibitor of the NOSs [5] while SDMA inhibits NO synthesis indirectly by depleting the intracellular L-arginine as it is transported and exchanged against intracellular L-arginine. SDMA also inhibits renal tubular L-arginine uptake and consequently enhancing its excretion [6].

The current study results showed that the serum concentrations of ADMA and SDMA in CAD patients were significantly higher than controls. These results are consistent with those of Valkonen et al. who reported a 3.9-fold increased risk of acute coronary events in subjects with highest quartile of ADMA compared to other quartiles [7]. Lu et al. confirmed that increased risk of cardiovascular events was noted with increasing levels of ADMA in stable angina patients undergoing percutaneous coronary intervention [33]. On the same line, 24 months follow up study of 880 healthy women showed that a 0.15 µmol/L increase in baseline ADMA levels was associated with an approximately 30% increase in incident cardiovascular risk and 30% increase in fatal cardiovascular disease after adjustment for conventional cardiovascular risk factors [34].

Other various studies had shown that ADMA contributes to promoting vascular damage and hypertension-induced cardiovascular risk [35]. Imbalance of arginine and ADMA was an independent risk factor in atherosclerosis progression [36]. Plasma levels of ADMA are

Table 3 Serum ADMA and SDMA concentrations in various genotypes of studied SNPs

SNP	Genotypes	CAD patients n = 100		Controls n = 50	
		Serum ADMA (μmol/L)	Serum SDMA (μmol/L)	Serum ADMA (μmol/L)	Serum SDMA (μmol/L)
AGXT2 rs37369	CC	0.98 ± 0.06	1.31 ± 0.05	0.59 ± 0.03	0.96 ± 0.05
	CT	1.42 ± 0.03	1.81 ± 0.04	0.92 ± 0.02	1.37 ± 0.02
	TT	1.79 ± 0.03	2.18 ± 0.05	1.41 ± 0.01	1.59 ± 0.02
		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
AGXT2 rs16899974	CC	0.9 ± 0.08	1.34 ± 0.04	0.44 ± 0.05	0.86 ± 0.06
	CA	1.34 ± 0.03	1.8 ± 0.04	0.88 ± 0.03	1.31 ± 0.02
	AA	1.7 ± 0.03	2.14 ± 0.04	1.3 ± 0.04	1.51 ± 0.03
		$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$

Results are expressed as mean ± SEM

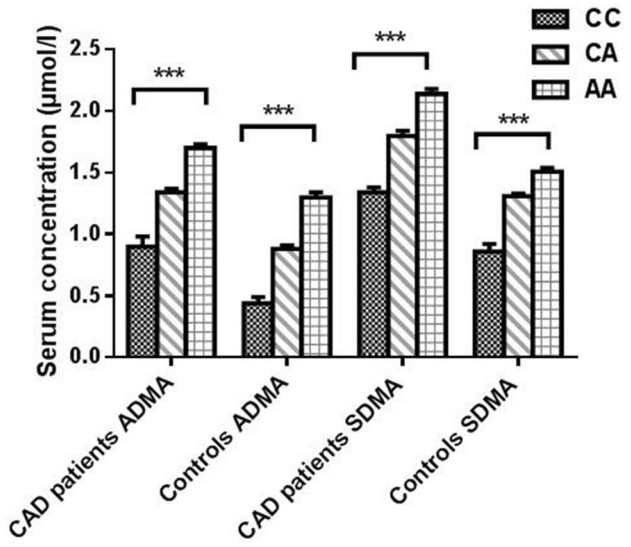

Fig. 4 Serum ADMA and SDMA concentrations in various AGXT2 rs16899974 genotypes in CAD patients and controls subjects. Results are expressed as mean ± SEM. ***Significant difference from the control group at $p < 0.0001$

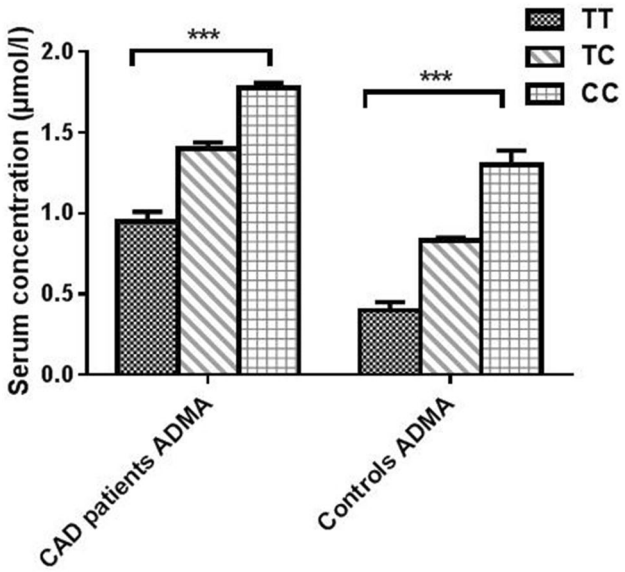

Fig. 5 Serum ADMA and SDMA concentrations in various DDAH-1 rs997251genotypes in CAD patients and controls subjects. Results are expressed as mean ± SEM. ***Significant difference from the control group at $p < 0.0001$

elevated in chronic cardiac failure (CHF) patients [37] and positively correlated with disease severity in CHF [38]. Mahran and coworkers reported that ADMA is linked to predisposition of restenosis following coronary stenting [39].

Bode-Böger et al. reported that SDMA might be a useful parameter for detecting patients in very early stages of chronic kidney disease and for determining their risk for developing cardiovascular disease [40].. Serum SDMA level could also predict all-cause mortality in patients with coronary heart disease [41], ischemic stroke [42] and symptomatic peripheral arterial disease [43]. In contrast to all these studies, Wang et al. reported that ADMA and SDMA levels were not associated with CAD severity [44].

Table 4 Serum ADMA concentrations in various genotypes of DDAH-1 rs997251 SNP

SNP	Genotypes	Serum ADMA (μmol/L)	
		CAD patients n = 100	Controls n = 50
DDAH-1 rs997251	TT	0.95 ± 0.06	0.4 ± 0.05
	TC	1.4 ± 0.04	0.83 ± 0.02
	CC	1.78 ± 0.03	1.3 ± 0.09
		$p < 0.0001$	$p < 0.0001$

Results are expressed as mean ± SEM

The current study investigated the clinical association of AGXT2 rs37369 and rs16899974 and DDAH1 rs997251 gene polymorphisms with incidence of cardiovascular disease in Egyptians. Results showed significant differences in the genotype distribution and allelic frequencies between CAD patients and controls in all the three studied SNPs. Subjects having genotypes CT + TT for AGXT2 rs37369 had a significantly higher risk for development of CAD than CC subjects. Similar observation was noticed for AGXT2 rs16899974 CA + AA genotypes compared to CC. Thus, C allele for both AGXT2 SNPs represents a "low" risk allele for the development of CAD.

These results are consistent with a Japanese study that demonstrated that the AGXT2 rs37369 TT and rs16899974 AA genotypes possess lowest AGXT2 activity and highest mean carotid intimal media thickness, and consequently the highest risk of carotid atherosclerosis [45]. Another study reported that the rs37369 T and rs16899974 A alleles are involved in the pathogenesis of atrial fibrillation and its age-related thromboembolic complications [46]. Other studies linked the AGXT2 rs37369 SNP to increased risk of coronary heart disease [47] and chronic heart failure [48]. Seppälä et al. reported an association between rs16899974 and increased risk of atrial fibrillation and ischemic stroke [49].

In contrast, our results were inconsistent with those of a Chinese study that reported that homozygous rs37369 GG subjects (correspond to CC in the present study) were more susceptible to coronary heart diseases than AG and AA genotypes (correspond to CT and TT genotypes in the present study). They related these results to the predominance of the A allele in the Chinese population and explained this difference in genotype by ethnic differences [47].

In the current study, TT genotype of AGXT2 rs37369 SNP was associated with the highest serum ADMA and SDMA, followed by CT then CC in both CAD patients and controls. These results are consistent with a meta-analysis carried out on 5110 individuals of European descent drawn from two large cohorts; the YFS and the LURIC study. It was found that the most significant factor for increasing SDMA levels was the AGXT2 rs37369 (V140I) SNP variant on chromosome 5p13; the T allele was associated with higher levels of SDMA. The same meta-analysis also demonstrated that the rs16988874 A allele is highly associated with the increase in SDMA levels [46]. Luneburg et al. suggested that rs37369 SNP variant of AGXT2 plays a role in the modulation of its enzyme activity. Computer-based 3D structure modeling and analysis of AGXT2 predicted that the presence of isoleucine at position 140 for AGXT2 rs37369 (V140I) has an effect on loop conformation and substrate access to the active site. In addition, a large clash of I140 is observed with one methyl group of SDMA, which was predicted to dramatically reduce the affinity for this substrate. In HEK293 cells, overexpression of the AGXT2 rs37369 C-allele resulted in a significantly enhanced SDMA-metabolizing activity. A reversal effect was observed when the AGXT2 rs37369 T-allele was over-expressed [50]. Kittel et al. confirmed that amino acid exchange Val140Ile in the AGXT2 protein results in decreased enzyme activity [16]. The same study predicted that rs16988874 SNP leads to the replacement of valine at position 498 with leucine in the helix area of the AGXT2. The larger L498 side chain forms steric clashes that result in local structural rearrangement and might also cause a decreased stability of the enzyme [16].

With regard DDAH1, very few studies investigated the correlation between DDAH1 gene variants and incidence of CVD. Our study showed a significant difference in the genotype distribution and allele frequencies of DDAH1 rs997251 SNP between CAD patients and controls. Subjects possessing genotypes CC + CT had a significantly higher risk for development of CAD than TT subjects. Valkonen et al. reported that the occurrence of CHD was 50-fold higher among the carriers for a DDAH-1 mutation [14]. Another study concluded that the DDAH1 polymorphism that leads to DDAH1 loss of function is associated with both increased risk of thrombosis stroke and CHD in the Chinese Han population [51]. However, on the other hand, a study showed that none of the investigated SNPs in DDAH1, DDAH2, and AGXT2 was associated with CVD [52].

In the current study, the highest serum levels of ADMA were observed in subjects with CC genotype of DDAH1 SNP (rs997251) followed by CT then TT. These results were consistent with the results of a study that aimed to identify novel genetic variants influencing circulating ADMA. It was found in this study that the C allele of DDAH1 rs997251 SNP was significantly associated with elevated serum ADMA levels [46]. Another study performed on 26 SNP for DDAH suggested that the majority of DDAH1 genetic variations were significantly associated with high serum ADMA levels [53].

In conclusion, this study confirmed the association between CAD with high serum concentrations of ADMA and SDMA. AGXT2 rs37369-T, AGXT2 rs16899974-A and DDAH1 rs997251-C represent high risk alleles for CAD among Egyptians. Carriers of these alleles had significantly higher serum levels of ADMA and SDMA. This study shed the light on possible genetic determinants of ADMA and SDMA levels, which might be used ti identify high risk individuals among those with family history of CAD, then serum ADMA and SDMA levels should be carefully monitored as follow up to help in early discovery of cardiovascular diseases. However more studies are still needed to confirm these findings.

Limitations

No follow up records are available for the recruited patients.

Author contributions Concept—MZG, SIH; design—MZG, SIH, MA; supervision—MZG, SIH; fundings—MZG, SIH; materials—HAS; data collection and/or processing—MA, SIH; analysis and/or interpretation—MZG, SIH, MFA, MA; literature review—MA, MFA; writing—MZG, SIH, MFA, MA; critical review—MZG.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval German University Cairo ethics committee [Chair of Committee Prof. Dr. Hans-Georg Breitinger, Hans-Georg Breitinger (hans.breitinger@guc.edu.eg)], approved the study protocols.

Informed consent Consent by all participants (patients and healthy controls).

References

1. Lluch P, Segarra G, Medina P (2015) Asymmetric dimethylarginine as a mediator of vascular dysfunction in cirrhosis. World J Gastroenterol 21:9466
2. Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. Physiol Rev 87:315–424
3. Tain Y-L, Huang L-T, Lin I-C, Lau Y-T, Lin C-Y (2010) Melatonin prevents hypertension and increased asymmetric dimethylarginine in young spontaneous hypertensive rats. J Pineal Res 49:390–398
4. McDermott JR (1976) Studies on the catabolism of Ng-methylarginine, Ng, Ng-dimethylarginine and Ng, Ng-dimethylarginine in the rabbit. Biochem J 154:179–184
5. Böger RH, Sydow K, Borlak J, Thum T, Lenzen H, Schubert B, Tsikas D, Bode-Böger SM (2000) LDL cholesterol upregulates synthesis of asymmetrical dimethylarginine in human endothelial cells: involvement of S-adenosylmethionine-dependent methyltransferases. Circ Res 87:99–105
6. Closs EI, Basha FZ, Habermeier A, Förstermann U (1997) Interference of L-arginine analogues with L-arginine transport mediated by the y+ carrier hCAT-2B. Nitric Oxide 1:65–73
7. Valkonen V-P, Päivä H, Salonen JT, Lakka TA, Lehtimäki T, Laakso J, Laaksonen R (2001) Risk of acute coronary events and serum concentration of asymmetrical dimethylarginine. Lancet 358:2127–2128
8. Böger RH, Zoccali C (2003) ADMA: a novel risk factor that explains excess cardiovascular event rate in patients with end-stage renal disease. Atheroscler Suppl 4:23–28
9. Leong T, Zylberstein D, Graham I, Lissner L, Ward D, Fogarty J, Bengtsson C, Bjorkelund C, Thelle D (2008) Asymmetric dimethylarginine independently predicts fatal and nonfatal myocardial infarction and stroke in women: 24-year follow-up of the population study of women in Gothenburg. Arterioscler Thromb Vasc Biol 28:961–967
10. Ogawa T, Kimoto M, Watanabe H, Sasaoka K (1987) Metabolism of NG, NG- and NG, N'G-dimethylarginine in rats. Arch Biochem Biophys 252:526–537
11. MacAllister RJ, Rambausek MH, Vallance P, Williams D, Hoffmann KH, Ritz E (1996) Concentration of dimethyl-L-arginine in the plasma of patients with end-stage renal failure. Nephrol Dial Transplant 11:2449–2452
12. Zoccali C, Benedetto FA, Maas R, Mallamaci F, Tripepi G, Malatino LS, Böger R, CREED Investigators (2002) Asymmetric dimethylarginine, C-reactive protein, and carotid intima-media thickness in end-stage renal disease. J Am Soc Nephrol 13:490–496
13. Jones LC, Tran CTL, Leiper JM, Hingorani AD, Vallance P (2003) Common genetic variation in a basal promoter element alters DDAH2 expression in endothelial cells. Biochem Biophys Res Commun 310:836–843
14. Valkonen V-P, Tuomainen T-P, Laaksonen R (2005) DDAH gene and cardiovascular risk. Vasc Med 10:S45–S48
15. Suhre K, Wallaschofski H, Raffler J, Friedrich N, Haring R, Michael K, Wasner C, Krebs A, Kronenberg F, Chang D, Meisinger C, Wichmann H-E, Hoffmann W, Völzke H, Völker U, Teumer A, Biffar R, Kocher T, Felix SB, Illig T, Kroemer HK, Gieger C, Römisch-Margl W, Nauck M (2011) A genome-wide association study of metabolic traits in human urine. Nat Genet 43:565–569
16. Kittel A, Müller F, König J, Mieth M, Sticht H, Zolk O, Kralj A, Heinrich MR, Fromm MF, Maas R (2014) Alanine-glyoxylate aminotransferase 2 (AGXT2) polymorphisms have considerable impact on methylarginine and β-aminoisobutyrate metabolism in healthy volunteers. PLoS ONE 9:e88544
17. Gad MZ, Abdel Rahman MF, Hashad IM, Abdel-Maksoud SM, Farag NM, Abou-Aisha K (2012) Endothelial nitric oxide synthase (G894T) gene polymorphism in a random sample of the Egyptian population: comparison with myocardial infarction patients. Genet Test Mol Biomark 16:695–700
18. Abu el Maaty MA, Hassanein SI, Sleem HM, Gad MZ (2015) Vitamin D receptor gene polymorphisms (TaqI and ApaI) in relation to 25-hydroxyvitamin D levels and coronary artery disease incidence. J Recept Signal Transduct 35:391–395
19. Abu El Maaty MA, Hassanein SI, Sleem HM, Gad MZ (2013) Effect of polymorphisms in the NADSYN1/DHCR7 locus (rs12785878 and rs1790349) on plasma 25-hydroxyvitamin D levels and coronary artery disease incidence. J Nutrigenet Nutrigenomics 6:327–335
20. Abu el Maaty MA, Hassanein SI, Gad MZ (2016) Genetic variation in vitamin D receptor gene (Fok1:rs2228570) is associated with risk of coronary artery disease. Biomarkers 21:68–72
21. Rahman MFA, Hashad IM, Abou-Aisha K, Abdel-Maksoud SM, Gad MZ (2015) Addressing the link between paraoxonase-1 gene variants and the incidence of early onset myocardial infarction. Arch Med Sci 11:513–520
22. Hassanein SI, Abu el Maaty MA, Sleem HM, Gad MZ (2014) Triangular relationship between single nucleotide polymorphisms in the CYP2R1 gene (rs10741657 and rs12794714), 25-hydroxyvitamin d levels, and coronary artery disease incidence. Biomarkers 19:488–492
23. Hashad IM, Abdel Rahman MF, Abdel-Maksoud SM, Amr KS, Effat LK, Shaban GM, Gad MZ (2014) C242T polymorphism of NADPH oxidase p22phox gene reduces the risk of coronary artery disease in a random sample of Egyptian population. Mol Biol Rep 41:2281–2286
24. Gaunt TR, Rodríguez S, Day IN (2007) Cubic exact solutions for the estimation of pairwise haplotype frequencies: implications for linkage disequilibrium analyses and a web tool "CubeX". BMC Bioinform 8:428
25. Seppala I, Kleber ME, Lyytikainen L-P, Hernesniemi JA, Makela K-M, Oksala N, Laaksonen R, Pilz S, Tomaschitz A, Silbernagel G, Boehm BO, Grammer TB, Koskinen T, Juonala M, Hutri-Kahonen N, Alfthan G, Viikari JSA, Kahonen M, Raitakari OT,

Marz W, Meinitzer A, Lehtimaki T (2014) Genome-wide association study on dimethylarginines reveals novel AGXT2 variants associated with heart rate variability but not with overall mortality. Eur Heart J 35:524–531
26. Martens-Lobenhoffer J, Westphal S, Awiszus F, Bode-Böger SM, Luley C (2005) Determination of asymmetric dimethylarginine: liquid chromatography-mass spectrometry or ELISA? Clin Chem 51:2188–2189
27. Schulze F, Maas R, Freese R, Schwedhelm E, Silberhorn E, Boger RH (2005) Determination of a reference value for NG, NG-dimethyl-L-arginine in 500 subjects. Eur J Clin Invest 35:622–626
28. Atzler D, Schwedhelm E, Nauck M, Ittermann T, Böger RH, Friedrich N (2014) Serum reference intervals of homoarginine, ADMA, and SDMA in the Study of Health in Pomerania. Clin Chem Lab Med 52:1835–1842
29. Sobczak A, Goniewicz ML, Szoltysek-Boldys I (2009) ADMA and SDMA levels in healthy men exposed to tobacco smoke. Atherosclerosis 205:357–359
30. Gad MZ, Hassanein SI, Abdel-Maksoud SM, Shaban GM, Abou-Aisha K, Elgabarty HA (2010) Assessment of serum levels of asymmetric dimethylarginine, symmetric dimethylarginine and L-arginine in coronary artery disease. Biomarkers 15:746–752
31. Boelaert J, Schepers E, Glorieux G, Eloot S, Vanholder R, Lynen F (2016) Determination of asymmetric and symmetric dimethylarginine in serum from patients with chronic kidney disease: UPLC-MS/MS versus ELISA. Toxins (Basel) 8:149
32. Sitia S, Tomasoni L, Atzeni F, Ambrosio G, Cordiano C, Catapano A, Tramontana S, Perticone F, Naccarato P, Camici P, Picano E, Cortigiani L, Bevilacqua M, Milazzo L, Cusi D, Barlassina C, Sarzi-Puttini P, Turiel M (2010) From endothelial dysfunction to atherosclerosis. Autoimmun Rev 9:830–834
33. Lu T, Ding Y-A, Lin S-J, Lee W-S, Tai H-C (2003) Plasma levels of asymmetrical dimethylarginine and adverse cardiovascular events after percutaneous coronary intervention. Eur Heart J 24:1912–1919
34. Leong T, Zylberstein D, Graham I, Lissner L, Ward D, Fogarty J, Bengtsson C, Bjorkelund C, Thelle D, Swedish-Irish-Norwegian Collaboration (2008) Asymmetric dimethylarginine independently predicts fatal and nonfatal myocardial infarction and stroke in women: 24-year follow-up of the population study of women in Gothenburg. Arterioscler Thromb Vasc Biol 28:961–967
35. Hu XL, Li MP, Song PY, Tang J, Chen XP (2017) AGXT2: An unnegligible aminotransferase in cardiovascular and urinary systems. J Mol Cell Cardiol 113:33–38
36. Notsu Y, Yano S, Shibata H, Nagai A, Nabika T (2015) Plasma arginine/ADMA ratio as a sensitive risk marker for atherosclerosis: Shimane CoHRE study. Atherosclerosis 239:61–66
37. Usui M, Matsuoka H, Miyazaki H, Ueda S, Okuda S, Imaizumi T (1998) Increased endogenous nitric oxide synthase inhibitor in patients with congestive heart failure. Life Sci 62:2425–2430
38. Hsu C-P, Lin S-J, Chung M-Y, Lu T-M (2012) Asymmetric dimethylarginine predicts clinical outcomes in ischemic chronic heart failure. Atherosclerosis 225:504–510
39. Khalifa NM, Gad MZ, Hataba AA, Mahran LG (2012) Changes in ADMA and TAFI levels after stenting in coronary artery disease patients. Mol Med Rep 6:855–859
40. Bode-Böger SM, Scalera F, Kielstein JT, Martens-Lobenhoffer J, Breithardt G, Fobker M, Reinecke H (2006) Symmetrical dimethylarginine: a new combined parameter for renal function and extent of coronary artery disease. J Am Soc Nephrol 17:1128–1134
41. Siegerink B, Maas R, Vossen CY, Schwedhelm E, Koenig W, Böger R, Rothenbacher D, Brenner H, Breitling LP (2013) 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 102:193–202
42. Schulze F, Carter AM, Schwedhelm E, Ajjan R, Maas R, von Holten R-A, Atzler D, Grant PJ, Böger RH (2010) Symmetric dimethylarginine predicts all-cause mortality following ischemic stroke. Atherosclerosis 208:518–523
43. Staniszewska A, Rajagopalan S, Al-Shaheen A, Thies F, Brittenden J (2015) Increased levels of symmetric dimethyl-arginine are associated with all-cause mortality in patients with symptomatic peripheral arterial disease. J Vasc Surg 61:1292–1298
44. Wang J, Sim AS, Wang XL, Salonikas C, Moriatis M, Naidoo D, Wilcken DEL (2008) Relations between markers of renal function, coronary risk factors and the occurrence and severity of coronary artery disease. Atherosclerosis 197:853–859
45. Yoshino Y, Kohara K, Abe M, Ochi S, Mori Y, Yamashita K, Igase M, Tabara Y, Mori T, Miki T, Ueno S (2014) Missense variants of the alanine: glyoxylate aminotransferase 2 gene correlated with carotid atherosclerosis in the Japanese population. J Biol Regul Homeost Agents 28:605–614
46. Seppälä I, Kleber ME, Bevan S, Lyytikäinen L-P, Oksala N, Hernesniemi JA, Mäkelä K-M, Rothwell PM, Sudlow C, Dichgans M, Mononen N, Vlachopoulou E, Sinisalo J, Delgado GE, Laaksonen R, Koskinen T, Scharnagl H, Kähönen M, Markus HS, März W, Lehtimäki T (2016) Associations of functional alanine-glyoxylate aminotransferase 2 gene variants with atrial fibrillation and ischemic stroke. Sci Rep 6:23207
47. Zhou J-P, Bai Y-P, Hu X-L, Kuang D-B, Shi R-Z, Xiong Y, Zhang W, Xia J, Chen B-L, Yang T-L, Chen X-P (2014) Association of the AGXT2 V140I polymorphism with risk for coronary heart disease in a Chinese population. J Atheroscler Thromb 21:1022–1030
48. Hu X-L, Zhou J-P, Kuang D-B, Qi H, Peng L-M, Yang T-L, Li X, Zhang W, Zhou H-H, Chen X-P (2016) Considerable impacts of AGXT2 V140I polymorphism on chronic heart failure in the Chinese population. Atherosclerosis 251:255–262
49. Seppälä I, Kleber ME, Bevan S, Lyytikäinen L-P, Oksala N, Hernesniemi JA, Mäkelä K-M, Rothwell PM, Sudlow C, Dichgans M, Mononen N, Vlachopoulou E, Sinisalo J, Delgado GE, Laaksonen R, Koskinen T, Scharnagl H, Kähönen M, Markus HS (2016) Associations of functional alanine-glyoxylate aminotransferase 2 gene variants with atrial fibrillation and ischemic stroke. Sci Rep. https://doi.org/10.1038/srep23207
50. Luneburg N, Lieb W, Zeller T, Chen M-H, Maas R, Carter AM, Xanthakis V, Glazer NL, Schwedhelm E, Seshadri S, Ikram MA, Longstreth WT, Fornage M, Konig IR, Loley C, Ojeda FM, Schillert A, Wang TJ, Sticht H, Kittel A, Konig J, Benjamin EJ, Sullivan LM, Bernges I, Anderssohn M, Ziegler A, Gieger C, Illig T, Meisinger C, Wichmann H-E, Wild PS, Schunkert H, Psaty BM, Wiggins KL, Heckbert SR, Smith N, Lackner K, Lunetta KL, Blankenberg S, Erdmann J, Munzel T, Grant PJ, Vasan RS, Boger RH (2014) Genome-Wide Association Study of L-arginine and dimethylarginines reveals novel metabolic pathway for symmetric dimethylarginine. Circ Cardiovasc Genet 7:864–872
51. Ding H, Wu B, Wang H, Lu Z, Yan J, Wang X, Shaffer JR, Hui R, Wang DW (2010) A novel loss-of-function DDAH1 promoter polymorphism is associated with increased susceptibility to thrombosis stroke and coronary heart disease. Circ Res 106:1145–1152
52. Anderssohn M, McLachlan S, Lüneburg N, Robertson C, Schwedhelm E, Williamson RM, Strachan MWJ, Ajjan R, Grant PJ, Böger RH, Price JF (2014) Genetic and environmental determinants of dimethylarginines and association with cardiovascular disease in patients with type 2 diabetes. Diabetes Care 37:846–854
53. Abhary S, Burdon KP, Kuot A, Javadiyan S, Whiting MJ, Kasmeridis N, Petrovsky N, Craig JE (2010) Sequence variation in DDAH1 and DDAH2 genes is strongly and additively associated with serum ADMA concentrations in individuals with type 2 diabetes. PLoS ONE 5:e9462