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CLINICAL RESEARCH
Atrial fibrillation

Genome-wide association study on dimethylarginines reveals novel *AGXT2* variants associated with heart rate variability but not with overall mortality

Ilkka Seppälä[1†*], Marcus E. Kleber[2,3†], Leo-Pekka Lyytikäinen[1], Jussi A. Hernesniemi[1,4], Kari-Matti Mäkelä[1], Niku Oksala[1,5], Reijo Laaksonen[1], Stefan Pilz[6,7], Andreas Tomaschitz[8,9], Günther Silbernagel[10], Bernhard O. Boehm[11,12], Tanja B. Grammer[3], Tuomas Koskinen[13], Markus Juonala[13,14], Nina Hutri-Kähönen[15], Georg Alfthan[16], Jorma S.A. Viikari[14], Mika Kähonen[17], Olli T. Raitakari[13,18], Winfried März[3,19,20], Andreas Meinitzer[20‡], and Terho Lehtimäki[1‡], for AtheroRemo Consortium

[1]Department of Clinical Chemistry, Fimlab Laboratories, Tampere University School of Medicine, Finn-Medi 2, 3rd floor, PO Box 2000, Tampere FI-33521, Finland; [2]Department of Internal Medicine II—Cardiology, University of Ulm Medical Centre, Ulm, Germany; [3]Institute of Public Health, Social and Preventive Medicine, Mannheim Medical Faculty, University of Heidelberg, Mannheim, Germany; [4]Department of Internal Medicine, North Karelia Central Hospital, Joensuu, Finland; [5]Division of Vascular Surgery, Department of Surgery, Tampere University Hospital, Tampere, Finland; [6]Department of Epidemiology and Biostatistics, EMGO Institute for Health and Care Research, VU University Medical Center, Amsterdam, The Netherlands; [7]Department of Internal Medicine, Division of Endocrinology and Metabolism, Medical University of Graz, Graz, Austria; [8]Department of Cardiology, Medical University of Graz, Graz, Austria; [9]Specialist Clinic for Rehabilitation PV Bad Aussee, Bad Aussee, Austria; [10]Department of Angiology, Swiss Cardiovascular Center, Inselspital, University of Bern, Bern, Switzerland; [11]Division of Endocrinology and Diabetes, Department of Internal Medicine, Ulm University, Ulm, Germany; [12]LKC School of Medicine, Imperial College London and NTU, Singapore; [13]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland; [14]Department of Medicine, Turku University Hospital and University of Turku, Turku, Finland; [15]Department of Paediatrics, University of Tampere and Tampere University Hospital, Tampere, Finland; [16]Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland; [17]Department of Clinical Physiology, Tampere University Hospital and University of Tampere, Tampere, Finland; [18]Department of Clinical Physiology and Nuclear Medicine, Turku University Hospital, Turku, Finland; [19]Synlab Services GmbH, Mannheim, Germany; and [20]Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria

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Aims The purpose of this study was to identify novel genetic variants influencing circulating asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) levels and to evaluate whether they have a prognostic value on cardiovascular mortality.

Methods and results We conducted a genome-wide association study on the methylarginine traits and investigated the predictive value of the new discovered variants on mortality. Our meta-analyses replicated the previously known locus for ADMA levels in *DDAH1* (rs997251; $P = 1.4 \times 10^{-40}$), identified two non-synomyous polymorphisms for SDMA levels in *AGXT2* (rs37369; $P = 1.4 \times 10^{-40}$ and rs16899974; $P = 1.5 \times 10^{-38}$) and one in *SLC25A45* (rs34400381; $P = 2.5 \times 10^{-10}$). We also fine-mapped the *AGXT2* locus for further independent association signals. The two non-synonymous *AGXT2* variants independently associated with SDMA levels were also significantly related with short-term heart rate variability (HRV) indices in young adults. The major allele (C) of the novel non-synonymous rs16899974 (V498L) variant associated with decreased SDMA levels and an increase in the ratio between the low- and high-frequency spectral components of HRV ($P = 0.00047$). Furthermore, the SDMA decreasing allele (G) of the non-synomyous *SLC25A45* (R285C) variant was associated with a lower resting mean heart rate during the HRV measurements ($P = 0.0046$), but not with the

† I.S. and M.E.K. equally contributed.

‡ A.M. and T.L. share senior authorship.

* Corresponding author. Tel: +358 3 3117 4048, Fax: +358 3 31174168, Email: ilkka.seppala@uta.fi

HRV indices. None of the studied genome-wide significant variants had any major effect on cardiovascular or total mortality in patients referred for coronary angiography.

Conclusions AGXT2 has an important role in SDMA metabolism in humans. AGXT2 may additionally have an unanticipated role in the autonomic nervous system regulation of cardiac function.

Keywords Genome-wide association study • Asymmetric dimethylarginine • Symmetric dimethylarginine • Genetics • Heart rate variability • Mortality • Sudden cardiac death

Introduction

Increasing evidence shows that both asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) might have independent roles in cardiovascular diseases[1] and the prediction of cardiovascular events.[2,3] Elevated circulating SDMA levels have been reported to be associated with increased mortality in stroke patients,[4] an increased risk of cardiac death in patients with non-ST-segment elevation myocardial infarction,[5] and worse prognosis in patients referred for coronary angiography.[3] Indeed, increasing *in vitro* and *in vivo* evidence support the concept that SDMA may have an independent role in the pathogenesis of cardiovascular diseases. Symmetric dimethylarginine induces reactive oxygen species production of monocytes[6] and promotes inflammation in chronic kidney disease.[7] Moreover, recent data show that SDMA may promote endothelial dysfunction.[8]

Previous candidate gene studies have identified a limited number of single-nucleotide polymorphisms (SNPs) associated with plasma ADMA levels in the dimethylarginine dimethylaminohydrolase-1 and -2 (*DDAH1* and *DDAH2*) loci,[9] whereas the genetic variants regulating SDMA levels are not known. There is evidence that AGXT2 plays a role in both ADMA and SDMA metabolisms *in vivo*,[10] but association data between genetic variants and circulating methylarginine levels in humans are currently lacking. Furthermore, AGXT2 knockout mice show a hypertensive phenotype, but the underlying mechanisms behind this observation are not currently well understood.[11] To investigate the role of AGXT2 in health and disease in humans, the identification of the functional genetic variants affecting the enzymatic activity of the AGXT2 protein and the regulation of its expression in different human tissues is of major importance.

Therefore, to identify additional new loci for ADMA, and especially for SDMA, we performed the first meta-analyses of genome-wide association (GWA) studies for the methylarginines using data from 5110 participants from the Young Finns Study (YFS) and the Ludwigshafen Risk and Cardiovascular Health (LURIC) study. Secondly, we fine-mapped the *AGXT2* locus, that showed the strongest association with SDMA, for variants independently affecting circulating SDMA levels. Finally, we tested the association of these variants with mortality endpoints in LURIC and heart rate variability (HRV) parameters in YFS to be able to characterize the pathophysiological role of these new *AGXT2* variants.

Methods

The study flow is summarized in *Figure 1*.

Study populations

We conducted a meta-analysis of GWA data on 5110 individuals of European descent drawn from two large cohorts: the YFS and the LURIC study. The YFS cohort is a Finnish longitudinal population study sample on the evolution of cardiovascular risk factors from childhood to adulthood.[12] In the present study, we used the variables measured in 2001. The LURIC study consists of 3316 Caucasian patients hospitalized for coronary angiography between 1997 and 2000 at a tertiary care centre in Southwestern Germany.[13] Clinical indications for angiography were chest pain or a positive non-invasive stress test suggestive of myocardial ischaemia. To limit clinical heterogeneity, individuals suffering from acute illnesses other than acute coronary syndrome, chronic non-cardiac diseases, and a history of malignancy within the 5 past years were excluded. See further description and characteristics in Supplementary material online, Methods S1 and *Table S1*.

Biochemical

Asymmetric dimethylarginine and symmetric dimethylarginine were measured from frozen serum using the same reversed-phase high-performance liquid chromatography method in both studies. For

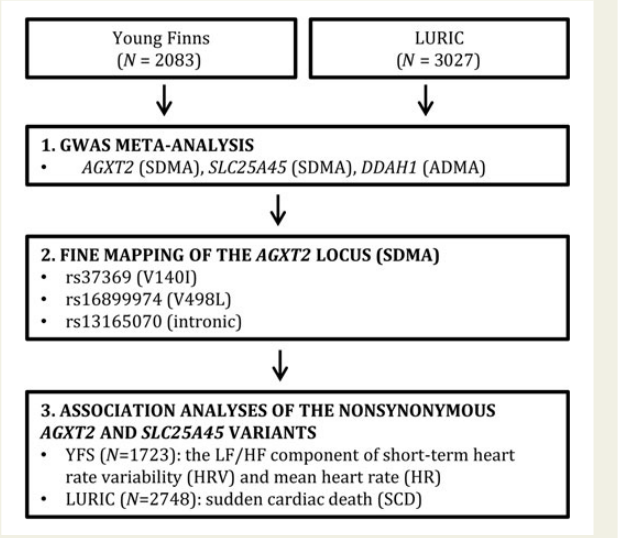

Figure 1 Study population and flow. Flow diagram depicting the cohorts and numbers of participants. The associations of the *AGXT2* variants identified in the conditional analysis with heart rate variability and sudden cardiac death were investigated in the Young Finns Study and Ludwigshafen Risk and Cardiovascular Health cohorts, respectively.

details and other biochemical measurements, see Supplementary material online, Methods S2.

Genotyping and quality control

In LURIC, genotyping was done by using the Affymetrix Human SNP Array 6.0 at the LURIC Study facility. In the YFS, genotyping was done using a custom-built Illumina Human 670k BeadChip at the Welcome Trust Sanger Institute (Supplementary material online, Methods S3). Genotype imputation was performed using minimac[14] and IMPUTE2[15] in LURIC and YFS, respectively. Genotype imputation in both studies was performed using 1000 Genomes Phase I Integrated Release Version 3 (March 2012) samples as a reference. We excluded imputed SNPs and insertion–deletion polymorphisms with low imputation quality (minimac Rsq <0.3 or IMPUTE info <0.4) and allele frequency of $<0.1\%$. After the exclusions, 10 085 758 imputed genetic variants were available in both studies.

Short-term heart rate variability

We used the ratio between low- and high-frequency components (LF/HF) of spectral HRV to estimate sympathovagal balance in the YFS participants. The mean heart rate during HRV measurement was used as an estimate of the resting heart rate. Breathing was metronome controlled at the frequency of 0.25 Hz. We excluded subjects with current antihypertensive medications, Type 1 diabetes, pregnant women, and breastfeeding women. After these exclusions, genotype data were available for 1723 subjects and were included in the association analyses. For details, see Supplementary material online, Methods S4.

Classification of SCD cases

In LURIC, sudden cardiac death (SCD) was defined as sudden unexpected death either within 1 h of symptom onset or within 24 h of having been observed alive and symptom-free. Patients who suffered from any non-cardiac chronic and terminal disease (e.g. cancer), so that their death was not unexpected, and those whose sudden death was most likely attributed to a non-cardiac cause were not classified as SCD cases. See Supplementary material online, Methods S4 for more details about the cause of death classifications.

Statistical analysis

To identify and correct for population stratification, we performed an MDS analysis as implemented in PLINK 1.07.[16] In both studies, residual ADMA and SDMA concentrations or residuals of ADMA/SDMA ratio were determined after regression adjustment using the R software. Outliers ($\geq$4 SD from the mean) were removed from the dimethylarginine phenotype values before covariate adjustment to avoid spurious associations (typically 5–20 data points for any one outcome). Residuals were obtained using linear regression analysis in which the dimethylarginine phenotypes were adjusted for sex, age, body mass index, and serum creatinine to account for variation in renal function, as well as the first three MDS components to control for population stratification. As circulating ADMA is a major determinant of SDMA levels and vice versa,[3] the model for ADMA was additionally adjusted for SDMA and that for SDMA was adjusted for ADMA. Residuals were normalized to have a mean of 0 and SD of 1 using inverse-normal transformation. The association analysis of the imputed SNPs and indels assuming an additive genetic model was carried out utilizing the SNPTEST v2.4.1[17] and ProbABEL v. 0.3.0[18] softwares in YFS and LURIC, respectively. Quantile-quantile and Manhattan plots were drawn for the analysis of the results. To estimate how well the distribution was calibrated, for each phenotype in both studies, we calculated the genomic inflation factor (λ) from all imputed variants. The λ for each trait in both studies ranged from 0.993 to 1.012, suggesting that there was no major residual confounding by population stratification, and little evidence of cryptic relatedness. We created the regional association plots using LocusZoom.[19] The summary results from both cohorts were meta-analyzed with the random-effects meta-analysis method implemented in GWAMA.[20] A stringent genome-wide significance level of 1.66×10^{-8} was set to correct for multiple testing of the three phenotypes.

We fine-mapped the *AGXT2* locus using a series of sequential conditional analyses by adding the most strongly associated SNP into the regression model as a covariate and using the inverse-normal transformed residuals as an outcome variable to test all remaining regional SNPs for association. The results from both studies were combined using a random-effects meta-analysis. We repeated this procedure until the strongest SNP showed a conditional *P*-value of $>10^{-4}$.

Further statistical analyses were performed using R 3.0.0 (http://www.r-project.org). A Cox regression proportional hazards analysis was performed to test whether the *AGXT2* and *SLC25A45* genotypes were associated with mortality endpoints. The risk of mortality was quantified as hazard ratios (HRs). The proportional hazards assumption was checked with Schoenfeld's residuals and was met for all models. The screening tests were conducted using age and sex as covariates and an additive genetic model. Further analyses were performed adjusting for well-established SCD risk factors. Secondly, to investigate the possible mechanism underlying the associations with SCD, analyses were stratified according to the median value of left ventricular systolic pressure (LVSP). Finally, the associations with HRV variables were tested using linear regression analysis. Genotyped data were used for rs37369 in LURIC. For all other SNPs and analyses, imputed data were used. Imputation quality parameters and minor allele frequencies for *AGXT2* and *SLC25A45* SNPs are provided in Supplementary material online, *Table S2*.

Results

Genome-wide association studies for dimethylarginines and their ratio

We carried out GWA meta-analyses on ADMA, SDMA, and their ratio using data from the YFS and LURIC populations, involving a total of 5110 individuals of European ancestry. The strongest associations for each locus from the meta-analyses are summarized in *Table 1*, with an index SNP representing the most significant association labelled for each independent signal. Across the genome, the most significant association for ADMA levels ($P = 1.4 \times 10^{-40}$) was on chromosome 1p22 harbouring *DDAH1*, which encodes the key regulatory enzyme of ADMA metabolism DDAH. The most significant signal for SDMA levels ($P = 1.4 \times 10^{-40}$) was on chromosome 5p13 at rs37369 (V140I) in the coding sequence of alanine–glyoxylate aminotransferase 2 (*AGXT2*). Another genome-wide significant signal for SDMA levels ($P = 2.5 \times 10^{-10}$) was identified in the coding sequence of a mitochondrial carrier protein *SLC25A45* at rs34400381 (R285C). A list of suggestive signals for each trait not reaching genome-wide significance is provided in Supplementary material online, *Tables S4* and *S5*.

Fine-mapping of the *AGXT2* region

Next, we fine-mapped the newly identified *AGXT2* region showing the strongest association with SDMA for additional independent signals using sequential conditional analysis. Of the methylarginine traits, SDMA was used as an outcome variable in this analysis,

Table I Genome-wide significant associations for each methylarginine trait in the meta-analyses

Trait	Variant	Locus	Chr.	Position	EA	NEA	EAF (%)	*n*	β (SE)	*P*-value
ADMA	rs28489187	Intron of *DDAH1*	1	85 797 110	G	A	21.6	5105	0.37 (0.028)	1.39×10^{-40}
SDMA	rs37369	CDS of *AGXT2* (V140I)	5	35 037 115	T	C	8.8	5103	0.47 (0.035)	1.42×10^{-40}
SDMA	rs1884139	Intron of *DDAH1*	1	85 845 998	T	G	37.1	5103	−0.18 (0.020)	9.65×10^{-19}
SDMA	rs34400381	CDS of *SLC25A45* (R285C)	11	65 143 892	A	G	4.3	5102	0.36 (0.056)	2.48×10^{-10}
ADMA/SDMA	rs37369	CDS of *AGXT2* (V140I)	5	35 037 115	T	C	8.8	5102	−0.41 (0.035)	2.08×10^{-32}
ADMA/SDMA	chr1:85897175:D	Intron of *DDAH1*	1	85 897 175	A	ATCC	17.4	5102	0.27 (0.027)	3.85×10^{-24}

Chr., chromosome; position, variant position in NCBI human build 37; EA and NEA, effect allele and non-effect allele; EAF, effect allele frequency; CDS, coding sequence; β, effect size in standard deviations per copy of the effect allele, ADMA, asymmetric dimethylarginine; SDMA, symmetric dimethylarginine.

because it showed the strongest association with *AGXT2* variants in the original meta-analysis. Interestingly, another independent genome-wide significant association was pinpointed at rs16899974 (V498L) when conditioning on the top SNP at rs37369 (V140I) (*Figure* 2A and B). In the next step, when adjusting for the two missense mutations (rs37369 and rs16899974), a number of intronic variants showed significant association ($P < 10^{-4}$) with SDMA after correcting for multiple testing in the *AGXT2* region (*Figure* 2C). No other informative ($P < 10^{-4}$) intronic or coding sequence *AGXT2* variants were identified when further adjusting for the top intronic variant rs13165070 (*Figure* 2D).

Association of non-synonymous *AGXT2* variants with heart rate variability in young adults (Young Finns Study)

The SDMA decreasing alleles of the studied *AGXT2*-coding variants were associated with an increase in the ratio between LF and HF spectral components of the short-term HRV in the YFS (*Table* 2). The association was especially marked between the novel non-synonymous rs16899974 (V498L) variant and the LF/HF ratio ($P = 0.00047$). None of the variants showed an association with the LF component (always $P > 0.20$). Instead, rs16899974 (V498L) variant showed a significant association ($P = 0.028$) with the HF component (estimate of vagal tone). The non-synonymous *SLC25A45* rs34400381 (R285C) variant was not associated with the LF/HF component ($P = 0.39$), but the SDMA decreasing allele (G) was associated with a decrease in the resting mean heart rate during the HRV measurements independent of the LF/HF ratio (*Table* 2). Further adjustment for serum ADMA or SDMA levels did not change our results.

Associations between non-synonymous *AGXT2* and *SLC25A45* variants with mortality in Ludwigshafen Risk and Cardiovascular Health

Finally, we investigated whether the non-synonymous *AGXT2*- and *SLC25A45*-coding variants independently associated with AGXT2 substrate levels could also predict mortality among patients referred for coronary angiography. None of the studied non-synonymous *AGXT2* or *SLC25A45* SNPs were associated with all-cause or cardiovascular mortality (always $P > 0.05$). However, the SDMA decreasing major allele (G) of the *SLC25A45* polymorphism (rs34400381, R285C) was associated with an increased risk for SCD with per allele HR of 13.0 (95% CI 1.54–110, $P = 0.018$). The two non-synonymous *AGXT2* variants were not associated with SCD in the whole study population. However, both variants showed a significant interaction with LVSP on SCD (*Table 3*). *AGXT2* rs16899974 (V498L) showed a nominally significant associated with SCD in the low LVSP group ($P = 0.021$), whereas there was a trend towards an association for rs37369 ($P = 0.10$). Analogously to the association for the *SLC25A45* variant (R285C), the SDMA decreasing alleles were associated with an increased risk for SCD. No associations were observed between the *AGXT2* variants and SCD in the high LVSP group (all $P > 0.20$), and there was no significant interaction between *SLC25A45* rs34400381 and the LVSP status on SCD ($P = 0.63$). Additional adjustments for circulating ADMA or SDMA did not materially change our results.

Discussion

We report the first GWAS results on circulating ADMA and SDMA levels based on meta-analyses of 5110 individuals of European ancestry, and provide strong evidence that two common non-synonymous-coding variants in *AGXT2* and one non-synonymous-coding variant in *SLC25A45* (R285C) are independently associated with circulating SDMA levels. Moreover, we document that the non-synonymous *AGXT2* and *SLC25A45* variants showed associations with the LF/HF component of spectral HRV and resting heart rate in young adults, respectively.

The polymorphism (rs37369) with the strongest association with circulating SDMA codes for a non-synonymous valine-to-isoleucine (V140I) substitution in the AGXT2 protein. Another non-synomyous *AGXT2* rs16899974 (V498L) SNP and a number of intronic variants were also associated independently with SDMA levels. The lead intronic variant (rs13165070) identified in the conditional analysis is in a high linkage disequilibrium ($r^2 = 0.9$) with intronic rs10521021 that has been previously associated with *AGXT2* mRNA levels in the human liver.[21] The T allele of rs37369 is associated with both increased circulating SDMA levels in the present study and increased levels of urinary 3-aminoisobutyrate (BAIB) metabolite.[22] These results together refer to the possibility that the two novel non-synomyous *AGXT2* variants may contribute to plasma SDMA levels through the enzymatic decomposition of SDMA in the kidneys. Indeed, *AGXT2*

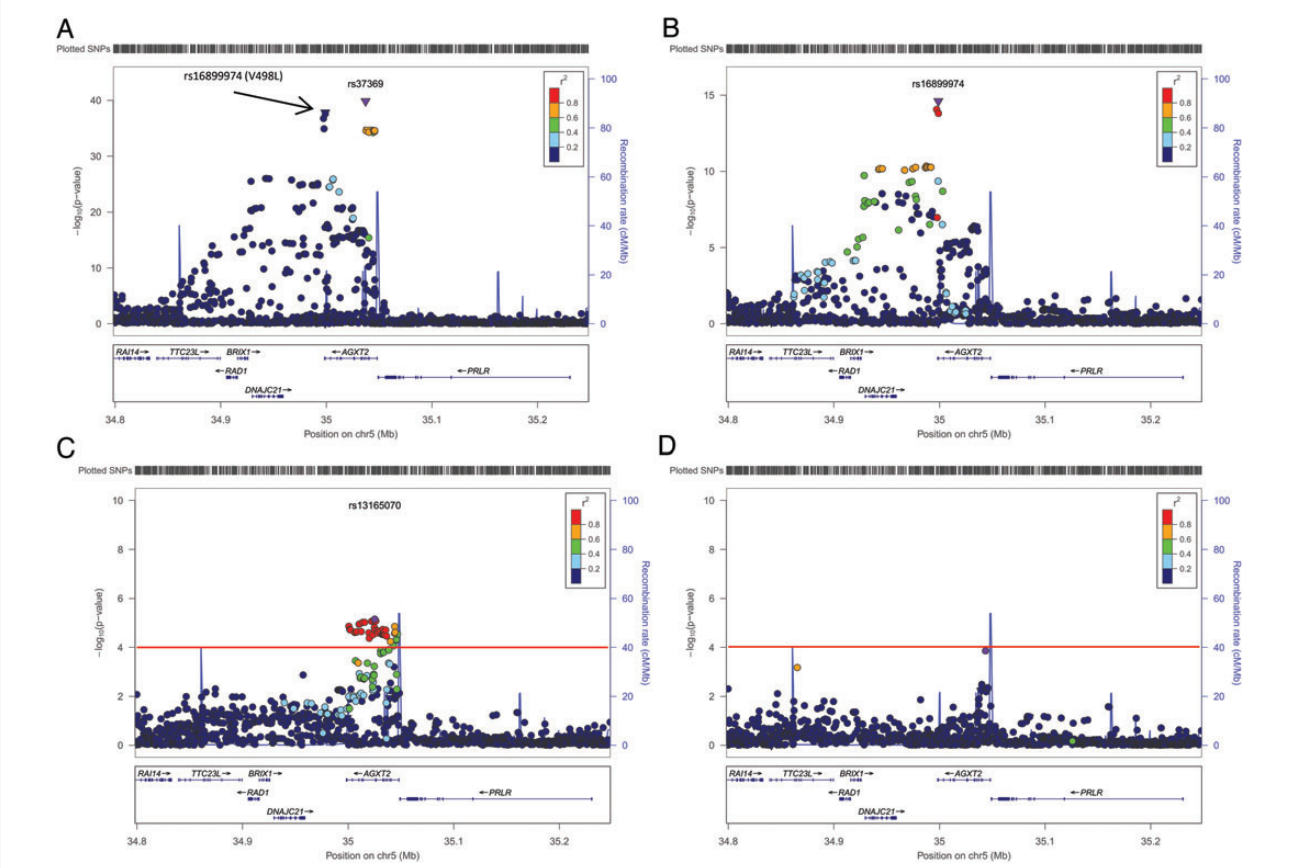

Figure 2 Regional plots for fine-mapping results in the *AGXT2* region. Fine-mapping of the *AGXT2* region for independent association signals using sequential conditional analysis and circulating symmetric dimethylarginine levels as an outcome variable. Original meta-analysis (*A*), adjusted for rs37369 (V140I) (*B*), additionally adjusted for rs16899974 (V498L) (*C*), and additionally adjusted for rs13165070 (*D*). Signals above the red line ($P < 1 \times 10^{-4}$) were considered to exhibit evidence of association in the *AGXT2* region.

Table 2 Associations between non-synonomyous *AGXT2* and *SLC25A45* variants with symmetric dimethylarginine, heart rate variability, and resting heart rate

SNP	Gene	Effect allele	EAF (%)	GWAS meta-analysis ($n = 5110$)		Young Finns ($n = 1723$)			
				SDMA		lnLF/HF		Mean HR (b.p.m.)	
				β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
rs37369 (V140I)	*AGXT2*	C	91.2	−0.47 (0.035)	1.4×10^{-40}	0.10 (0.052)	0.045	−0.54 (0.58)	0.36
rs16899974 (V498L)	*AGXT2*	C	77.2	−0.32 (0.024)	1.5×10^{-38}	0.13 (0.036)	0.00047	−0.73 (0.41)	0.073
rs34400381 (R285C)	*SLC25A45*	G	95.7	−0.36 (0.056)	2.5×10^{-10}	0.055 (0.064)	0.39	−2.02 (0.71)	0.0046

Per effect allele β (SE) from conditional linear regressions. Effect alleles are SDMA decreasing alleles from the GWAS meta-analysis. Models for the ln-transformed LF/HF component of short-term HRV are adjusted for age, sex, and heart rate. Models for the mean heart rate are adjusted for age, sex, and lnLF/HF.
EAF, effect allele frequency; HR, heart rate; b.p.m., beats per minute; SE, standard error; SNP, single-nucleotide polymorphisms; SDMA, symmetric dimethylarginine; LF/HF, the ratio between the low- and high-frequency spectral components of heart rate variability.

expression in the kidney has recently been shown to be inversely correlated with both circulating ADMA and SDMA levels in humans.[11] SDMA is transaminated to α-keto-δ-(*N*,*N*′-dimethylguanidino)valeric acid (DMGV′) by AGXT2.[23] Our findings support the hypothesis that this mitochondrial[24] AGXT2-related pathway can effectively metabolize SDMA in humans. Moreover, measurable quantities of

Table 3 Associations between non-synonomyous *AGXT2* variants and sudden cardiac death

SNP	Gene	Effect allele	EAF (%)	All ($n = 2748$, 173 SCDs)		LVSP < 145 mmHg ($n = 1220$, 66 SCDs)		LVSP ≥ 145 mmHg ($n = 1199$, 83 SCDs)		*P* for interaction
				HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	
rs37369	*AGXT2*	C	91.2	1.04 (0.70–1.55)	0.83	1.98 (0.88–4.45)	0.10	0.72 (0.44–1.19)	0.20	0.041
rs16899974	*AGXT2*	C	77.2	1.17 (0.87–1.55)	0.30	1.84 (1.10–3.09)	0.021	0.81 (0.55–1.21)	0.31	0.035

Per effect allele HRs (95% CI) from conditional Cox regressions. Effect alleles are SDMA decreasing alleles from the GWAS meta-analysis. All models are adjusted for age, sex, Cystatin C, HbA1c, beta blocker use, left ventricular hypertrophy (ECG), and cardiomyopathy.
SCD, sudden cardiac death; EAF, effect allele frequency; HR, hazard ratio per effect allele increase; CI, confidence interval; LVSP, left ventricular systolic pressure in coronary angiography; SNP, single-nucleotide polymorphism.

α-keto-δ-(*N*,*N*-dimethylguanidino)valeric acid (DMGV), the transamination product of ADMA by AGXT2, was recently reported being present in human plasma and urine.[25]

We observed a genome-wide significant association for SDMA at rs34400381 (R285C) in *SLC25A45* encoding a mitochondrial inner-membrane transporter protein. Interestingly, SLC25A45 has the same intracellular localization than AGXT2, although its function is not well known.[26] The association is a highly genome-wide significant in the YFS ($P = 2.4 \times 10^{-9}$), but only nominally significant in LURIC ($P = 0.026$). This association clearly needs replication in an independent population.

Our data suggests that non-synonymous *AGXT2* variants may have an effect on the autonomic balance mainly by modulating the vagal tone, because *AGXT2* rs16899974 was associated with both the LF/HF ratio and the vagal component HF of HRV alone. The physiological interpretation of LF/HF is not straightforward. The LF component was observed to be correlated with baroreflex function.[27] Moreover, both HF and LF have been shown to be reduced by atropine administration, indicating that both spectral components of HRV are under vagal control,[28] in line with the idea that under resting conditions variations in the heart rate are largely dependent on vagal modulation.[29] Nevertheless, increased LF/HF and decreased HF have been previously associated with increased prevalence of the metabolic syndrome in the YFS cohort.[30] Moreover, decreased HRV appears to be associated with hypertension, dyslipidaemia, impaired glucose metabolism, increased ventricular arrhythmias, and mortality.[31–35] In the present study, we also showed that a non-synonymous *SLC25A45* variant (R285C) was associated with a decreased resting heart rate during the HRV measurements, but not with HRV indices. The observation that the adjustment for circulating methylarginine levels did not change the results suggests that the associations between the *AGXT2*- and *SLC25A45*-coding variants and heart rate regulation could be mediated through transamination of other AGXT2 substrates than methylarginines. Although further studies are needed to understand the molecular mechanisms underlying the associations between heart rate regulation and non-synonymous variants within *AGXT2* and *SLC25A45*, it is tempting to speculate that transamination activity of AGXT2 on γ-aminobutyric acid isomers glycine and/or β-aminoisobutyric acid (BAIB), a partial glycine receptor agonist[36] could play a role explaining the associations. The striking association of *AGXT2* rs37369 with urinary BAIB levels[22] and the presence of glycinergic synaptic inputs to cardiac vagal neurons in the brainstem nuclei[37] support this hypothesis.

None of the genome-wide significant variants were associated with cardiovascular or overall mortality. Interestingly, the AGXT2 substrate (SDMA) decreasing major allele (G) of the non-synonymous *SLC25A45* (R285C) variant was associated with an increased risk for SCD in the whole LURIC population independent of circulation methylarginine levels. However, this association should be interpreted with conscious due to the moderate imputation quality for the variant (Rsq= 0.43). Moreover, we observed significant interactions between the pressure creation capacity of the left ventricle and the two non-synonymous *AGXT2* variants on SCD. We used LVSP to quantify the degree of the systolic dysfunction and heart failure. Both the increased sympathic drive[38] and the parasympathic withdrawal[39] are integral components of congestive heart failure. It has been known for decades that sympathetic activation can trigger fatal ventricular arrhythmias, whereas vagal activity may exert a protective effect.[38] Interestingly, the HF HRV decreasing major allele (C) of rs16899974 was associated with an increased risk for SCD in patients with impaired systolic function, but not in those with preserved LVSP. Although the AGXT2 substrate levels decreasing alleles of these variants are consistently associated with an increased risk for SCD, these results should be considered as explanatory and a replication in an independent population is warranted.

Based on our results, it may be worth investigating whether AGXT2 −/− animals have altered vagal tone compared with wild-type animals, and whether they are protected against ventricular arrhythmia in an experimental model of myocardial infarction (MI)-induced systolic heart failure. The associations with the HRV parameters and SCD should also be investigated in an independent population of MI and heart failure patients.

The present study has several strengths and limitations that warrant consideration. The strengths include large, well-characterized cohorts, the prospective design, and long-term follow-up with prospectively adjudicated SCD cases. The potential limitations include the possibility of some degree of misclassification of SCD cases. However, this kind of bias is likely to lead to an underestimation rather than an overestimation of the true genetic effects. Moreover, the generalizability of the SCD association with the general population and other ethnic groups may be limited. Finally, although we attempted to adjust for

all relevant covariates, we could not rule out the possibility of residual confounding.

In summary, our data clearly indicate that two coding sequences SNPs rs37369 (V140I) and rs16899974 (V498L) in *AGXT2* are associated with circulating SDMA levels in two large cohorts of healthy adults and patients referred to coronary angiography, supporting the idea that AGXT2 has a physiological role in the SDMA metabolism in humans. The two non-synomyous *AGXT2* variants showed associations with the LF/HF component of spectral HRV, whereas the novel non-synomyous *SLC25A45* variant was associated with the resting heart rate in young adults. AGXT2 and SLC25A45 may have unanticipated roles in the regulation of autonomic balance and heart rate in health and disease.

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