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Original research

Association between protein arginine *N*-methyltransferase 1 polymorphism and overt diabetic nephropathy: Role of asymmetric dimethylarginine in vascular tone

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ABSTRACT

Background: ω-N^G,N^G-asymmetric dimethylarginine (ADMA) regulates vascular tone and may participate in the pathogenesis of diabetic nephropathy (DN).
Objective: To investigate whether single-nucleotide polymorphisms (SNPs) around the protein arginine *N*-methyltransferase 1 gene (*PRMT1*) influence ADMA dynamics and DN incidence and severity.
Methods: This study utilized a hospital-based database containing 310 Japanese patients with type 2 diabetes mellitus (T2DM). The association of *PRMT1*-related tagged SNPs with DN stage distribution was examined using a dominant model of minor alleles. *PRMT1* mRNA, serum ADMA, reactive hyperemia-peripheral arterial tonometry index (RHI), and brachial-ankle pulse wave velocity (baPWV) were compared between the genotype-based subgroups of causal SNP, and correlations between these variables were evaluated.
Results: The composition of DN stages significantly differed between the GG and GA + AA subgroups of rs892151 ($p = 0.026$). In a propensity-matching cohort of rs892151, the GA + AA subgroup had an increased incidence of overt DN (odds ratio 2.92, 95 % confidence interval 1.12–7.62, $p = 0.028$), along with higher *PRMT1* mRNA, serum ADMA levels, and baPWV than the GG subgroup ($p < 0.001$, $p = 0.023$ and 0.047, respectively). There were correlations between *PRMT1* mRNA and serum ADMA levels, between serum ADMA levels and RHI, and between baPWV and urinary albumin excretion ($r = 0.335$, $p < 0.001$, $r = -0.221$, $p = 0.029$, and $r = 0.254$, $p = 0.004$, respectively).
Conclusions: T2DM patients carrying the *PRMT1*-related variant rs892151 were susceptible to overt DN. ADMA-mediated endothelial dysfunction and arterial stiffness may be involved in the variant-related pathogenesis of overt DN.

Introduction

Kidneys are terminal organs with low resistance and high flow, and their afferent arterioles undergo hemodynamic stress from upstream large arteries [1]. Systemic endothelial dysfunction and arterial stiffness damage these strain vessels, causing glomerular hypertension followed by disturbed barrier function and impaired permeability of the glomeruli, which leads to albumin leakage into the urine [1]. Excessive albuminuria represents an early manifestation of diabetic nephropathy (DN) and widespread vascular damage [1,2]. DN is a leading cause of end-stage renal disease (ESRD) and the strongest predictor of morbidity and mortality associated with cardiovascular disease (CVD), suggesting that the two pathologies are closely affiliated [3].

Protein arginine *N*-methyltransferase 1 (PRMT1) transfers methyl groups from S-adenosyl-L-methionine to the guanidine nitrogen atom of the arginine side chain of proteins, such as histones and RNA-binding proteins [4]. The methylation reaction yields S-adenosylhomocysteine and two forms of methylarginine, ω-N^G-monomethyl-L-arginine and ω-N^G,N^G-asymmetric dimethylarginine (ADMA) [4]. Upon proteolysis of arginine-methylated proteins, ADMA is released into the cytoplasm and

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then migrates into the extracellular space and the bloodstream. Dimethylarginine dimethylaminohydrolases (DDAHs) are primarily responsible for the degradation of ADMA into L-citrulline [4].

ADMA, an endogenous competitive inhibitor of endothelial nitric oxide synthase (eNOS), exhibits strong potency and long-term stability in the blood (>20 min of half-life) [4]. ADMA reduces endothelium-derived nitric oxide (NO) production and initiates the onset of arteriosclerosis through endothelial dysfunction and arterial stiffness, which are relevant to increasing renovascular resistance and CVD risk [5,6]. Furthermore, ADMA may also compromise the integrity of the glomerular filtration barrier [7]. Type 2 diabetes mellitus (T2DM) patients with higher circulating ADMA levels have a greater chance of DN progressing more quickly [8].

An accumulation of overt DN in a single family suggests possible genetic factors responsible for DN exacerbation, and single-nucleotide polymorphisms (SNPs) play a critical role in determining susceptibility to the disease [9]. This study aimed to explore whether *PRMT1*-related SNP(s) could influence DN incidence and severity by modulation of ADMA dynamics.

Material and methods

Study design and data source

The current investigation utilized a dataset of 310 Japanese T2DM patients (204 men and 106 women aged 40 to 75 years at study entry) who completed the previous study [10]. Briefly, the dataset included comprehensive clinical and laboratory results, information regarding gender, age, duration of T2DM, family history, smoking habits, and alcohol consumption, along with concomitant hypertension, dyslipidemia, and DN, and the genotypes of ten tagged SNPs surrounding human *PRMT1* (ENSG00000126457) (Fig. 1) [10]. The urinary albumin-to-creatinine (Cr) ratio (UACR) and the estimated glomerular filtration ratio (eGFR) were used to categorize DN severity: stage 0 (normoalbuminuria, UACR $<$ 30 mg/g·Cr), stage 1 (microalbuminuria, UACR 30–299 mg/g·Cr), stage 2 (overt DN, UACR $\geq$ 300 mg/g·Cr and eGFR $\geq$ 30 mL/min/1.73 m^2), stage 3 (renal failure, eGFR $<$ 30 mL/min/1.73 m^2), and stage 4 (ESRD with continuous hemodialysis). The Institutional Review Board of Toshiba Rinkan Hospital approved this research project (Approval No. H29-013). The study protocols followed the Declaration of Helsinki, revised in Fortaleza, Brazil, in October 2013. All the participants provided written informed consent prior to the commencement of the study.

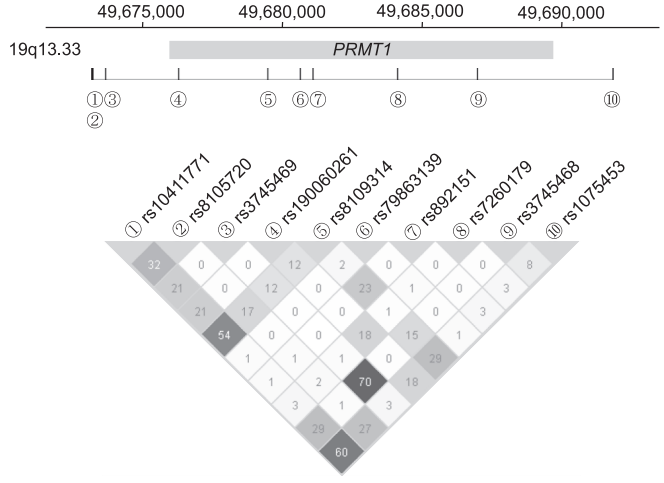

Fig. 1. Schematic diagram of the human *PRMT1* (ENSG00000126457, *grey square*) and the ten tagged single-nucleotide polymorphisms (SNPs) (*vertical bars*) with rs numbers under investigation. The SNPs' linkage disequilibrium (LD) of the study is indicated by diamonds representing the magnitude of LD for a single pair of markers. A standard scheme displays LD with a solid black diamond for absolute LD ($\gamma^2 = 1$), a solid white diamond for no LD ($\gamma^2 = 0$), and a solid grey diamond for intermediate LD. The number inside the diamond indicates the γ^2 value (×100). *Abbreviations*: PRMT1, protein arginine *N*-methyltransferase 1; 19q13.33, chromosome 19q13.33.

Quantification of mRNA expression levels

Total RNA samples from peripheral blood mononuclear cells (PBMCs) were reverse-transcribed to generate complementary DNA, and the reaction products were subjected to a quantitative real-time polymerase chain reaction using specific primers. Detailed primer sequences for *PRMT1*, *glyceraldehyde-3-phosphate dehydrogenase*, *DDAH1*, and *β-actin* are shown in Table S1 [11,12]. A comparative CT method was employed to analyze mRNA expression.

Measurement of serum ADMA levels

Serum ADMA levels were measured using an enzyme-linked immunosorbent assay kit (Elabscience Biotechnology Co., Ltd, Houston, TX, USA) according to the manufacturer's instructions. This analysis excluded subjects with an ESRD history.

Assessment of endothelial function

Endothelial function was evaluated using a peripheral reactive hyperemia-peripheral arterial tonometry (RH-PAT) device (Endo-PAT2000, Itamar Medical Ltd, Caesarea, Israel), which was equipped with finger probes for tracking pulse waves in digital arterioles. RH was induced by compressing the upper arm using a brachial sphygmomanometer cuff for 5 min and then releasing it. RH-PAT index (RHI) was calculated by multiplying the rate of increase in finger plethysmogram after reperfusion by that of the non-ischemic hand. This analysis excluded subjects with an ESRD or surgically treated breast cancer history.

Assessment of arterial stiffness

Arterial stiffness was assessed using a brachial-ankle pulse wave velocity (baPWV) device (BP-203RPE, Omron Colin, Kyoto, Japan), which comprised cuffs with piezoelectric sensors attached to the upper arms and ankles. baPWV was calculated as $(D_1–D_2)/T$. D_1 and D_2 are the distances from the aortic valve orifice to the left ankle (D_1) and the right upper arm (D_2). T is the difference in pulse wave latency between the right upper arm and the left ankle. An average of the right and left baPWV was used in the analysis. This analysis excluded subjects with an ESRD or peripheral arterial disease history.

Statistical analysis

Fisher's exact test was applied to determine the association of each tagged SNP variant with DN severity. A logistic regression analysis was conducted using a cohort of casual SNP(s) derived from propensity score matching (PSM). The chi-squared test was used to compare qualitative clinical and laboratory characteristics, and the Mann-Whitney *U* test was conducted to compare parametric and nonparametric continuous variables. Spearman's rank correlation rho (r) was used to evaluate the relationship between two continuous nonparametric variables. Nonparametric continuous variables with lognormal distributions were logarithmically transformed, and normal distribution methods were applied. The Smirnov–Grubbs test was used to exclude outliers. A two-sided p-value less than 0.05 was considered to be statistically significant. Microsoft R Open version 3.5.3 (Microsoft Corporation, Redmond, WA, USA) and the EZR software (https://www.jichi.ac.jp/saitama-sct/SaitamaHP.files/statmed.html) were used for the statistical analysis.

Results

Effect of the PRMT1-related variant rs892151 on DN

Univariate analysis of the composition of DN stages showed a significant difference between the GG and GA + AA subgroups of rs892151, based on a dominant model of minor alleles ($p = 0.026$) (Table 1). The GA + AA subgroup had a higher incidence of stage 2 DN versus stage 1 DN than the GG subgroup ($p = 0.034$). However, the incidences of stage 1 DN versus stage 0 DN ($p = 1.000$) and stage ≥ 3 DN versus stage 2 DN ($p = 1.000$) were similar between the two genotype-based subgroups. The post hoc (1-β) was 0.77 to detect a difference in the incidence of stage ≥ 2 overt DN between the two genotype-based subgroups of rs892151. The other nine loci did not influence the distribution of DN stages. Using the PSM method, a new cohort was created to correct the imbalances in the two genotype-based subgroups of rs892151 in terms of duration of diabetes, glycated hemoglobin levels, and body mass index (Table 2). The two genotype-based subgroups in the PSM cohort did not differ regarding the other potential confounders. A logistic regression analysis of the PSM cohort revealed that the incidence of stage ≥ 2 overt DN in the GA + AA subgroup was more likely than that of the GG subgroup (odds ratio: 2.92, 95 % confidence interval: 1.12–7.62, $p = 0.028$) (Table 3).

Table 3
Relationship between the rs892151 genotype and the incidence of stage ≥ 2 overt diabetic nephropathy.

rs892151 genotype	Overall *n*	Overt DN− *n* (%)	Overt DN+ *n* (%)	OR	95 % CI	*p*
GG	66	59 (89.4)	7 (10.6)			
GA + AA	66	49 (74.2)	17 (25.8)	2.92	1.12–7.62	0.028*

Multiple logistic regression analysis was performed between the genotype-based subgroups of rs892151 after adjusting for duration of diabetes, glycated hemoglobin levels, and body mass index by propensity score matching. *$p < 0.05$. *Abbreviations*: Overt DN, stage ≥ 2 overt diabetic nephropathy; OR, odds ratio; CI, confidence interval.

Effects of the rs892151 variant on PRMT1 and DDAH1 mRNA and circulating ADMA in vivo

The *PRMT1* mRNA levels in the GA + AA subgroup were 19 % higher than those in the GG subgroup ($p < 0.001$) (Fig. 2a). The *DDAH1* mRNA levels did not differ significantly between the two genotype-based subgroups ($p = 0.167$) (Fig. 2b). In addition, the serum ADMA levels in the GA + AA were also 7 % higher than those in the GG subgroup when

Table 1
Relationship between the rs892151 genotype and the distribution of diabetic nephropathy stages.

rs892151 genotype	stage 0 *n* (%)	stage 1 *n* (%)	stage 2 *n* (%)	stage ≥ 3 *n* (%)	*p*	p^a	p^b	p^c
GG	143 (58.6)	72 (29.5)	19 (7.8)	10 (4.1)				
GA + AA	36 (54.5)	13 (19.7)	13 (19.7)	4 (6.1)	0.026*	1.000	0.034*	1.000

The composition of diabetic nephropathy stages was initially compared between the genotype-based subgroups of rs892151 (*p*). The incidence of stage 1 versus stage 0 (p^a), that of stage 2 versus stage 1 (p^b), and that of stage ≥ 3 versus stage 2 (p^c) was then compared between the two genotype-based subgroups. Bonferroni correction was used for multiple testing corrections. *p, $p^b < 0.05$.

Table 2
Clinical and laboratory characteristics of the genotype-based subgroups of rs892151 in the propensity score matching cohort.

Variable	GG ($n = 66$)	GA + AA ($n = 66$)	*p*
Male, *n* (%)	41 (62)	44 (67)	0.716
Age (years)	63 (58–70)	67 (59–71)	0.390
Body mass index (kg/m^2)	24.5 (21.9–26.5)	23.5 (21.8–26.2)	0.560
Duration of diabetes (years)	10 (6–19)	10 (6–20)	0.964
Family history of diabetes, *n* (%)	45 (68)	46 (70)	1.000
Current or past smoker, *n* (%)	41 (62)	39 (59)	0.859
Current or past drinker, *n* (%)	30 (46)	24 (36)	0.376
Systolic blood pressure (mmHg)	130 (122–136)	132 (122–138)	0.660
Diastolic blood pressure (mmHg)	75 (69–81)	73 (68–79)	0.183
Glycated hemoglobin (mmol/mol)	53 (50–60)	54 (51–60)	0.594
(%)	7.0 (6.7–7.7)	7.1 (6.8–7.7)	
Total cholesterol (mmol/L)	4.5 (3.9–4.9)	4.2 (3.8–4.7)	0.211
LDL cholesterol (mmol/L)	2.4 (2.0–2.9)	2.3 (2.0–2.7)	0.568
HDL cholesterol (mmol/L)	1.4 (1.2–1.7)	1.3 (1.1–1.6)	0.200
Triglycerides (mmol/L)	1.5 (1.2–2.2)	1.5 (1.1–2.3)	0.634
Creatinine (μmol/L)	74 (59–93)	71 (59–83)	0.577
eGFR (mL/min/1.73 m^2)	66 (54–80)	71 (56–81)	0.471
Alanine aminotransferase (IU/L)	21 (16–31)	19 (15–30)	0.374
γ-glutamyl transpeptidase (IU/L)	35 (22–61)	24 (18–35)	0.050
C-reactive protein (μmol/L)	700 (300–1200)	700 (300–1375)	0.745
Hypertension, *n* (%)	52 (79)	53 (80)	1.000
Dyslipidemia, *n* (%)	53 (80)	58 (88)	0.341
Obesity (body mass index ≥ 25), *n* (%)	30 (46)	22 (33)	0.212

All data are presented as median (interquartile range) or *n* (%). The characteristics were compared between the two genotype-based subgroups. *Abbreviations*: LDL, low-density lipoprotein; HDL, high-density lipoprotein; eGFR, estimated glomerular filtration ratio.

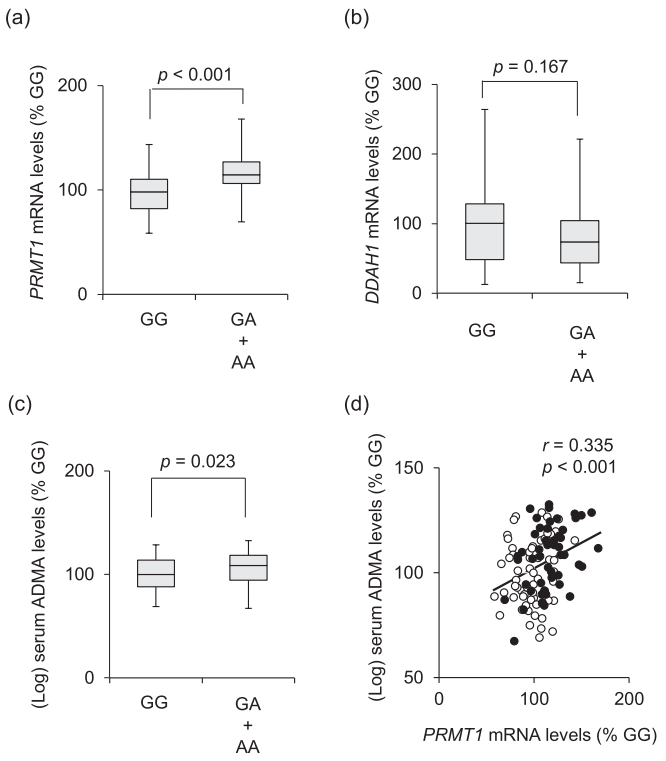

Fig. 2. The effects of the rs892151 variant on *PRMT1* and *DDAH1* mRNA and serum ADMA *in vivo*. Values indicate the changes in (a) *PRMT1* relative to *GAPDH* mRNA levels, (b) *DDAH1* relative to *β-actin* mRNA levels, and (c) (Log) serum ADMA levels normalized to the mean levels of the GG subgroup. Box plots represent medians, interquartile ranges, and 95 % confidence intervals. (d) The relationship between *PRMT1* mRNA and serum ADMA levels. Values are depicted in the GG (*open circles*) and GA + AA subgroups (*closed circles*). A line indicates linear regression of the parameters. *Abbreviations*: PRMT1, protein arginine *N*-methyltransferase 1; DDAH1, dimethylarginine dimethylaminohydrolase 1; ADMA, ω-N^G,N^G-asymmetric dimethylarginine; (Log), logarithm-transformed; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

logarithm-transformed ($p = 0.023$) (Fig. 2c). The participants in the PSM cohort showed a positive correlation between *PRMT1* mRNA and logarithm-transformed serum ADMA levels ($r = 0.335$, $p < 0.001$) (Fig. 2d), suggesting that the rs892151 variant may affect *PRMT1* transcription and methylation reaction *in vivo*, resulting in the change in circulating ADMA levels.

Relationships between circulating ADMA, endothelial function, arterial stiffness, and albuminuria

The participants in the PSM cohort exhibited a negative correlation between logarithm-transformed circulating ADMA levels and RHI representing endothelial function ($r = -0.221$, $p = 0.029$) (Fig. 3a). The baPWV in the GA + AA subgroup was 8 % higher than that in the GG subgroup ($p = 0.047$) (Fig. 3b), suggesting that the rs892151 variant may be functionally linked to arterial stiffness, which occurs as a result of endothelial dysfunction. Furthermore, baPWV and logarithm-transformed UACR showed a positive correlation ($r = 0.254$, $p = 0.004$) (Fig. 3c), suggesting that the rs892151 variant-modified circulating ADMA may be associated with the pathogenesis of overt DN through endothelial dysfunction and arterial stiffness (Fig. 4).

Discussion

The present study demonstrated that the rs892151 variant in *PRMT1* enhanced the enzyme transcription and ADMA biosynthesis, resulting in endothelial dysfunction, arterial stiffness, and an increased rate of overt DN among Japanese patients with T2DM. Concerning the variations surrounding *PRMT1*, the NHGRI-EBI Catalogue of human genome-wide association study (GWAS) (https://www.ebi.ac.uk/gwas/) noted that the rs1045567 variant has an association with general cognitive abilities and that the rs8109314 variant is associated with heights. Several studies have linked the rs975484 variant to the expression of immune checkpoint programmed cell death-ligand 1 (PD-L1) and PD-L2 genes [13], as well as the rs10415880 variant to malfunction of the arteriovenous shunt in male hemodialysis patients [14]. Recent research has demonstrated that the rs3745468 variant is involved in proliferative diabetic retinopathy, influencing the hypoxic inducible factor-1 pathway [10].

According to the HaploReg v4.1 database (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php), the rs892151 variant occurs in an open chromatin region that is highly susceptible to DNase and contains an active histone H3 mark across a wide range of tissues. The rs892151 variant augmented *PRMT1* transcription in PBMCs, whereas the rs3745468 variant decreased it [10]. A similar pattern of the variant-dependent modulation of *PRMT1* transcription was observed in macrophages associated with hepatocellular carcinoma: *PRMT1* mRNA

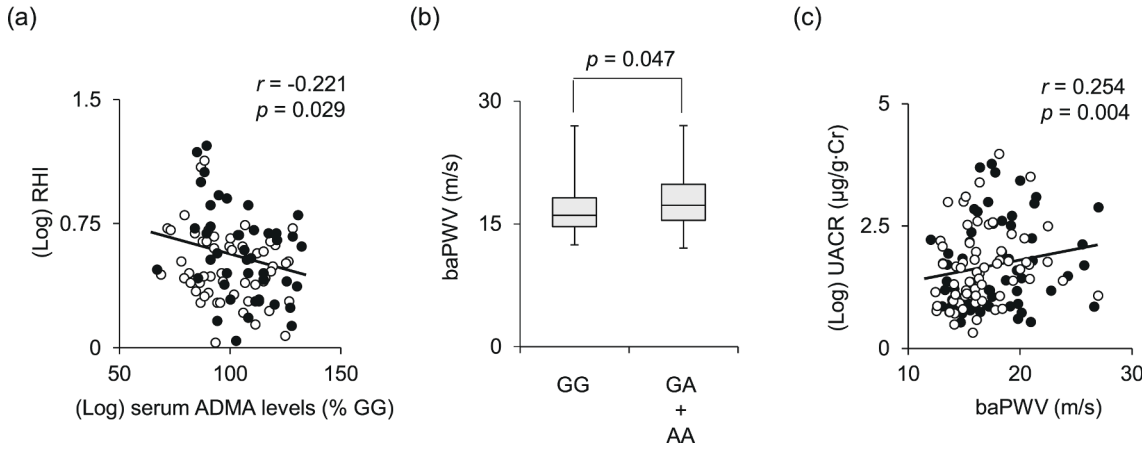

Fig. 3. The relationships (a) between serum ADMA and endothelial dysfunction and (c) between arterial stiffness and albuminuria. Values are depicted in the GG (*open circles*) and GA + AA subgroups (*closed circles*). Lines indicate linear regression of the parameters. (b) The effect of the rs892151 variant on arterial stiffness. Box plots represent medians, interquartile ranges, and 95 % confidence intervals. *Abbreviations*: (Log), logarithm-transformed; ADMA, ω-N^G,N^G-asymmetric dimethylarginine; RHI, reactive hyperemia-peripheral arterial tonometry index; baPWV, brachial-ankle pulse wave velocity; UACR, urinary albumin to creatinine ratio; Cr, creatinine.

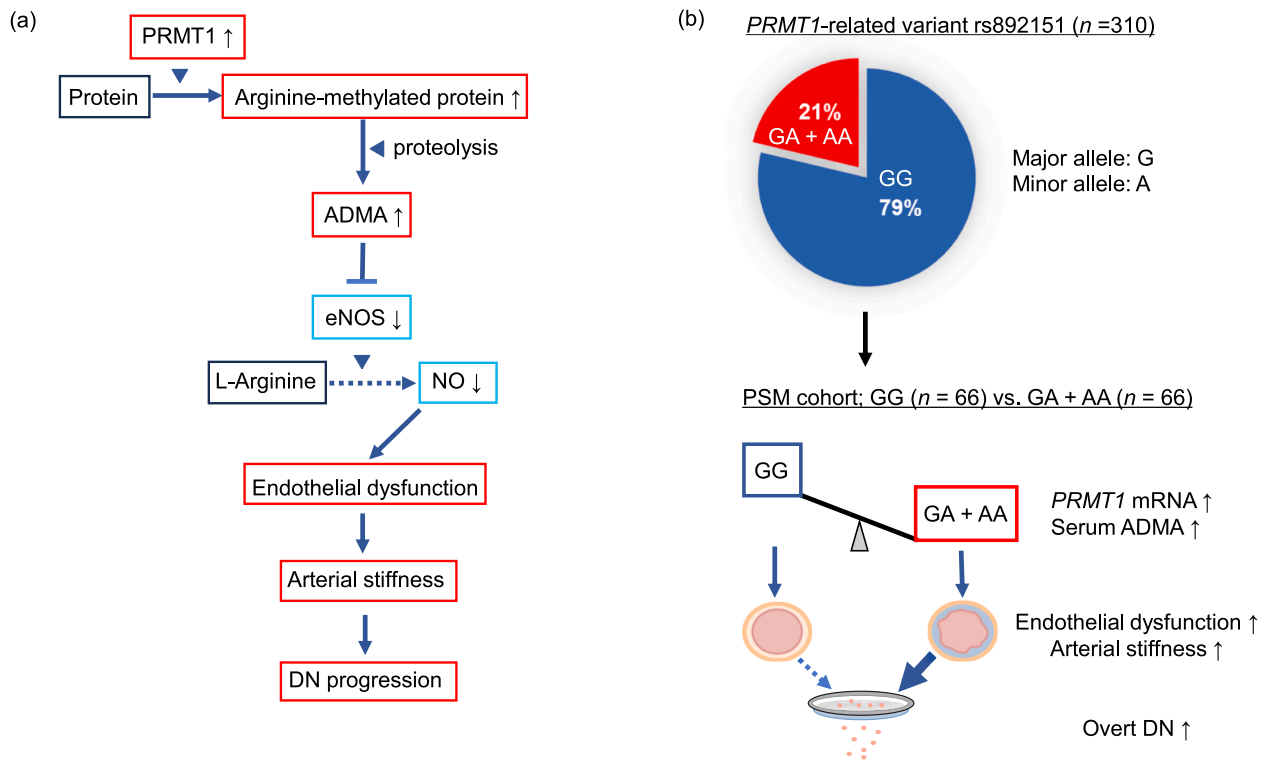

Fig. 4. Potential mechanism of how the *PRMT1*-related variant rs892151 influences the incidence of overt DN. (a) The increased PRMT1 activity may increase circulating ADMA levels and impair eNOS activity, leading to endothelial dysfunction and arterial stiffness followed by possible DN progression. (b) The GA + AA subgroup of rs892151 may be associated with an increased incidence of overt DN than the GG subgroup via ADMA-mediated endothelial dysfunction and arterial stiffness. *Abbreviations*: PRMT1, protein arginine *N*-methyltransferase 1; ADMA, ω-N^G,N^G-asymmetric dimethylarginine; eNOS, endothelial nitric oxide synthase; NO, nitric oxide; DN, diabetic nephropathy; PSM, propensity score matching.

expression was higher with the rs975484 variant but lower with the rs8109314 variant [13]. The linkage disequilibrium between rs3745468 and rs975484 ($\gamma^2 = 0.36$) was more robust than that between rs892151 and rs975484 ($\gamma^2 = 0.02$) in the Japanese population of the 1000 Genome Project (https://www.internationalgenome.org/), suggesting a cell- or tissue-specific basis for the effect on gene transcription attributable to the variants.

The circulating ADMA levels in the present study ranged from 0.16 to 4.9 μmol/L. Considering that the κi value of ADMA for eNOS function is 0.9 μmol/L [15], the rs892151 variant likely modulates the vasculature's NO bioavailability by altering circulating ADMA levels. Various factors can affect circulating ADMA levels, including aging, smoking, T2DM, hypertension, hypercholesterolemia, chronic kidney disease (CKD), and CVD [4]; however, the PSM cohort could overcome these confounding factors. The rs233112 variant in *DDAH1*, but not the rs10415880 or rs975484 variants in *PRMT1*, altered circulating ADMA levels in unselected individuals [16]. However, a conflicting result indicated that ADMA metabolism by DDAH1 played a lesser role in circulating ADMA levels in patients with albuminuria [17]. When adenosine dialdehyde inhibited PRMT1 and decreased circulating ADMA levels, an experimental CKD model restored the acetylcholine-induced vasodilation of renal afferent and efferent arterioles [18], which supports the finding that the rs892151 variant modulates circulating ADMA levels and arterial stiffness. The previous study demonstrated a positive correlation between circulating ADMA and serum C-reactive protein (CRP) levels in patients with T2DM [17] despite the rs892151 genotype and circulating ADMA being independent of serum CRP levels in this study.

ADMA-induced inactivation of eNOS, followed by endothelial dysfunction, adversely affects the clinical outcomes of DN [19]. The increase in RHI values can be primarily attributed to endothelium-derived NO, which accounted for an estimated 50 % of the increase [20]. This study showed a negative correlation between RHI values and circulating ADMA levels, suggesting that T2DM patients carrying the rs892151 variant are susceptible to ADMA-mediated endothelial dysfunction. Microalbuminuria, which occurs during the early stages of DN, may be caused by damage to glomerular endothelial cells [21]. ADMA levels in renal tissue were more abundant than in blood [4]; therefore, endothelial dysfunction in glomerular capillaries may be alternatively responsible for the variant-mediated DN pathology. There is a contrasting notion that glomerular endothelial cells are unlikely to serve as the primary barrier to albumin in the early phase of the diseases that present proteinuria [1]. Future studies need to clarify the role of the rs892151 variant in the endothelial dysfunction of the glomerular capillaries.

Carotid-femoral (cf)PWV and baPWV show a strong correlation [22], reflecting the characteristics of elastic and muscular vessels composed of vascular smooth muscle fibers into which NO diffuses from nearby endothelium [23]. There was a positive correlation between cfPWV and circulating ADMA levels among prediabetic patients [24]. Both cfPWV and circulating ADMA levels have been shown to predict the occurrence of albuminuria in patients with T2DM [8,25]. There was a higher incidence of albuminuria in T2DM patients with increased baPWV [26], whereas a reduction in baPWV was associated with an improvement in albuminuria, regardless of the systolic blood pressure [27]. Together, baPWV offers a valuable tool for evaluating the influence of ADMA-mediated arterial stiffness on DN progression. According to this study, overt DN was more common in the patients carrying the rs892151 variant with a 1.2 m/s increase in baPWV, which is consistent with the earlier finding that a 2.0 m/s increase in the baPWV corresponded to a 19 % higher risk of albuminuria [28]. Under the stiffness of the upstream arterial tree characterized by higher baPWV, afferent arterioles, which

are small in diameter and short in overall length from the arcuate arteries, are subjected to a solid hemodynamic stress with a more significant pressure gradient [1]. This unique feature of renal circulation may partially explain why the rs892151 variant-mediated arterial stiffness resulted in severe kidney deterioration.

There are several limitations of this study. Firstly, this study was conducted within a single institution in an ethnically and socially homogeneous population. In our dataset, rs892151 had a minor allele frequency (MAF) of 0.11 [10]. A comprehensive Japanese genetic variation database (https://togovar.biosciencedbc.jp/) and a dsSNP database (https://www.ncbi.nlm.nih.gov/snp/) indicate that rs892151 is more common among East Asians (MAF = 0.12) than Caucasian Europeans and Africans (MAF < 0.01). Consequently, East Asians, including Japanese, may experience a more substantial impact from the rs892151 variant than other ethnic groups. Secondly, the cross-sectional design of this study precluded the conclusion about the rs892151 variant being a predictor of overt DN progression. In the PSM cohort population, a retrospective evaluation showed that the median follow-up period in our hospital was 7.7 years (interquartile range: 3.8–11.6 years) in the GG subgroup and 7.8 years (interquartile range: 5.1–11.2 years) in the GA + AA subgroup. During the follow-up period, the risk of worsening DN stage 1 or more was 31.8 % in the GA + AA subgroup and 21.2 % in the GG subgroup. The odds ratio of the event was 1.73 between the GG and GA + AA subgroups, but there was no statistically significant difference. Third, the post hoc power of this study was 0.77 to detect a difference between the two genotype-based subgroups of rs892151 in terms of overt DN incidence using the chi-squared test with a two-sided α of 0.05. The low prevalence of overt DN and the rs892151 variant with the relatively small cohort size resulted in a high odds ratio and a wide 95 % confidence interval. Finally, the two Japanese GWAS found no significant association between the *PRMT1* variant and DN phenotype [29,30]. According to these studies, DN susceptibility, but not the disease severity, was associated with the rs2268388 variant in the acetyl-coenzyme A carboxylase beta gene and the rs56094641 variant in the fat-mass and obesity-associated gene. An analysis of prospective cohorts with multi-center, multi-ethnic, and large-scale sampling sizes is needed to verify the validity of the current findings.

Conclusions

The *PRMT1*-related variant rs892151, as a possible predisposing factor for overt DN, may assist in extracting a subgroup of patients with T2DM who require close monitoring. ADMA-mediated endothelial dysfunction followed by arterial stiffness may explain the effect of the variant on DN severity; therefore, the pathway may be a potential target for tailored treatment for DN, particularly when carrying the variant. Whole genome sequencing (WGS) using next-generation sequencing technologies allows for rapid and large-scale DNA sequence analysis, enabling comprehensive screening of multiple variants in the entire genome that contributes to the prevention and early detection of chronic common diseases and is expected to become more popular shortly [31,32]. The rs892151 may be genotyped by WGS analysis as a personalized genomic/precision medicine component.

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CRediT authorship contribution statement

Hiroaki Iwasaki: Writing – review & editing, Writing – original draft, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The author declares that he has no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcte.2024.100351.

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