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ORIGINAL ARTICLE

Protein arginine *N*-methyltransferase 1 gene polymorphism is associated with proliferative diabetic retinopathy in a Japanese population

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Abstract

Aims To investigate the effects of single-nucleotide polymorphisms (SNPs) around the protein arginine *N*-methyltransferase 1 (PRMT1) gene on the incidence and severity of diabetic retinopathy (DR).

Methods A total of 310 Japanese patients with type 2 diabetes mellitus (T2DM) were investigated. Genotyping of ten tagged SNPs were performed by quantitative real-time polymerase chain reaction (qRT-PCR). The association between each SNP genotype and diabetic microangiopathy was assessed using univariate analysis in a dominant model of the minor alleles followed by multivariate logistic regression analysis with the propensity score matching (PSM) method. The effect of disease-related SNP on PRMT1 and hypoxia-inducible factor-1α (HIF-1α) mRNA levels in vivo was evaluated by qRT-PCR.

Results In the univariate analysis, the minor A allele at rs374569 and the minor C allele at rs3745468 were associated with DR severity ($P=0.047$ and $P=0.003$, respectively), but not diabetic nephropathy and peripheral polyneuropathy severity. Multivariate analysis showed that the rs3745468 variant caused an increased incidence of proliferative DR (PDR) (odds ratio 9.37, 95% confidence interval 1.12–78.0, $P=0.039$). In the PSM cohort, the patients carrying the rs3745468 variant had lower PRMT1 mRNA levels compared to those without the variant ($P=0.037$), and there was an inverse correlation between PRMT1 and HIF-1α mRNA levels ($r=-0.233$, $P=0.035$).

Conclusions The rs3745468 variant in the PRMT1 gene was associated with an increased incidence of PDR in Japanese patients with T2DM and might be involved in the HIF-1-dependent hypoxic pathway through altered PRMT1 levels.

Keywords Type 2 diabetes mellitus · Diabetic retinopathy · Single-nucleotide polymorphism · Protein arginine *N*-methyltransferase 1 · Hypoxia-inducible factor-1

Introduction

Diabetic retinopathy (DR) is a microvascular complication of diabetes that develops in approximately 60% of patients with type 2 diabetes mellitus (T2DM) within 20 years of the disease onset [1]. DR is triphasically staged as simple (SDR), pre-proliferative (pre-PDR) and proliferative DR (PDR) [1]. Pre-PDR is the vaso-obliterative phase characterised by retinal ischaemia due to microvascular occlusion, which leads to impaired contrast sensitivity and visual fields, thereby causing difficulties in daily life such as reading and driving [1–3]. PDR is the vaso-proliferative phase characterised by retinal neovascularisation and increased vascular permeability, resulting in vitreous haemorrhage from new vessels and tractional retinal detachment from progressive fibrosis followed by vision loss and blindness [1–3]. Several interventions are used for treating severe DR, such as pan-retinal laser photocoagulation and intravitreal injections of corticosteroids and antibody agents against vascular endothelial growth factor (VEGF), a master regulator of ocular angiogenesis [2–4].

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Hypoxia-inducible factor-1 (HIF-1) is a transcriptional factor that is tightly regulated by oxygen and plays a pivotal role in the adaptive responses of tissues to hypoxia due to vessel closure and ischaemia [5]. HIF-1 upregulates the transcription of VEGF and other angiogenic factors, which are implicated in ocular pathologies underlying oxygenation perturbation including severe DR and glaucoma [6, 7]. Protein *N*-arginine methyltransferase 1 (PRMT1), a predominant type I PRMT in mammalians, catalyses protein arginine methylation to form ω-N^G,N^G-asymmetric dimethylarginine (ADMA) residues on various substrates such as histones, structural proteins and transcriptional factors, resulting in altered gene expression, RNA metabolism and genome stability [8]. It has been shown that PRMT1 changes HIF-1α transcription and VEGF expression under hypoxia [9]. PRMT1-mediated arginine methylation is present in the retina [10], which, if dysregulated, may affect oxygen-dependent ocular pathologies through the HIF-1-dependent pathway [11].

Large-scale epidemiological studies have shown that diabetes duration, inadequate glycaemic control and uncontrolled hypertension are the strongest risk factors for DR onset and severity [2, 4], although they account for only 20% of the overall risk [12]. The familial clustering of early onset DR and/or severity, independently of metabolic status, suggests the presence of genetic factors that presumably account for 25% of the disease risk [13], and intense efforts have been made to identify the causative single-nucleotide polymorphisms (SNPs) as genetic predispositions for DR [14]. This study aimed to investigate whether SNPs located around the PRMT1 gene affect the incidence and severity of DR, and are capable of altering the HIF-1-dependent pathway.

Methods

Subjects

Patients with T2DM who visited Toshiba Rinkan Hospital three or more times as outpatients from March 2018 to September 2020 were enrolled if they met the following criteria: (i) aged 40–75 years, (ii) diagnosed with T2DM more than five years ago according to the Japanese Clinical Practice Guideline for Diabetes [15] and/or (iii) having a history of diabetic microangiopathy. Patients were excluded from the study if they fulfilled one of the following criteria: (i) type 1 diabetes, (ii) glucose intolerance due to other specific disorders including liver, exocrine pancreas, endocrine, immune and infectious disorders, (iii) drug- or chemical-induced diabetes, (iv) the presence of currently treated malignant disease(s) and/or (v) the presence of hyperglycaemic symptoms. Finally, a total of 310 patients (204 men and 106 women) were included in our study. All participants reported to be of Japanese nationality. The present study was approved by the Ethic Committee of Toshiba Rinkan Hospital on 26 February 2018. The protocols were in compliance with the Declaration of Helsinki. Written informed consent for being included in the study was obtained from all subjects before the commencement of the study.

Clinical assessment

DR was classified into four stages according to Davis classification based on detailed eye examination: no DR (NDR), SDR, pre-PDR and PDR. Treatment history of laser photocoagulation, intravitreal injections of corticosteroids, anti-VEGF agents or surgical vitrectomy for DR, confirming the information provided to ophthalmologists, was defined as an indicator of previous progression to pre-PDR or PDR. Diabetic nephropathy (DN) was classified into five stages: normoalbuminuria (stage 0), microalbuminuria (stage 1), macroalbuminuria/overt proteinuria (stage 2), renal failure (stage 3) and end-stage renal failure (stage 4), based on the average of the three urinary albumin to creatinine ratio measurements and the estimated glomerular filtration ratio (eGFR) according to the definition of the Japanese Clinical Practice Guideline for Diabetes [15]. Diabetic peripheral polyneuropathy (DPN) was classified into five stages according to the Baba classification based on a nerve conduction velocity study for bilateral sural and tibial nerves (Supplementary Fig. 1) [16], except for one who was not tested.

All participants underwent standardised clinical and laboratory evaluations according to the recommended configuration [17]. Systolic and diastolic blood pressure (SBP and DBP), weight, body mass index (BMI), glycated haemoglobin (HbA_{1c}), total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, creatinine, eGFR, alanine aminotransferase and γ-glutamyl transpeptidase in blood were measured at three consecutive visits, and the average values were calculated. HbA_{1c} levels were determined by high-performance liquid chromatography (HLC723GHb; Tosoh Bioscience, Tokyo, Japan) and presented as equivalents to the National Glycohaemoglobin Standardisation Programme (%) and International Federation of Clinical Chemistry and Laboratory Medicine (mmol/mol) values. The clinical laboratory at Toshiba Rinkan Hospital carried out all the laboratory measurements.

Smoking habit was defined as a current or past smoker if they had smoked ≥ 100 cigarettes and/or ≥ 6 months in their lifetime. Alcohol use was defined as positive for ≥ 20 g of ethanol per day during ≥ 1 year in their lifetime. Hypertension was defined as either receiving antihypertensive medications or an average office BP of ≥ 130/80 mmHg according to the Japanese Society of Hypertension Guidelines for

the Management of Hypertension 2019 [18]. Dyslipidaemia was defined as either receiving lipid-lowering agents or an average LDL cholesterol, HDL cholesterol or triglycerides levels of ≥ 3.1 mmol/l, < 1.0 mmol/l or ≥ 1.7 mmol/l, respectively, according to the Japan Atherosclerosis Society Guideline for the Diagnosis and Prevention of Atherosclerotic Cardiovascular Diseases 2017 [19].

DNA extraction and genotyping

Genomic DNA was isolated from peripheral venous blood (2 ml) using the QIAamp® Blood Midi Kit (Qiagen GmbH, Hilden, Nordrhein-Westfalen, Germany). Genotyping of genomic DNA (20 ng per sample) was performed using TaqMan® SNP Genotyping Assays, consisting of allele-specific probes labelled with FAM™ or VIC™ reporter dyes and corresponding polymerase chain reaction (PCR) primers ordered with the Assay-on-demand™ service (Thermo Fisher Scientific, Waltham, MA, USA), and the Type-it Fast SNP Probe PCR kit (Qiagen) with quantitative real-time PCR (qRT-PCR) by the CFX96 system (Bio-Rad Laboratories, Inc., Hurcules, CA, USA). The successful typing rate was > 99%, and 2% of the samples were randomly selected and genotyped in duplicates. The discrepancy between them was 0%.

RNA isolation and quantification of mRNA expression levels

Total RNA was isolated from peripheral blood mononuclear cells using the Nucleospin® RNA blood kit (Takara Bio Inc., Kusatsu, Shiga, Japan), and each sample (100 ng) was reverse transcribed using PrimeScript™ RT Master Mix (Perfect Real Time) (Takara Bio Inc.). qRT-PCR was performed using the specific primers (Supplementary Table 1) [9, 20] and KAPA SYBR® FAST qPCR Master Mix (2×) Kit (KAPA Biosystems, Wilmington, MA, USA) with the CFX96 system.

Statistical analysis

Hardy–Weinberg equilibrium (HWE) was checked for the tagged SNPs using PLINK version 1.9 (The Broad Institute, Cambridge, MA, USA). Clinical characteristics were compared among the subgroups classified by DR stages using the Kruskal–Wallis test for quantitative characteristics, chi-squared test for qualitative characteristics and Mann–Whitney U-test for parametric and non-parametric continuous variables. The association between each SNP variant and microangiopathy severity was initially determined by univariate analysis using the Fisher's exact test. In the logistic regression analysis, a cohort of each SNP was extracted by the propensity score matching (PSM) method [21] using the EZR software [22] to adjust for potential confounders. The common logarithm transformation was used if normality or equal variance assumptions were not met. All reported P values (P) are two-sided, and $P < 0.05$ was considered statistically significant. Statistical analysis was performed using Microsoft R Open version 3.5.3 (Microsoft Corporation, Redmond, WA, USA).

Results

Baseline characteristics

The clinical and laboratory characteristics of the NDR, SDR, pre-PDR and PDR (pre-PDR + PDR) study participants are shown in Supplementary Table 2. The pre-PDR + PDR group had a significantly longer diabetes duration, lower DBP levels and lower renal function representing higher serum creatinine and lower eGFR levels than the NDR group. The SDR group had significantly higher HbA_{1c} levels compared to the NDR group. The frequencies of DN and DPN mergers tended to increase, corresponding to the severity of DR. There were no significant differences in other characteristics among the three groups.

Selection of genetic variants

Human PRMT1 gene (ENSG00000126457) is located on chromosome 19q13.33 (49,675,897–49,688,447) (Fig. 1a). To better cover the common variations across the gene, SNPs around the PRMT1 gene with 5 kb added to both ends of 5′ and 3′ were reviewed in the JPN population (Japanese in Tokyo) enrolled in the 1000 Genome Project (https://www.internationalgenome.org/home). There were 60 SNPs with a minor allele frequency ≥ 0.05 in the region, and ten loci were selected as tagged SNPs with a minimum γ^2 threshold of 0.80 by the pairwise option of Haploview version 4.2 (The Broad Institute) (Fig. 1b).

Effects of rs3745469 and rs3745468 variants on DR severity

Ten tagged SNPs of all participants were genotyped. The genotype frequency, minor allele frequency and HWE expectations P value at each SNP are presented in Supplementary Table 3. All loci were in HWE. To investigate the association between tagged SNP genotype and the incidence and severity of DR, the composition of the DR stages was compared in a dominant model of the minor alleles. Univariate analysis showed differences in the distribution of the DR stages between genotype-based subgroups of rs3745469 and rs3745468 ($P = 0.047$ and 0.003, respectively) (Table 1). The minor C allele at rs3745468 (GC + CC) ($P = 0.004$), but

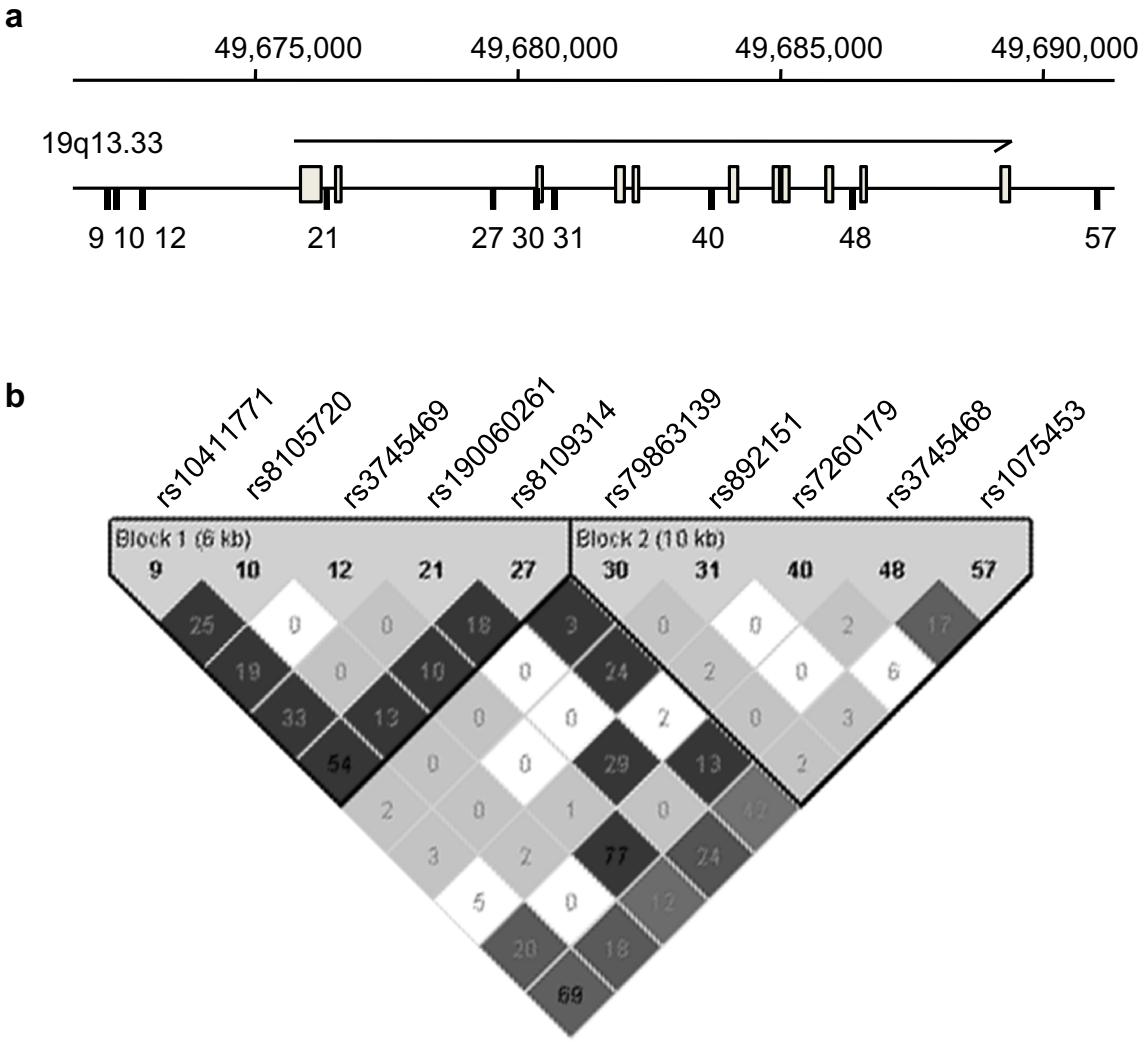

Fig. 1 Schematic representation of the human PRMT1 gene and LD of the tagged SNPs in the study. **a** The relative positions of the 11 exons of the human PRMT1 gene (ENSG00000126457, *an arrow*) and the tagged SNPs under investigation are indicated by grey boxes and black squares with the corresponding numbers in Fig. 1b, respectively. **b** LD of the SNPs with rs numbers in the 1000 Genome Project (Japanese in Tokyo) are 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 ($\times 100$). *PRMT1*—protein arginine *N*-methyltransferase 1, *LD*—linkage disequilibrium, *SNP*—single-nucleotide polymorphism

not the A allele at rs3745469 (GA) ($P=0.082$), increased the incidence of PDR relative to pre-PDR. There were no differences in the incidence of SDR relative to NDR and pre-PDR relative to SDM between the two genotype-based subgroups of each SNP ($P=1.000$ in all comparisons). The strong linkage disequilibrium (LD) between rs3745469 and rs3745468 ($\gamma^2=0.77$) was thought to contribute to the simultaneous consequence of the associations of these variants with the severity of DR (Fig. 1b). The published databases from the FANTOM 5 project (https://fantom.gsc.riken.jp/5/) and RefEx (https://refex.dbcls.jp/) have shown that PRMT1 gene expression vary greatly depending on the tissue, being much higher in the eye or retina than in the kidney (Supplementary Fig. 2) and peripheral nervous system, suggesting that the retina is potentially one of the target tissues where the effect of the rs3745468 variant on the PRMT1 gene expression is more pronounced. In this context, DN and DPN frequently coexist with DR [12, 23]; however, the rs3745469 and rs3745468 variants did not affect the severity of either DN ($P=0.259$ and 0.546, respectively) or DPN ($P=0.621$ and 0.606, respectively) (Table 1).

To adjust the imbalances in diabetes duration and HbA_{1c} levels between the genotype-based subgroups of rs3745468, a cohort was extracted using the PSM method. In the PSM cohort, the genotype-based subgroups of the variant were balanced in terms of diabetes duration and

Table 1 Relationship between the rs3745469 or rs3745468 genotype and the severity of diabetic microangiopathy

SNP genotype	NDR *n* (%)	SDR *n* (%)	Pre-PDR *n* (%)	PDR *n* (%)	*P*	P^a	P^b	P^c
Retinopathy								
rs3745469								
GG	176 (64.2)	47 (17.2)	40 (14.6)	11 (4.0)				
GA	25 (69.4)	4 (11.1)	2 (5.6)	5 (13.9)	0.047*	1.000	1.000	0.082
rs3745468								
GG	170 (65.1)	44 (16.9)	39 (14.9)	8 (3.1)				
GC + CC	31 (63.3)	7 (14.3)	3 (6.1)	8 (16.3)	0.003*	1.000	1.000	0.004*
SNP genotype	Stage 0 *n* (%)	Stage 1 *n* (%)	Stage 2 *n* (%)	Stage 3 + 4 *n* (%)	*P*			
Nephropathy								
rs3745469								
GG	160 (58.4)	75 (27.4)	29 (10.6)	10 (3.6)				
GA	19 (52.8)	10 (27.8)	3 (8.3)	4 (11.1)	0.259			
rs3745468								
GG	152 (58.2)	71 (27.2)	28 (10.7)	10 (3.8)				
GC + CC	27 (55.1)	14 (28.6)	4 (8.2)	4 (8.2)	0.546			
Peripheral neuropathy								
rs3745469								
GG	134 (49.1)	68 (24.9)	39 (14.3)	32 (11.7)				
GA	16 (44.4)	11 (30.6)	3 (8.3)	6 (16.7)	0.621			
rs3745468								
GG	127 (48.8)	67 (25.8)	37 (14.2)	29 (11.2)				
GC + CC	23 (46.9)	12 (24.5)	5 (10.2)	9 (18.4)	0.606			

The composition of diabetic retinopathy, nephropathy and peripheral neuropathy stages was initially compared between the genotype-based subgroups of each SNP (*P*). The incidence of SDR relative to NDR (P^a), that of pre-PDR relative to SDR (P^b) and that of PDR relative to pre-PDR (P^c) was compared between the genotype-based subgroup of each SNP. Bonferroni correction was used for multiple testing correction. **P*, P^a, P^b, $P^c < 0.05$. *SNP*—single-nucleotide polymorphism, *NDR*—no diabetic retinopathy, *SDR*—simple diabetic retinopathy, *pre-PDR*—pre-proliferative diabetic retinopathy, *PDR*—proliferative diabetic retinopathy

HbA_{1c} levels, as well as the characteristics of other well-known confounders, such as BMI, SBP, LDL cholesterol, triglycerides levels and concomitant overt nephropathy [12, 24] (Supplementary Table 4). Logistic regression analysis of the PSM cohort revealed that the C allele at rs3745468 (GC + CC) (odds ratio [OR]: 9.37, 95% confidential interval [CI]: 1.12–78.0, $P = 0.039$) was associated with an increased incidence of PDR (Table 2).

Effect of the rs3745468 variant on PRMT1 and HIF-1α mRNA levels in vivo

To determine whether the rs3745468 variant exhibits functionality in the transcription of the PRMT1 gene in vivo, PRMT1 mRNA levels in peripheral blood mononuclear cells were compared between the genotype-based subgroups in the PSM cohort of rs3745468. PRMT1 mRNA levels were 7.6% lower in the subgroup carrying the C allele (GC + CC) compared to the subgroup not carrying that allele (GG)

Table 2 Relationship between rs3745468 genotype and the incidence of proliferative diabetic retinopathy

SNP genotype	Overall	No PDR *n* (%)	PDR *n* (%)	OR	95% CI	*P*
GG	49	48 (98.0)	1 (2.0)			
GC + CC	49	41 (83.7)	8 (16.3)	9.37	1.12, 78.0	0.039*

Multiple logistic regression analysis was performed between the genotype-based subgroups after adjusting for diabetes duration and glycated haemoglobin by the propensity score matching. **P* < 0.05. *SNP*—single-nucleotide polymorphism, *PDR*—proliferative diabetic retinopathy, *OR*—odds ratio, *CI*—confidence interval

($P=0.037$) (Fig. 2a). The severity of DR of the patients carrying the C allele at rs3745468 did not affect PRMT1 and HIF-1α mRNA levels ($P=0.848$ and $P=0.261$ for NDR + SDR vs pre-PDR + PDR, respectively) (Supplementary Fig. 3a and b). There was a negative correlation between the mRNA levels of PRMT1 and HIF-1α throughout the PSM cohort ($r=-0.233$, $P=0.035$) (Fig. 2b), suggesting that the rs3745468 variant may be involved in changing in HIF-1-dependent hypoxic pathway by altered PRMT1 levels regardless of the DR status.

Effect of the rs3745468 variant on serum C-reactive protein levels in vivo

To examine whether the rs3745468 variant affects inflammatory process, serum C-reactive protein (CRP) levels in the PSM cohort were measured. No significant difference in serum CRP levels was found in the subgroup carrying the C allele (GC + CC) compared to the subgroup not carrying the allele (GG) ($P=0.974$) (Supplementary Fig. 4a). Furthermore, no statistical correlation was found between PRMT1 mRNA levels and serum CRP levels throughout the PSM cohort ($r=0.019$, $P=0.865$) (Supplementary Fig. 4b), suggesting that the rs3745468 variant is unlikely to be associated with the systemic inflammatory status.

Discussion

Expression quantitative trait loci (eQTL) mapping shows that SNPs lying in the non-coding regions may alter transcriptional regulation [25]. The HaploReg database (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) shows that the rs3745468 variant is located in the intron, but is presented at the DNA hypersensitive site by an enhancer, histone H3 acetylation at lysine 27 residue in various tissues, supporting the result that the minor C allele at rs3745468 affected the transcription of the PRMT1 gene. PRMT1 has a profound effect on the chromatin structure by depositing dimethylarginine for histone H4 at arginine 3 residue (H4R3me2) [8]. Inhibition of PRMT1 in monocytes facilitates open chromatin conformation at the cyclic responsive cis-element site and then increases the MHC class I gene expression [26]. Attenuation of H4R3me2 by the rs3745468 variant may alter the local chromatin environment around

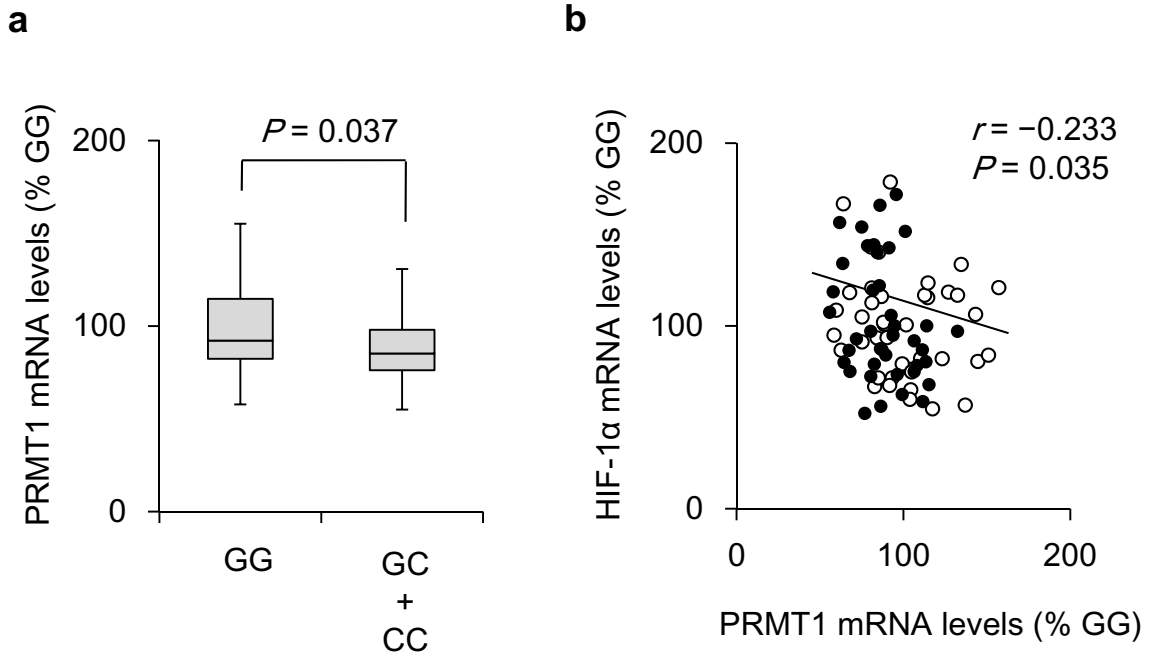

Fig. 2 Effect of the rs3745468 variant on PRMT1 mRNA levels and the relationship between PRMT1 and HIF-1α mRNA levels in vivo. **a** Comparison of PRMT1 mRNA levels in peripheral blood mononuclear cells between the genotype-based subgroup not carrying the C allele (GG, $n=43$) and the subgroup carrying the C allele (GC + CC, $n=42$) at rs3745468. Values are changes in PRMT1 mRNA levels relative to GAPDH mRNA levels normalised to the mean levels of the GG subgroup. Boxes extend from the 25th to the 75th percentile of each subgroup's distribution of values and horizontal lines within each box denote median values. *P* is presented above the box plots. **b** Correlation analysis of the relationship between PRMT1 and HIF-1α mRNA levels. Values of the parameters are depicted from GG (*open circles*, $n=42$) and GC + CC (*closed circles*, $n=40$). The line shows the linear regression of the parameters. Spearman's rank correlation rho (*r*) and *P* for correlation are indicated in the graph. Outliers were excluded by the Smirnov-Grubbs test. *PRMT1*—protein arginine *N*-methyltransferase 1, *HIF-1α*—hypoxia-inducible factor-1α, *GAPDH*—glyceraldehyde-3-phosphate dehydrogenase

the gene(s) associated with the HIF-1α transcription, such as Sp family of transcriptional factors genes [9].

A comprehensive Japanese genetic variation database (https://togovar.biosciencedbc.jp/) and 1000 Genome Project show that the frequency of the rs3745468 variant is higher in Africans (26–28%) and South Asians (16–17%) compared to Caucasian Europeans (6–8%), which is consistent with the result that the prevalence of PDR was higher in Africans and South Asians compared to Caucasian Europeans in the UK population [27]. In the current work, the incidence of PDR increased in the subgroup carrying the rs3745468 variant, whereas there was no difference in the ratio of NDR and SDR between the two subgroups with or without the variant. PRMT1 and HIF-1α mRNA levels were independent of the DR severity of the patients carrying the C allele at rs3745468, suggesting that the rs3745468 variant upregulated HIF-1α expression regardless of the DR status. However, under normal retinal oxygenation during the NDR and SDR stages, the immediate proteasomal degeneration process may cancel the effect of the rs3745468 variant on HIF-1α expression [5]. Once pre-PDR and PDR develops, HIF-1α levels in the retina carrying the rs3745468 variant may remain high in the areas of vascular occlusion and ischaemic lesions of the nerve fibre layer, resulting in worsening DR and impeding the recovery from PDR.

The tissue-specific eQTL analysis revealed that the effects of SNPs on gene expression vary from tissue to tissue [28]. However, it appears to be difficult to determine whether the rs3745468 variant alters retinal PRMT1 and HIF-1α mRNA levels, especially due to the histological peculiarity of the retina. The published databases from the FANTOM 5 project (https://fantom.gsc.riken.jp/5/) and RefEx (https://refex.dbcls.jp/) show that PRMT1 mRNA levels are much higher in the eye or retina than in the kidney and peripheral nervous system, whereas HIF-1α is expressed in a wide range of tissues without a large difference. The present study demonstrated that 28% and 17% of the patients with pre-PDR and PDR remained deficient in DN and DPN, respectively. Regarding the qualification of pre-PDR and PDR, the frequency of the absence of both DN and DPN was three times as high in the subgroup carrying the rs3745468 variant compared to the subgroup not carrying the variant. Taken together, the tissue specificity of PRMT1 expression may explain that the rs3745468 variant was one of specific determinants of DR severity, but not of DN and DPN severity. To confirm the role of the rs3745468 variant on the tissue-specific PRMT1 and HIF-1α mRNA expression, a cell or tissue model where the variant has been introduced using genome editing techniques would be useful.

Previous genome-wide association studies (GWAS) have reported several DR- and PDR-related SNPs in T2DM, such as rs9896052 near the GRB2 gene in Europeans [29]; rs391353 of the NOX4 gene in the Scottish [30]; rs12906891, rs3081219, rs11070992 and rs67619978 in WD repeat-containing protein 72 in Africans [31] and rs9362054 in long intergenic non-coding RNA RP1-90L14.1 adjacent to the CEP162 gene in the Japanese [32]. The significance of the rs3745468 variant on PDR has not been identified according to the NHGRI-EBI catalogue of human GWAS (https://www.ebi.ac.uk/gwas/). GWAS predominates as an initial groundwork for assessing millions of SNPs throughout the genome and helps unravel the genetic complexity of diseases [14]. However, SNP arrays for GWAS do not directly determine all variations in the genome, and their ability to extract disease-related SNPs with a low OR is not always high [33]. A candidate gene approach focused upon small numbers of SNPs within a particular region may provide a supplement to GWAS for identifying causative SNPs and underlying actual effector transcripts.

The previous study demonstrated that an endogenous competitive inhibitor of nitric oxide synthase, ADMA levels in the aqueous humour are similar to circulating ADMA levels and are increased in diabetic patients, especially those with severe DR [10]. ADMA can affect cell proliferation, reactive oxygen species production, intercellular adhesion molecule 1 and tight junction-associated protein occludin expression in bovine retinal capillary endothelial cells, contributing to impaired endothelial permeability and barrier function of PDR [34]. Increased PRMT expression, but not renal clearance of ADMA, results in higher circulating ADMA levels [35], and PRMT1 is one of the major enzymes responsible for ADMA production [8]. These findings suggest the involvement of PRMT1-mediated ADMA modulation in the development of PDR. However, a decrease in PRMT1 mRNA expression in the patients carrying the rs3745468 variant may potentially cause lower circulating and intraocular ADMA levels. Future studies aimed at elucidating the circulating biomarkers that show significant differences between the two subgroups with or without the rs3745468 variant may help identify novel candidates associated with worsening DR.

Chronic retinal inflammation in DR enhances VEGF production, recruits inflammatory mediators in retinal endothelial and neural cells and then contributes to DR progression [2, 3]. PRMT1 has shown to be a regulator of inflammatory process [36]. This study demonstrated that serum CRP levels were unaffected by the rs3745468 variant and were independent of the PRMT1 mRNA levels. CRP is a systemic inflammatory marker whose levels represent the sum of the effects of various inflammatory factors including infections, atherosclerosis, periodontitis, dermatitis and chronic obstructive lung disease, in each subject. Whether the rs3745468 variant modulates the local inflammation as well as oxidative stress, another PRMT1-related factor of the disease progression [34], should be addressed in the future.

Several limitations of our study should be acknowledged. The study population was racially homogeneous, with all the participants being Japanese, and the findings may not be applicable to other populations. Furthermore, this cross-sectional study limits the interpretation of the association between the PRMT1 variant and PDR and is insufficient to conclude the PRMT1 mRNA level as a predictor of PDR. Longitudinal epidemiologic studies by multi-centre and multi-ethnic collaborative efforts are needed to validate our findings. In post hoc power analysis, (1-β) was calculated to be 0.83 for comparing the incidence of PDR between the two genotype-based subgroups of rs3745468, when the two-sided α was set to 0.05. However, given the low frequency of PDR and the rs3745468 variant, our study had a relatively small number of participants, resulting in a high OR and wide 95% CI. The functional consequence of the rs3745468 variant on PRMT1 and HIF-1α transcription presumes the strength of the results of the relationship between the variant and PDR.

In conclusion, the rs3745468 variant may be a genetic marker to predict the PDR risk and help identify a subset of T2DM patients who prioritise fine-grained follow-up and personalised treatment for DR. Future experimental-based research to elucidate the pathological importance of PRMT1 in DR may develop new therapies that contribute to the delay or prevention of sight-threatening DR.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00592-021-01808-5.

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Authors' contributions All authors contributed to the conception and design of the study. Hiroaki Iwasaki performed the acquisition of data and the interpretation of the results by statistical analysis, and wrote the manuscript. Masayoshi Shichiri was involved in the research initiative, supervised research works in the laboratory and reviewed the manuscript. All authors have given their final approval of the posted version. Hiroaki Iwasaki is responsible for the integrity of the work as a whole.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Ethical approval The present study was approved by the Ethic Committee of Toshiba Rinkan Hospital held on 26 February 2018. The protocols were in compliance with the Declaration of Helsinki. All procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008.

Informed consent Written informed consent for being included in the study and regarding the publication of the study content was obtained from all subjects before the commencement of the study.

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