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Original Article

High frequency of type 2 diabetes and impaired glucose tolerance in Japanese subjects with the angiopoietin-like protein 8 R59W variant

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KEYWORDS:
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BACKGROUND: Angiopoietin-like protein 8 (ANGPTL8) is considered to be metabolically multifunctional. One notable function still to be elucidated definitively is a betatrophic role in protecting and preserving pancreatic beta-cell function. There is, however, a paucity of data regarding the role of ANGPTL8 in the etiology of type 2 diabetes (T2D), but some findings of human research have suggested the potential for significant involvement.

OBJECTIVE: To examine the frequency of T2D and impaired glucose tolerance (IGT) in Japanese subjects with the ANGPTL8 R59W variant.

METHODS: ANGPTL8 R59W (Rs2278426, c.194C > T) was determined by polymerase chain reaction–restriction fragment length polymorphism using the restriction enzyme FokI in 797 consecutive Japanese individuals. Subjects with triglyceride levels greater than or equal to 150 mg/dL were considered to be hypertriglyceridemic.

RESULTS: Genotype frequencies of ANGPTL8 R59W were as follows: wild-type RR (C/C) 53.5%, RW (C/T) 36.6%, and WW (T/T) 9.9%. T2D and IGT were significantly prevalent in WW and RW subjects relative to RR among all 797 subjects ($P = .0138$) and also in hypertriglyceridemic subjects ($P = .0015$). In multiple logistic regression models for the existence of T2D and IGT in hypertriglyceridemic subjects, the odds ratio for heterozygote RW and homozygote WW genotypes to wild-type RR was 2.406 ($P = .0017$) after controlling the risk factors of age, gender, and body mass index as covariates.

CONCLUSIONS: The frequency of ANGPTL8 R59W is significantly higher in Japanese subjects than in other ethnic groups. The rates of T2D and IGT were greater in subjects with the R59W variant. These findings indicate that ANGPTL8 is a participant in diabetes and a potential therapeutic target for T2D prevention, especially in East Asians.

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Introduction

Type 2 diabetes (T2D) is a serious public health problem especially among Asians because it occurs with a relatively lower body mass index (BMI) than in Caucasians. The number of T2D is still growing in Asia in accordance with the explosive population increase in the region. Because this is a global health problem that is not limited to Asians, it is clear that a critical goal should be to elucidate the pathogenesis underlying the occurrence of Asian T2D and impaired glucose tolerance (IGT).

Angiopoietin-like protein 8 (ANGPTL8) was considered to be metabolically multifunctional in previous studies.[1,2] One established function is to increase triglyceride (TG) levels through the suppression of lipoprotein lipase (LPL) activity by promoting ANGPTL3 cleavage.[2–5] Although LPL inhibition of ANGPTL8 is ANGPTL3-dependent, circulating ANGPTL8 concentration has more impact on serum lipids than the concentration of ANGPTL3.[6] Lipolysis of TG-rich lipoprotein with LPL is considered to play a pivotal role in insulin resistance and T2D. LPL activation therapy targeting the ANGPTL3-4-8 axis is expected to reduce not only atherogenic lipoproteins but also may decrease the risk of T2D.

To identify the gene encoding ANGPTL8 as a risk factor for T2D and to evaluate the relevance in lipid metabolism, we herein examined the frequency of T2D and IGT in Japanese subjects with R59W. Because East Asians, including the Japanese, show insulin secretion defect from the early stage of T2D and even in IGT, a beta-cell protective role of ANGPTL8 could be apparent in this study. Accordingly, we hypothesized that subjects with R59W would show a high frequency of T2D and IGT and an anti-atherogenic lipid profile.

Subjects and methods

Study design and study subjects

In total, 797 consecutive outpatients in the Department of Internal Medicine, Kanazawa University Hospital, and the Hokuriku Electric Power Clinic were recruited. This study was approved by the Ethics Committee of the Kanazawa University Graduate School of Medical Science, and written informed consent was obtained from each participant. Fasting plasma samples were collected, and plasma lipid and glucose levels, and hemoglobin A1c were measured. To exclude LPL deficiency, subjects with TG greater than 1000 mg/dL were excluded. Moreover, subjects undergoing regular hemodialysis therapy were also excluded. Subjects with a BMI greater than or equal to 25 were considered to be obese, and subjects with TG greater than or equal to 150 mg/dL were considered to have hypertriglyceridemia. The diagnoses of T2D and IGT were based on the American Diabetes Association's diagnostic criteria, 2010.[7]

PCR-RFLP assay

A coding variant in ANGPTL8 (Rs2278426, c.194C > T) responsible for an amino acid change, R59W, was identified with polymerase chain reaction–restriction fragment length polymorphism. The polymerase chain reaction–restriction fragment length polymorphism detection was validated with DNA sequencing. Genomic DNA was extracted from peripheral leukocytes and amplified by polymerase chain reaction using forward (5′-GACCCTCAGTCATGCCAGTG-3′) and reverse (5′-GAAGTTCCTGGGCTGCATCC-3′) primers. The amplified 285-bp DNA fragment was digested with FokI and separated by agarose electrophoresis into 196- and 89-bp fragments (Fig. 1). All the subjects were divided into 3 genotypes: wild-type (RR), heterozygous (RW), and homozygous (WW) (Fig. 1).

Statistical analysis

Data were reported as means ± standard deviation. A P-value $<.05$ was considered statistically significant. Continuous variables were compared with the Student's t-test or Wilcoxon nonparametric statistics and categorical variables with chi-square statistics. Baseline variables with $P < .20$ in univariate analysis were included in the multivariable models. Multiple logistic regression models yielding odds ratios and the 95% confidence interval were used to identify predictors. All statistical analyses were performed using the JMP software program version 13.1 for Macintosh (SAS Institute Inc. Cary, NC).

Results

Clinical characteristics of the 797 subjects (age: mean 53 ± 16 years, BMI: mean 23.5 ± 3.9 kg/m^2) are shown in Table 1. The study subjects were stratified according to their

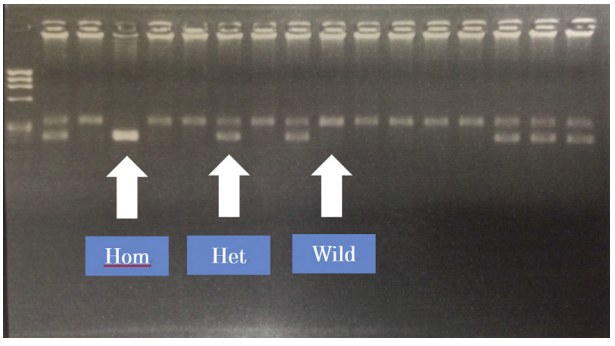

Figure 1 Agarose electrophoresis showing the ANGPTL8 R59W variant analyzed by PCR-RFLP. DNA samples were digested with restriction enzyme FokI and the resulting restriction fragments are separated according to their lengths by gel electrophoresis. ANGPTL8, angiopoietin-like protein 8; Hom, homozygote; Het, heterozygote; PCR-RFLP, polymerase chain reaction–restriction fragment length polymorphism; Wild, wild type.

Table 1 Baseline characteristics

Variable	Total (n = 797)	RR (n = 426)	RW (n = 292)	WW (n = 79)	*P*-value
Age (y)	53 ± 16	52 ± 17	54 ± 16	53 ± 15	.4268
BMI (kg/m^2)	23.5 ± 3.9	23.4 ± 3.8	23.7 ± 3.8	23.5 ± 4.3	.6630
TC (mg/dL)	230 ± 72	232 ± 79	228 ± 65	225 ± 57	.6162
TG (mg/dL)	150 ± 119	145 ± 128	152 ± 103	166 ± 122	.3459
HDL-C (mg/dL)	53 ± 18	53 ± 18	53 ± 18	51 ± 17	.5097
LDL-C (mg/dL)	160 ± 74	167 ± 83	154 ± 62	152 ± 59	.1034
PG (mg/dL)	121 ± 41	119 ± 41	121 ± 41	127 ± 45	.3509
HbA1c (%)	6.43 ± 1.45	6.33 ± 1.39	6.51 ± 1.48	6.59 ± 1.58	.2607

BMI, body mass index; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PG, plasma glucose; RR, ANGPTL8 R59W wild type; RW, ANGPTL8 R59W heterozygote; SD, standard deviation; TC, total cholesterol; TG, triglyceride; WW, ANGPTL8 R59W homozygote.

Data are indicated in means ± SD.

genotype: wild-type RR (C/C) 426 (53.5%), heterozygote RW (C/T) 292 (36.6%), and homozygote WW (T/T) 79 (9.9%). The frequency of the R59W genotype in the control group without both T2D and IGT was consistent with Hardy-Weinberg equilibrium: wild-type RR 283 (56.7%), heterozygote RW 175 (35.1%), and homozygote WW 41 (8.2%) (χ^2 test $P = .0641$). The frequency of each genotype was compared with other ethnic groups (Table 2).

The prevalence of T2D and IGT was significantly higher in subjects with heterozygote RW and homozygote WW genotypes than in those with the wild-type RR genotype ($P = .0138$*) (Table 3). Similar analyses were conducted in subjects stratified by plasma TG levels. In hypertriglyceridemic subjects, the frequencies of T2D and IGT were significantly higher in subjects with heterozygote RW and homozygote WW genotypes than in those with the wild-type RR genotype ($P = .0015$*) (Table 3). In multiple logistic regression models for the existence of T2D and IGT in hypertriglyceridemic subjects, the odds ratio for heterozygote RW and homozygote WW genotypes to wild-type RR was 2.406 (95% confidence interval: 1.380–4.200: $P = .0017$) after setting the risk factors of age, gender, and BMI as covariates. (Table 4).

Low-density lipoprotein to high-density lipoprotein (HDL) ratio in IGT subjects was significantly higher in wild-type RR subjects than in those with heterozygote RW and homozygote WW genotypes ($P = .0018$*) (Fig. 2). Because a longitudinal study is required to determine the exact age of nonobese T2D onset, we estimated the onset age and found it to be significantly higher in wild-type RR subjects than in those with heterozygote RW and homozygote WW genotypes ($P = .0373$*) (Fig. 3).

Discussion

This study found a high frequency of the ANGPTL8 R59W variant in Japanese subjects in comparison with the other previously reported ethnic groups. It also found a high frequency of T2D and IGT in subjects with heterozygote RW and homozygote WW genotypes in comparison with the wild-type RR genotype, especially in hypertriglyceridemic subjects. IGT subjects with R59W showed an antiatherosclerotic lipid profile.

As shown in Table 2, the frequency of Japanese subjects with R59W (47%) was significantly higher than in European Americans (5%), Arabs (12.4%), African-Americans (18%), and Hispanics (26%).[5,8] ANGPTL8 was initially introduced as a circulating adipokine that is secreted from adipocytes and hepatocytes and principally modulates lipid metabolism.[2,9–11] The ethnic differences in genotypes are also observed in other adipokines.[12–14] A thrifty gene hypothesis could explain the current distribution of genetic variants in East Asia affected by the historical times of nutrient scarcity.[15] Genetic selection could explain the ethnic differences in the frequency of R59W due to the variance in diet and the distribution of adipose tissues.

While a genome-wide association study has identified genetic variants associated with T2D, including the KCNQ1, UBE2E2, C2CD4A–C2CD4B, ANK1, SLC16A13, LEP-MIR129, and *GPSM1* genes in the Japanese,[9,16–19]

Table 2 Frequency of ANGPTL8 R59W in different ethnic groups

Race	RR (%)	WW and RW (%)	Study
European-Americans	95	5	Victor RG, et al. (Am J Cardiol. 2004)
African-Americans	82	18	Victor RG, et al. (Am J Cardiol. 2004)
Arabs	88	12	Abu-Farha M, et al. (Lipids Health Dis. 2016)
Japanese	53	47	JH Liu, et al. (current data)

ANGPTL8, angiopoietin-like protein 8; RR, ANGPTL8 R59W wild type; RW, ANGPTL8 R59W heterozygote; WW, ANGPTL8 R59W homozygote.

Table 3 Frequencies of T2D and IGT subjects in genotype variations of ANGPTL8 (Rs2278426, c.194 C > T)

Patient characteristics	T2DM + IGT (%)	OR (95% CI)	*P*-value
All subjects			
RR	134/344 (38.95)	1.00	–
RW + WW	149/307 (48.53)	1.478 (1.092–2.019)	.0138
Subjects with hypertriglyceridemia (TG ≥150)			
RR	42/103 (40.78)	1.00	–
RW + WW	71/114 (48.53)	2.398 (1.389–4.139)	.0015

ANGPTL8, angiopoietin-like protein 8; CI, confidence interval; IGT, impaired glucose tolerance; OR, odds ratio; RR, ANGPTL8 R59W wild type; RW, ANGPTL8 R59W heterozygote; WW, ANGPTL8 R59W homozygote; TG, triglyceride.

ANGPTL8 has not been nominated as an etiological candidate for T2D. However, our observations strongly suggest that reduced ANGPTL8 activity could lead to a diabetes-prone state with the coexistence of hypertriglyceridemia. Lifestyle changes such as higher fat intake and less physical activity accelerate the prevalence of T2D also in East Asians. Fujimoto et al reported that Japanese-Americans who have adopted Western dietary habits, including a higher consumption of animal fat, showed higher rates of T2D. Further increase in hypertriglyceridemia was observed along with IGT and T2D with lifestyle changes,[20] possibly reflecting oxidative insults derived from lipotoxicity.

T2D is a serious public problem especially in East Asians because it occurs with a relatively lower BMI than in Europeans. A previous study by Hsu et al[21] also showed that a typical Western diet might be associated with impaired insulin sensitivity in nonobese Asian-Americans, but not in nonobese Caucasian-Americans. Accordingly, East Asians are sensitive to changes in diet patterns. The findings of our present study lead us to speculate that racial difference in sensitivity to diet change could be explained by existence of the ANGPTL8 R59W variant. Induction of ANGPTL8 expression can be observed with a high-fat diet and hyperinsulinemia in the insulin-resistant state.[22–24] Our finding of a higher frequency of T2D and IGT in subjects with heterozygote RW and homozygote WW genotypes in comparison with the wild-type RR genotype, especially in hypertriglyceridemic subjects, might reflect the vulnerability of preserving beta cells against excessive fatty acids due to the presence of the ANGPTL8 R59W variant. It is important to note that elucidating the pathogenic backgrounds of Asian T2Ds is critical to dealing with the burgeoning global health problem of diabetes because the number of diabetic patients is expected to increase dramatically in Asia due to the population explosion in the region.[15] Moreover, ANGPTL8 R59W is prevalent in the Japanese and possibly throughout East Asians, which could be affecting the incidence of glucose intolerance in hypertriglyceridemic states, especially given the proliferating lifestyle changes including a Western diet.[25–28]

Another notable function that still requires unequivocal clarification is a betatrophic role in protecting and preserving pancreatic beta-cell function. In May 2013, Yi et al[29] controversially reported that ANGPTL8 overexpression resulted in improved glucose tolerance. They showed a 17-fold increase in beta-cell proliferation and a threefold increase in beta-cell mass and lower fasting blood glucose in their mice models. However, subsequent research findings negated their initial findings, and this relationship remains obscure. The controversy surrounding a betatrophic role of ANGPTL8 exists because of the difficulty in proving the hypothesis in a mouse model that only lacks ANGPTL8.[30–32] In humans, several studies reported an increase in circulating ANGPTL8 levels in patients with T2D[33–38] and obesity,[33,39,40] whereas others found no difference[32,41] or even a decrease[42] in these metabolic disorders. Genome-wide association studies for T2D risk have identified a significant number of genetic loci associated with disease susceptibility. Although ANGPTL8 has not been nominated as an etiological candidate for T2D, a previous study in Arabs showed the clinical significance of the ANGPTL8 R59W variant in glucose metabolism because

Table 4 Logistic regression analyses on T2D and IGT subjects with and without hypertriglyceridemia

Parameter	OR ratio	95% CI	r	*P*-value
T2DM and IGT subjects with hypertriglyceridemia (TG ≥150)				
Age	6.3089	1.5430–25.7952	2.6188	.0088
ANG R59W (RR)	2.4063	1.3799–4.1959	3.1366	.0017
T2DM and IGT subjects without hypertriglyceridemia (TG <150)				
Age	129.8739	27.6209–610.6689	6.8596	<.0001
Gender (M/F)	2.7614	1.5815–4.8214	3.7042	.0004
BMI	10.3472	1.4616–73.2512	2.3832	.0193

BMI, body mass index; CI, confidence interval; IGT, impaired glucose tolerance; OR, odds ratio; RR, ANGPTL8 R59W wild type; TG, triglyceride.

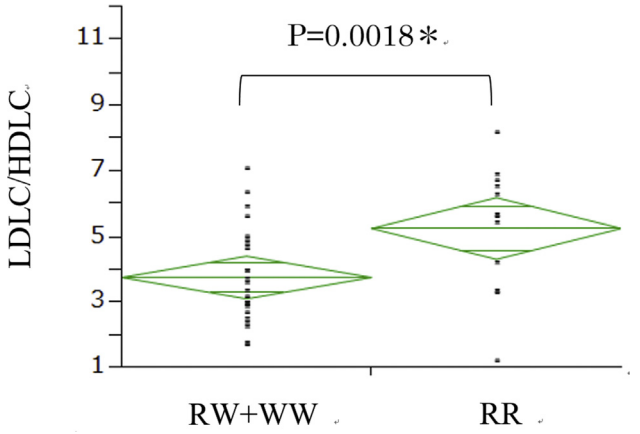

Figure 2 LDLC/HDLC ratio in IGT subjects. Ratio of LDL-C to HDL-C was compared between the subjects with wild-type RR and those with heterozygote RW and homozygote WW forms of the ANGPTL8 Rs.2278426 variants in 37 IGT subjects. ANGPTL8, angiopoietin-like protein 8; IGT, impaired glucose tolerance; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; RR, ANGPTL8 R59W wild type; RW, ANGPTL8 R59W heterozygote; WW, ANGPTL8 R59W homozygote.

subjects with R59W showed higher fasting glucose levels compared to the wild type.[8]

We also considered the clinical correlations with lipoproteins in subjects with the ANGPTL8 R59W variant. Low HDL is the major defect found with heterozygous LPL deficiency, and we assume that the lower LDL-C/HDL-C ratio in subjects with the variant (Fig. 2) could be due to greater LPL activity, leading to higher HDL. On the other hand, the age of nonobese diabetic subjects was lower in those with the variant than the wild type. This finding does not have a conclusive explanation, but given the beta-trophic role of ANGPTL8, it possibly reflects fragility of the less protected islet beta cells under the lipotoxic state of hypertriglyceridemia.

Because some reports have mentioned the importance of an optimal concentration of fatty acids in glucose-stimulated insulin secretion,[43–45] it is reasonable to explain the role of the ANGPTL8 R59W variant in insulin secretion from the viewpoint of LPL activity. The work of Pappan et al[46] may provide some insight into this discussion. In their study using beta cell–specific LPL transgenic mice, overexpression of LPL in the beta cell resulted in insulin secretion defect. This finding is compatible with our prediction that activation of LPL caused by a defect in ANGPTL8 would lead to insulin secretion defect. However, in their transgenic mice, glucose oxidation was impaired, but the islet adenosine triphosphate content paradoxically increased. If the overexpression of LPL resulted in a fatty acid concentration defect causing insulin secretion, islet adenosine triphosphate should have decreased. Accordingly, it is difficult to explain the insulin secretion defect arising from the lipid metabolism disturbance caused by LPL overexpression in the islets. In addition, they found that beta cell–specific LPL knockout mice also showed an insulin secretion defect, which is not compatible with our

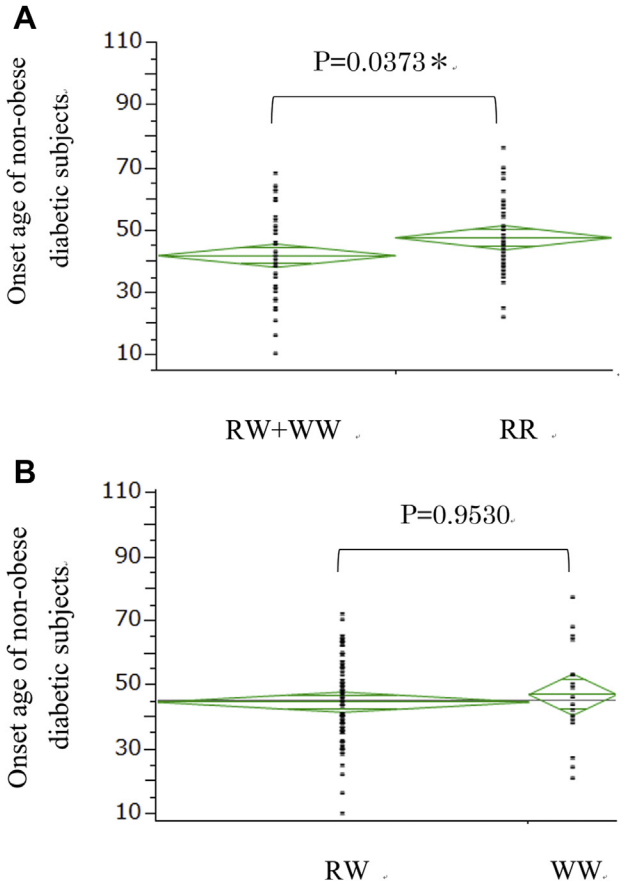

Figure 3 Onset age of non-obese diabetes subjects. (A) Onset age of T2D mellitus was compared between the subjects with wild-type RR and those with heterozygote RW and homozygote WW forms of the ANGPTL8 Rs.2278426 variants in 88 nonobese diabetes subjects. (B) Onset age of T2D mellitus was compared between the subjects with heterozygote RW and homozygote WW forms of the ANGPTL8 Rs.2278426 variants in 47 non-obese diabetes subjects. ANGPTL8, angiopoietin-like protein 8; T2D, type 2 diabetes; RR, ANGPTL8 R59W wild type; RW, ANGPTL8 R59W heterozygote; WW, ANGPTL8 R59W homozygote.

result. Thus, further investigations are required to explain the complex contribution of LPL in insulin secretion defect in subjects with the ANGPTL8 R59W variant.

It is important to note here that another common variant of ANGPTL8 has no effect on glucose metabolism. Clapham et al[47] reported an ANGPTL8 null variant (rs145464906; c.361C > T; p.Q121X) that results in nonsense-mediated RNA decay. Their subjects with p.Q121X showed no difference in glucose metabolism, but the variant was a viable LPL inhibitor. Nonsense-mediated RNA decay only alters the amount of protein without changing the protein's structure relating to function. The variance in the effects on glucose metabolism between R59W and p.Q121X could be explained depending on whether or not each variant accompanies alteration in the structure of ANGPTL8 that is critical for beta-cell protection.

There are some limitations in this study. First, it was performed with a cross-sectional design. Second, serum

ANGPTL8 concentrations of the subjects were not measured. Determining the level of ANGPTL8 would be useful to explore the relationship between lipid homeostasis and the development of diabetes in a prospective clinical study. To determine whether high TG predicts the subsequent development of T2D due to ANGPTL8 R59W, a longitudinal evaluation of ANGPTL8 R59W (vs wild-type) patients with normal fasting TG at baseline to assess the development of high TG over time (controlling for confounders) and incident T2D/IGT should be conducted in the future. In humans, it still remains to be demonstrated whether reductions in the risk of cardiovascular disease will be observed for TG-lowering variants such as those of ANGPTL8 and related proteins.[48] Nevertheless, one of the main strengths of our study is that the findings emphasize the significance of additional hypertriglyceridemia in clarifying an antidiabetogenic role of ANGPTL8.

Conclusions

Glucose intolerance was prevalent in Japanese subjects with the ANGPTL8 R59W variant, suggesting that ANGPTL8 might be a participant in diabetes and a potential therapeutic target for T2D prevention, especially in East Asians.

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Duality of interest: Authors' contributions: Jianhui Liu and Kunimasa Yagi designed and performed the study, analyzed the data, and wrote the article. Azusa Ohbatake, Aya Fujimoto, and Yukiko Miyamoto recruited the subjects. Atsushi Nohara and Daisuke Chujo contributed to the discussion. Junji Kobayashi and Masakazu Yamagishi reviewed and edited the article. Kunimasa Yagi is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Financial disclosure

The authors declare that there is no duality of interest associated with this article.

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