Original Research

Functional *AGXT2* SNP rs37369 Variant Is a Risk Factor for Diabetes Mellitus: Baseline Data From the Aidai Cohort Study in Japan

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Key Messages

- *AGXT2* rs37369 was independently related to diabetes mellitus.
- The CTA haplotype of rs37370, rs37369 and rs180749 was associated with diabetes.
- The interaction between rs37369 and smoking was not significant.

ARTICLE INFO

ABSTRACT

Objectives: The relationship between alanine-glyoxylate aminotransferase 2 (*AGXT2*) single-nucleotide polymorphisms (SNPs) and diabetes mellitus (DM) has not been investigated. Therefore, we performed a case−control study to examine this relationship.

Methods: The study subjects included 2,390 Japanese men and women aged 34 to 88 years. In total, 190 cases were defined as having a fasting plasma glucose level $\geq$126 mg/dL, hemoglobin (A1C) $\geq$6.5% or as currently using diabetic medication. The 2,200 remaining participants served as control subjects.

Results: Compared with study subjects with the CC genotype of *AGXT2* SNP rs37369, those with the TT, but not CT, genotype had a significantly increased risk of DM: the adjusted odds ratio (OR) for the TT genotype was 1.83 (95% confidence interval [CI], 1.04 to 3.47). *AGXT2* SNPs rs37370 and rs180749 were not significantly associated with the risk of DM. The CTA haplotype of rs37370, rs37369 and rs180749 was significantly positively associated with the risk of DM (crude OR, 1.25; 95% CI, 1.01 to 1.56), whereas the CCA haplotype was significantly inversely related to DM (crude OR, 0.53; 95% CI, 0.27 to 0.95). The multiplicative interaction between *AGXT2* SNP rs37369 and smoking status with regard to the risk of DM was not significant (p=0.32 for interaction).

Conclusions: This is the first study to show significant associations between *AGXT2* SNP rs37369, the CTA haplotype, and the CCA haplotype and DM. No interaction with regard to the risk of DM was observed between rs37369 and smoking.

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R É S U M É

Objectifs : La relation entre les polymorphismes mononucléotidiques (PMN) dans l'alanine-glyoxylate aminotransférase 2 (*AGXT2*) et le diabète sucré (DS) n'a pas fait l'objet d'études. Par conséquent, nous avons réalisé une étude cas témoins pour examiner cette relation.

Méthodes : Les sujets de l'étude étaient 2390 femmes et hommes japonais de 34 à 88 ans. Au total, nous avons recensé 190 cas qui avaient des concentrations plasmatiques de glucose à jeun ≥ 126 mg/dl, une hémoglobine (A1c) ≥ 6,5 % ou qui utilisaient actuellement des médicaments contre le diabète. Les 2200 participants restants constituaient les sujets témoins.

Résultats : Comparativement aux sujets de l'étude qui avaient un génotype CC de PMN rs37369 dans l'*AGXT2*, ceux qui avaient le génotype TT, mais non le génotype CT, avaient un risque significativement accru de DS : le rapport de cotes (RC) ajusté du génotype TT était de 1,83 (intervalle de confiance [IC] à 95 %, de 1,04 à 3,47). Les PMN rs37370 et rs180749 dans l'*AGXT2* n'étaient pas associés de façon significative au risque de DS. L'haplotype CTA du rs37370, du rs37369 et du rs180749 était de façon significative associé positivement au risque de DS (RC brut, 1,25 ; IC à 95 %, de 1,01 à 1,56), alors que l'haplotype CCA était de façon significative relié inversement (RC brut, 0,53 ; IC à 95 %, de 0,27 à 0,95). Quant au risque de DS, l'interaction multiplicative entre le PMN rs37369 dans l'*AGXT2* et le tabagisme n'était pas significative (valeur p de l'interaction = 0,32).

Conclusions : Il s'agit de la première étude à démontrer les associations significatives entre le PMN rs37369 dans l'*AGXT2*, l'haplotype CTA et l'haplotype CCA, et le DS. Quant au risque de DS, aucune interaction n'a été observée entre le rs37369 et le tabagisme.

Introduction

Asymmetric N^G,N^G-dimethyl-L-arginine (ADMA) is an endogenous inhibitor of nitric oxide synthase. Nitric oxide inhibits apoptosis and increases proliferation and migration of endothelial cells (1). ADMA inhibits endothelial cell proliferation and angiogenesis (2). ADMA and its enantiomer, symmetric N^G,N'^G-dimethyl-L-arginine (SDMA), are strong predictors of cardiovascular events and death in a range of illnesses (3,4). Not only individuals with type 2 diabetes mellitus (DM), but also those with prediabetes, have been shown to have significantly elevated concentrations of ADMA compared with healthy controls (5).

Alanine-glyoxylate aminotransferase 2 (AGXT2), first identified in 1969, is an important enzyme that regulates the metabolism of methylarginines, such as ADMA and SDMA (6). *AGXT2* is located on chromosome 5p13. In a study in Egypt, serum concentrations of ADMA and SDMA were higher in the TT genotype of *AGXT2* single-nucleotide polymorphism (SNP) rs37369 (V140I) compared with the CC genotype, and in the AA genotype of rs16899974 (V498L) compared with the CC genotype (7). In a German study, plasma concentrations of SDMA, but not ADMA, were higher in those heterozygous or homozygous for the minor allele of rs37369, whereas no such positive relationship was found between rs16899974 and plasma concentrations of ADMA or SDMA (8). *AGXT2* SNPs have also been shown to be related to the following vascular conditions and diseases and related biological data: coronary artery disease (7); heart rate variability (9); carotid atherosclerosis (10); atrial fibrillation (11); chronic heart failure (12); and blood pressure, blood sugar and blood creatinine (13).

AGXT2 activity is abolished among 30% to 40% of Japanese people, whereas <10% of individuals with European ancestry show a lack of AGXT2 activity (14). *AGXT2* SNPs rs37370 (S102N), rs37369, rs180749 (T212I) and rs16899974, and the CAAA haplotype decided by these SNPs, are associated with loss of function in AGXT2 activity (13,15).

In view of the lack of epidemiologic information regarding the relationship between *AGXT2* SNPs and the risk of DM, in this case–control study we investigated this issue in Japanese adults using baseline data from the Aidai Cohort Study (AICOS). In addition, haplotype analyses were performed, and the possibility of interaction between SNPs and smoking history was assessed. Tobacco products acutely increase the endothelial production of ADMA (16), and studies in China have reported a possible interaction between SNP rs37369 and smoking with regard to heart disease (12,17).

Methods

Study population

The AICOS is an ongoing population-based prospective study that started in 2015 (18–22). The present case–control study was conducted using baseline data from the AICOS collected in Yawatahama city in 2015, Uchiko town in 2016 and Seiyo city and Ainan town in 2017. Yawatahama city, Uchiko town, Seiyo city and Ainan town, with total populations of nearly 36,000, 17,000, 38,000 and 22,000, respectively, are 4 of the 20 municipalities in Ehime Prefecture on Shikoku Island, which is located south of Japan's main island. In Yawatahama city in 2015, Uchiko town in 2016, Seiyo city in 2017 and Ainan town in 2017, 798, 347, 524 and 755 study participants, respectively, were recruited from among those who had received health checkups conducted by their municipality of residence or through one of several alternative recruiting procedures. In addition, 223 employees of a manufacturing company (PHC Corp) in Toon city also participated in the AICOS. Ultimately, 2,647 participants, aged 33 to 89 years (1,069 men aged 35 to 89 years and 1,578 women aged 33 to 85 years), gave written informed consent and completed the baseline questionnaire. From among these participants, 257 were excluded because of missing or incomplete data on the factors under study, leaving data on 2,390 participants available for analysis (977 men aged 35 to 88 years and 1,413 women aged 34 to 85 years). The AICOS was approved by the ethics committee of the Ehime University Graduate School of Medicine (Approval No. 1504012).

Measurements

A self-administered questionnaire elicited information on age; sex; smoking habits; alcohol consumption habits; physical activity level; education; and current use of antihypertensive, cholesterol-lowering and diabetic medications. Never smoking was defined as having smoked <100 cigarettes over the lifetime. Former smoking was defined as having smoked >100 cigarettes over the lifetime, but having quit smoking by the time of the survey. Current smoking was defined as having smoked >100 cigarettes over the lifetime and still smoking at the time of the survey. Leisure-time physical activity was defined as present if the study participant had engaged in at least 30 minutes of any type of moderate-to-vigourous physical activity, such as brisk walking, golf, gardening, jogging or playing tennis, at least once a week. Body height and weight were measured in light clothing without shoes, and body mass index (BMI) was calculated as body weight (in kilograms) divided by height (in metres squared). An automated sphygmomanometer was used to take 2 blood pressure measurements, each with the study subject in the sitting position after at least 5 minutes of rest; the second measurement was used for the present study. Hypertension was defined as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or current use of antihypertensive medication. Blood samples were collected from an antecubital vein after overnight fasting. Serum low-density cholesterol, high-density lipoprotein cholesterol, triglyceride concentrations, plasma glucose concentrations and A1C levels were measured at an external laboratory (Shikoku Chuken, Ehime, Japan). Dyslipidemia was defined as a serum low-density lipoprotein cholesterol concentration ≥140 mg/dL, high-density lipoprotein cholesterol concentration <40 mg/dL, triglyceride concentration ≥150 mg/dL or current use of cholesterol-lowering medication. DM was defined as a fasting plasma glucose level ≥126 mg/dL, A1C level ≥6.5% or current use of diabetic medication. Among the 2,390 participants, 190 cases of DM were identified; the 2,290 remaining participants served as control subjects.

DNA extraction and genotyping

Genomic DNA was prepared from peripheral blood using a DNA extraction kit (QuickGene-610L; Fujifilm Wako Pure Chemical Corp, Osaka, Japan). Four *AGXT2* SNPs, rs37370 (C_1018750_1_), rs37369 (C_11162986_1_), rs180749 (C_1018735_1_) and rs16899974 (C_25742181_10), were genotyped using the pre-standardized and experimentally validated TaqMan SNP genotyping assay on a StepOnePlus machine (Applied Biosystems, Foster City, California, United States), according to the manufacturer's instructions.

Statistical analysis

Calculations of the Hardy−Weinberg equilibrium were performed among the controls using a chi-square test. Linkage disequilibrium was examined using Haploview software version 4.2 (Broad Institute, Cambridge, Massachusetts, United States) (23). Using logistic regression analysis, crude odds ratios (ORs) and 95% confidence intervals (CIs) for DM associated with the SNPs under investigation were calculated, with the reference category being the homozygote of the ancestral allele based on the SNP database of the National Center for Biotechnology Information. Multiple logistic regression analysis was performed to adjust for age, sex, smoking status, alcohol consumption, leisure-time physical activity, education, BMI, hypertension and dyslipidemia. The statistical power calculation was performed using QUANTO version 1.2.4 (24). Haplotypes and their frequencies were inferred by means of the expectation maximization algorithm using Stata/SE software version 13.1 (StataCorp, College Station, Texas, United States). For differences in haplotype frequency between the cases and controls, crude ORs and 95% CIs were estimated based on the frequency of each haplotype relative to all other haplotypes combined. The multiplicative interaction was estimated by introducing a multiplicative term into a multiple logistic regression model. All analyses excluding the calculation of linkage disequilibrium, statistical power and haplotype analysis, were conducted using SAS version 9.4 (SAS Institute, Cary, North Carolina, United States).

Results

Compared with the control subjects, the cases were more likely to be older, male and current alcohol drinkers, to report higher BMI, hypertension and dyslipidemia, and to report never having smoked (Table 1). No differences were found between cases and controls with respect to leisure-time physical activity and education.

Among the control subjects, *AGXT2* SNPs rs37370, rs37369 and rs180749 did not deviate from Hardy−Weinberg equilibrium (p=0.24, 0.06 and 0.30, respectively). On the other hand, *AGXT2* SNP rs16899974 deviated (p=0.04); therefore, *AGXT2* SNP rs16899974 was excluded from this study. *AGXT2* SNPs rs37370, rs37369 and rs180749 showed weak linkage disequilibrium (r^2≤0.13, 0.46≤D'≤0.80). The minor allele frequency was 0.47 in cases and 0.44 in controls for rs37370, 0.32 in cases and 0.36 in controls for rs37369 and 0.22 in cases and 0.21 in controls for rs180749.

Compared with study participants with the CC genotype of *AGXT2* SNP rs37369, those with the TT, but not the CT, genotype had a significantly increased risk of DM (Table 2). Adjustment for confounders under study did not materially change the result: the adjusted OR for the TT genotype was 1.83 (95% CI, 1.04 to 3.47). *AGXT2* SNPs rs37370 and rs180749 were not significantly associated with the risk of DM. The adjusted OR for SNP rs180749 under the additive model was 0.90 (95% CI, 0.70 to 1.17); the statistical power calculation revealed that, using our sample size, we could detect a gene−disease association for an OR of 0.703 with an accuracy of >80% at a significance level of 0.05 with a 2-sided alternative hypothesis under the log-additive model.

Table 1
Characteristics of the study population

Variable	Cases (N=190)	Controls (N=2,200)	p Value *
Age, years	66.4 ± 8.7	60.6 ± 10.6	<0.0001
Males	103 (54.2%)	874 (39.7%)	<0.0001
Smoking status			0.01
Never	107 (56.3%)	1,478 (67.2%)	
Former	63 (33.2%)	541 (24.6%)	
Current	20 (10.5%)	181 (8.2%)	
Alcohol consumption			0.006
Never	74 (39.0%)	910 (41.4%)	
Former	23 (12.1%)	134 (6.1%)	
Current	93 (49.0%)	1,156 (52.6%)	
Leisure-time physical activity	93 (49.0%)	941 (42.8%)	0.10
Education, years			0.06
<13	32 (16.8%)	285 (13.0%)	
13−14	97 (51.1%)	1,025 (46.6%)	
≥15	61 (32.1%)	890 (40.5%)	
Body mass index, kg/m²	25.0±3.9	23.3±3.3	<0.0001
Hypertension	121 (63.7%)	932 (42.4%)	<0.0001
Dyslipidemia	119 (62.6%)	1,176 (53.5%)	0.01

Note: Data expressed as number (%) or as mean ± standard deviation.
* Chi-square test or Wilcoxon's rank-sum test.

Table 2
Odds ratios and 95% confidence intervals for diabetes mellitus in relation to *AGXT2* polymorphisms

SNP	Genotype	Cases, n (%)	Controls, n (%)	Crude OR (95% CI)	Adjusted OR (95% CI) *
rs37370		(N=190)	(N=2,195)		
	CC	46 (24.2%)	440 (20.1%)	1.00	1.00
	CT	88 (46.3%)	1,055 (48.1%)	0.80 (0.55–1.17)	0.80 (0.54–1.18)
	TT	56 (29.5%)	700 (31.9%)	0.77 (0.51–1.15)	0.74 (0.48–1.13)
rs37369		(N=190)	(N=2,200)		
	CC	14 (7.4%)	266 (12.1%)	1.00	1.00
	CT	92 (48.4%)	1,057 (48.1%)	1.65 (0.96–3.07)	1.59 (0.91–3.00)
	TT	84 (44.2%)	877 (39.9%)	1.82 (1.05–3.39) †	1.83 (1.04–3.47) †
rs180749		(N=190)	(N=2,200)		
	GG	10 (5.3%)	102 (4.6%)	1.00	1.00
	GA	63 (33.2%)	704 (32.0%)	0.91 (0.47–1.94)	0.86 (0.43–1.86)
	AA	117 (61.6%)	1,394 (63.4%)	0.86 (0.46–1.79)	0.78 (0.41–1.66)

CI, confidence interval; *OR*, odds ratio.

* Adjusted for age, sex, smoking status, alcohol consumption, leisure-time physical activity, education, body mass index, hypertension and dyslipidemia.

† Statistically significant difference.

Seven haplotypes with a frequency of $\geq 1\%$ in both cases and controls were detected (Table 3). Given that the haplotype order was rs37370, rs37369 and rs180749, the CTA haplotype was significantly positively associated with the risk of DM compared with all other haplotypes combined: the crude OR was 1.25 (95% CI, 1.01 to 1.56). By contrast, the CCA haplotype was significantly inversely related to the risk of DM in comparison to all other haplotypes combined: the crude OR was 0.53 (95% CI, 0.27 to 0.95).

Compared with having never smoked, former and current smoking was not significantly related to the risk of DM (adjusted ORs: 1.12 [95% CI, 0.73 to 1.71] and 1.73 [95% CI, 0.94 to 3.07], respectively). We investigated the potential interaction between *AGXT2* SNP rs37369 and smoking status with respect to the risk of DM (Table 4). A positive association was observed between current smoking and risk of DM among participants with at least 1 C allele of *AGXT2* SNP rs37369, whereas no association was found between current smoking and risk of DM in participants with the TT genotype; the multiplicative interaction between *AGXT2* SNP rs37369 and current smoking was not statistically significant (p=0.32 for interaction).

Discussion

To our knowledge, this current case–control study is the first to report an association between *AGXT2* SNPs and the risk of DM. We have shown that, compared with the CC genotype of *AGXT2* SNP rs37369, the TT genotype, occurring in 39.9% of the control subjects, was independently positively related to DM, whereas the CT genotype was not associated with DM. No relationships were observed between *AGXT2* SNPs rs37370 or rs180749 and DM. Haplotype analyses identified a significant positive association

between the CTA haplotype of rs37370, rs37369 and rs180749, occurring in 36.5% of the control subjects, and DM, and a significant inverse relationship between the CCA haplotype, occurring in 5.8% of the control subjects, and DM.

A case–control study of 100 patients with coronary artery disease and 50 healthy controls conducted in Egypt showed that the rs37369 T allele and the rs16899974 A allele were significantly positively associated with coronary artery disease [7]. With respect to the relationship with SNP rs37369, this result is in partial agreement with our findings. The rs37369 GG genotype was significantly associated with an increased risk of chronic heart failure in a Chinese case–control study of 1,000 cases and 1,200 healthy controls [12]. The Young Finns Study showed that the rs37369 C and rs16899974 C alleles were associated with an increase in the ratio between the low- and high-frequency spectral components of short-term heart rate variability [9]. SNP rs37370, but not rs37369 or rs180749, and the aforementioned CAAA haplotype were significantly related to carotid atherosclerosis in a study of 1,426 Japanese individuals [10]. Another study of 750 older Japanese adults showed that SNP rs16899974 and the CAAA haplotype, but not rs37370, rs37369 or rs180749, were significantly associated with diastolic blood pressure, and that rs16899974, but not rs37370, rs37369, rs180749 or the CAAA haplotype, was significantly associated with casual blood sugar [13]. In another Chinese case–control study of 942 patients with coronary heart disease and 1,103 controls, rs37369 was not independently related to coronary heart disease [17]. SNP rs16899974, but not rs37369, was significantly associated with atrial fibrillation in a study of 1,834 individuals with atrial fibrillation and 7,159 unaffected individuals from Germany and Finland [11]. Regarding the presence or direction of the relationship with SNP rs37369, those results are at variance with our findings. On the other hand, the results related to the CAAA haplotype are in partial agreement with our finding regarding the CTA haplotype.

Hepatocyte nuclear factor 4α (HNF4α) directly binds to the *AGXT2* promoter and acts as a major regulator of AGXT2 expression [25]. Severe inborn HNF4α deficiency is rare and leads to maturity-onset diabetes of the young [26], and the mild *HNF4A* polymorphism is associated with an increased risk of type 2 DM [27,28]. HNF4α deficiency and subsequent downregulation of AGXT2 leads to an increase in plasma ADMA and SDMA concentrations and may be related to the risk of DM. Given the positive association between the TT genotype of SNP rs37369 and serum concentrations of ADMA and SDMA [7], and the positive relationship between the CAAA haplotype and plasma ADMA concentrations [13], the TT genotype of SNP rs37369 and the CTA haplotype may be associated with the development of DM via higher serum concentrations of ADMA and/or SDMA.

Table 3
Haplotype analysis of 3 *AGXT2* polymorphisms in relation to diabetes mellitus *

Haplotype †	Frequency, n (%)		Crude OR (95% CI) ‡
	Cases (2N = 380)	Controls (2N = 4,400)	
TTA	31 (8.2%)	483 (11.0%)	0.72 (0.48–1.06)
CTA	159 (41.8%)	1,604 (36.5%)	1.25 (1.01–1.56) §
TCA	96 (25.3%)	1,148 (26.1%)	0.96 (0.74–1.22)
CCA	12 (3.2%)	257 (5.8%)	0.53 (0.27–0.95) §
TTG	65 (17.1%)	706 (16.0%)	1.08 (0.80–1.43)
TCG	8 (2.1%)	129 (2.9%)	0.71 (0.30–1.46)
CCG	4 (1.1%)	55 (1.3%)	0.84 (0.22–2.29)

CI, confidence interval; *OR*, odds ratio.

* Rare haplotypes (frequency <1% in either cases or controls) were deleted.

† Haplotype order is rs37370, rs37369 and rs180749.

‡ Crude OR for each haplotype is relative to all other haplotypes combined.

§ Statistically significant difference.

Table 4
Interaction between *AGXT2* SNP rs37369 and smoking status with regard to risk of DM

| Smoking status | *AGXT2* SNP rs37369 | | | | p Value for interaction |
| | CC + CT | | TT | | |
	No. cases/controls	Adjusted OR (95% CI) *	No. cases/controls	Adjusted OR (95% CI) *	
Never	55/895	1.00	52/583	1.00	
Former	39/320	1.51 (0.87–2.63)	24/221	0.72 (0.36–1.42)	0.12
Current	12/108	2.20 (0.99–4.66)	8/73	1.22 (0.46–2.96)	0.32

CI, confidence interval; *DM*, diabetes mellitus; *OR*, odds ratio.

* Adjusted for age, sex, alcohol consumption, leisure-time physical activity, education, body mass index, hypertension and dyslipidemia.

In the present study, no significant multiplicative interaction was observed between *AGXT2* SNP rs37369 and smoking status with regard to risk of DM. In a previously cited case–control study in China, under the recessive model, a significant positive association was found between the rs37369 GG genotype and coronary heart disease among smokers, but not among nonsmokers, yet the interaction was not assessed ([17]). Similarly, in another previously cited case–control study in China, although the interaction was not examined, a significant positive association was found between the rs37369 GG genotype and chronic heart failure among smokers, but not among nonsmokers ([12]).

The present study had methodological advantages. The participants were homogeneous with respect to their residential area, and we controlled for several potential confounding factors.

Several limitations of our study should also be considered. In the baseline survey of the AICOS, the participation rate was low. Thus, our study participants were probably not representative of the Japanese general population. For example, the educational level of our participants was higher than that of the general population. According to a population census conducted in 2010 in Ehime prefecture ([29]), the proportions of persons aged 60 to 69 years with low, medium, high and unknown educational levels were 28.2%, 48.6%, 19.0% and 4.2%, respectively, in men, and 26.7%, 56.4%, 12.9% and 4.0%, respectively, in women. The corresponding figures in the present study for persons aged 60 to 69 years were 12.2%, 54.8%, 33.0% and 0.0%, respectively, in men, and 16.5%, 52.4%, 31.2% and 0.0%, respectively, in women. On the other hand, the distributions of all 3 SNPs under study were within the Hardy–Weinberg equilibrium and any selection bias in relation to genotype distribution was negligible.

Because no attempt was made to ascertain outcome status through reviews of medical records, we could not distinguish type 1 from type 2 DM. Nondifferential outcome misclassification may have biased the magnitude of the observed associations toward the null. In addition, the number of cases was rather small for a valid genetic association study, although a significant association was detected. The lack of significant relationships between *AGXT2* SNPs rs37370 or rs180749 and DM may be ascribed to insufficient statistical power. Residual confounding effects could not be ruled out.

In conclusion, in this case–control study we demonstrated that the TT genotype of *AGXT2* SNP rs37369 and the CTA haplotype of rs37370, rs37369 and rs180749 were significantly positively associated with the risk of DM, whereas the CCA haplotype was significantly inversely related to DM in Japanese adults. No significant interaction between *AGXT2* SNP rs37369 and smoking status was observed with respect to the risk of DM. These findings should be confirmed by further epidemiologic studies with larger numbers of subjects, especially patients with DM, in addition to functional studies.

Acknowledgments

Author Disclosures

Conflicts of interest: None.

Author Contributions

Conception or design of the study: H. Kumon, Y. Miyake, Y. Yoshino, J. Iga, K. Tanaka, E. Kimura, R. Kawamoto and S. Ueno. Drafting or revision of the manuscript: H.K., Y.M. and J.I. All authors were involved in acquisition, analysis, interpretation of the data and final approval of the manuscript submitted for publication.

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