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Correlations between lipoprotein(a) gene polymorphisms and calcific aortic valve disease and coronary heart disease in Han Chinese

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Abstract

Objective: To investigate the relationship between lipoprotein(a) gene (*LPA*) polymorphisms and calcific aortic valve disease (CAVD) and coronary heart disease (CHD) in Han Chinese.

Methods: A total of 148 patients were recruited (n = 71 with CAVD and n = 77 with CHD) based on a diagnosis achieved using color Doppler echocardiography, coronary angiography, or computed tomography angiography. Seventy-one control individuals without CAVD or CHD were also recruited. Biomarkers including levels of lipoprotein(a) [Lp(a)], low-density lipoprotein and high-density lipoprotein cholesterol, apolipoprotein A1, and apolipoprotein B were tested. *LPA* polymorphisms rs10455872, rs6415084, rs3798221, and rs7770628 were analyzed using SNaPshot SNP.

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Results: Lp(a) levels were significantly higher in CAVD and CHD groups compared with controls. There was no significant difference in the allelic frequency distribution of rs3798221, rs7770628, or rs6415084 between CHD, CAVD, and control groups. Linear regression showed that rs3798221, rs7770628, and rs6415084 were associated with increased Lp(a) concentrations. Two CAVD patients among the 219 participants carried AG minor alleles at rs10455872, while the remainder carried AA minor alleles.
Conclusion: rs3798221, rs6415084, and rs7770628 polymorphisms within *LPA* are associated with higher Lp(a) plasma levels, which correlate with increased CAVD and CHD risks.

Keywords
Lipoprotein(a) gene polymorphism, Han Chinese, calcific aortic valve disease, coronary heart disease, genotype, biomarker

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Introduction

Epidemiological and genetic studies provide strong evidence that lipoprotein(a) (Lp(a)) is a causal risk factor for cardiovascular disease (CVD).[1,2] Recent findings suggest that it is difficult to modify Lp(a) concentrations through lifestyle changes because levels are mainly determined by Lp(a) production rather than catabolism. Lp(a) levels are also highly heritable, with more than 50% of all variations caused by polymorphisms at the lipoprotein(a) gene (*LPA*) locus.[3–5] *LPA* was sequenced in 1987,[6] and since then several of its polymorphisms have been reported to be strong risk factors for CVD.

Lp(a) concentrations are particularly dependent on Apo A kringle-IV type 2 (KIV-2) copy numbers, with low KIV-2 copy numbers being associated with smaller Apo A isoforms and higher Lp (a) molar levels, leading to a higher risk of CVD.[7] Furthermore, Lp(a) is a major carrier of pro-inflammatory and pro-calcifying oxidized phospholipids (OxPL), supporting an important role for OxPL-mediated valvular interstitial cell calcification in CVD development.[8,9]

rs10455872 is a common *LPA* single nucleotide polymorphism (SNP) that has been intensively studied,[10] and which is significantly associated with Lp(a) concentrations in Caucasians. The minor allele (G; 7.0%) of rs10455872 is associated with increased Lp(a) levels.[11] rs3798221, rs7770628, and rs6415084 SNPs in *LPA* have also been investigated,[12–14] but these studies were mainly conducted in European Caucasian, African-American, or Hispanic-American populations; few studies have been carried out in the Han Chinese population.[15] Therefore, this cross-sectional study aimed to assess the associations between Lp(a) levels and the four *LPA* SNPs, to determine whether they are linked to the risks of CAD or CAVD development in Han Chinese.

Methods

Study population

A total of 148 patients were recruited from the Department of Cardiology, Tianjin Chest Hospital between January 2017 and September 2018. They underwent an

echocardiographic assessment, coronary angiography (CAG), or computed tomographic angiography (CTA) and were divided into two groups: the CAVD group (n = 71, of whom 23 patients received CTA and 48 received CAG) including patients with aortic valve stenosis and without CHD, and the CHD group (n = 77, of whom 11 patients received CTA and 66 received CAG) including patients with CHD but without aortic valve stenosis. CAVD was defined by an echocardiographic assessment as increased echogenicity, calcification, and thickening $\geq$1 mm of the aortic valve, and trans-aortic valve flow velocity >2.5 m/s. CHD was defined as luminal diameter stenosis $\geq$50% in a major epicardial coronary artery and its main branch. The control group (n = 71 who all received CTA) was selected from the physical examination center during the same time period, and included individuals without CAVD or CHD but with an aortic jet velocity < 1.5 m/s. Exclusion criteria for all participants were: aortic valve stenosis of rheumatic heart disease, infective endocarditis, severe renal failure, hyperparathyroidism, congenital bicuspid aortic valve, and lupus erythematosus.

The ethics committee of Tianjin Chest Hospital approved the study (approval no. 2019LW-008) and all study participants provide their written informed consent.

Characteristics and lipid profile determination

Characteristics such as age, sex, height, weight, body mass index (BMI), and blood pressure of each participant were recorded. After 12 hours of fasting, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), apolipoprotein A (Apo A), Apo B, triglycerides (TGs), and fasting blood glucose (FBG) were measured using commercial kits (Roche Diagnostics, Mannheim, Germany) and a Roche C701 analyzer. Information about participants' history of diabetes and hypertension was obtained from the medical history.

Lp(a) level determination

Lp(a) levels were measured by the latex-enhanced immunoturbidimetric assay. The Lp(a) detection kit (Roche Diagnostics, Mannheim, Germany) was used to determine the precipitation at 800/660 nm using latex particles coated with an anti-Lp(a) antibody to allow agglutination with human lipoprotein. Lp(a) levels of less than 75 mmol/L were considered to be within the normal range.

DNA extraction and genotyping

Lp(a) concentrations were shown to be particularly dependent on KIV-2 copy number, which is determined by *LPA*. The NCBI dbSNP database was searched for *LPA* SNPs and rs10455872 (high frequency: A, low-frequency: G), rs3798221 (high frequency: G, low-frequency: T), rs6415084 (high frequency: C, low frequency: T), and rs7770628 (high frequency: T, low frequency: C) genotypes, which were shown to associate with CHD or CAVD in white European, African-American, and Hispanic-American cohorts. 10. Participants fasted for 12 hours, then 2 ml of peripheral whole blood was collected in ethylenediaminetetraacetic acid tubes, stored at –20°C and DNA was extracted using the phenol-chloroform method. rs10455872, rs3798221, rs6415084, and rs7770628 genotypes were determined using the SNaPshot genotype discrimination assay. Genotyping was performed using the ABI PRISM® SNaPshot™ Multiplex Kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Primer sequences are shown in Table 1.

Table 1. Primer sequences for PCR.

Primer name	Primer sequence (5′ to 3′)	Product size (bp)
rs7770628-F	GAGTATTTTGAAATGCACCGTATG	291
rs7770628-R	CAGGCTAGAACAAATATGCAGAAA	
rs3798221-F	AAAGCATGGGTCTTCTAACCAATA	283
rs3798221-R	TGTTTCTCTGCTCATGCAATTACT	
rs6415084-F	ACTCTCTTGAGTCCCTACCCAATT	197
rs6415084-R	TTAAATAGCATGGATGGAGCTCA	
rs10455872-F	CCTGAATGTGTAACTCTTCAGCA	245
rs10455872-R	CATTCTAATCTCCAAGCCCTG	
rs7770628-YS (F)	CTCCATGCACACTTTAATGTGTA	52
rs3798221-YS (F)	GAGTTGGCTGTTGCTCCTCTTAT	44
rs6415084-YS (R)	TATACTCAGGAAAGAAGCCATGT	56
rs10455872-YS (F)	CAGACACCTTGTTCTCAGAACCCA	61

F: forward primer R: reverse primer.

Statistical analyses

Statistical analyses were performed using IBM SPSS software (version 22.0; IBM Inc., Armonk, NY, USA). The significance level was set at $P < 0.05$. Continuous variables (age, height, weight, and BMI) defined to follow a normal distribution were represented as means ± standard deviations, and compared using one-way analysis of variance. Those not following a normal distribution were represented as quartiles. Categorical data were reported as frequencies and percentages and compared using the Chi-square test. Indicators not following a normal distribution, such as Lp(a), underwent natural logarithm conversion and were compared using the Kruskal–Wallis test. Binary logistic regression was used to analyze the influence of different genotypes on the incidence of CAVD and CHD, and the odds ratio (OR) and 95% confidence interval (95% CI) were calculated. Linear regression was used to analyze the effects of different genotypes and APOB levels on the Lp(a) concentration, with a test level of $\alpha = 0.05$.

The four SNPs were also assessed for deviance from the Hardy–Weinberg equilibrium. Our results showed that there were no statistically significant differences between the actual genotype frequencies of the four SNPs and the expected value.

Results

Baseline characteristics and lipid profile level

Baseline characteristics. No significant differences were observed with respect to sex or BMI among the three groups; however, there was a significant difference in age ($P < 0.05$) with the average age of patients in the CHD group being significantly higher than that of control and CAVD groups. Alcohol consumption was not significantly different among the three groups, but there were significant differences in the history of diabetes and hypertension among the three groups ($P < 0.05$) (Table 2).

FBG and lipid profile level. Significant differences were found with respect to APOB and Lp(a) among the three groups, with LDL and FBG concentrations being significantly higher in CAVD and CHD groups compared with controls ($P < 0.05$). APOA and

Table 2. Baseline characteristics of participants.

Characteristic	Control group	CAVD group	CHD group	F/H/χ^2	P value
Number of participants	71	71	77		
Male sex, number (%)	30 (42.3%)	36 (50.7%)	45 (58.4%)	3.873	0.144
Age (years)	63.55 ± 8.86	63.89 ± 9.27	$67.22 \pm 6.82^{\#**}$	4.425	0.013
Body mass index (kg/m^2)	25.57 ± 3.64	25.36 ± 3.3	25.93 ± 2.98	0.580	0.561
Smokers, number (%)	21 (29.6%)	31 (43.7%)	36 (46.8%)	5.063	0.080
Consumers of alcohol, number (%)	16 (22.5%)	14 (19.7%)	12 (15.6%)	1.171	0.557
Diabetes mellitus, number (%)	11 (15.5%)	9 (12.7%)	28 (36.4%)	14.645	0.001
Hypertension, number (%)	37 (52.1%)	45 (63.4%)	58 (75.3%)	8.644	0.013
Creatinine (μmol/L)	69.70 ± 15.78	$78.15 \pm 22.95^{*}$	$80.79 \pm 23.90^{**}$	5.400	0.005
Fasting blood glucose (mmol/L)	6.00 ± 1.97	5.57 ± 1.14	$6.91 \pm 2.87^{\#**}$	7.695	0.001
Lp(a) (nmol/L)	23.6 (9.4, 48.6)	37.2 (16.5, 79.6)	46.7 (21.5, 104.6)	13.337	0.001
TC (mmol/L)	4.29 ± 0.87	4.52 ± 0.84	4.64 ± 1.47	1.915	0.150
TG (mmol/L)	1.58 ± 0.90	1.42 ± 0.58	$1.68 \pm 0.79^{\#}$	2.214	0.112
LDL (mmol/L)	2.74 ± 0.80	$3.07 \pm 0.81^{*}$	$3.14 \pm 1.18^{**}$	3.662	0.027
HDL (mmol/L)	1.24 ± 0.93	1.18 ± 0.30	$1.09 \pm 0.33^{**}$	4.281	0.015
APOA (g/L)	1.42 ± 0.25	$1.30 \pm 0.26^{*}$	$1.26 \pm 0.26^{**}$	7.399	0.001
APOB (g/L)	0.97 (0.82, 1.10)	1.04 (0.87, 1.26)	1.12 (0.88, 1.31)	7.426	0.024

F value was derived from one-way ANOVA; H value was derived from the Kruskal–Wallis test; χ^2 value was derived from the Chi-square test.

*CAVD group compared with control group, $p < 0.05$.

**CHD group compared with control group, $p < 0.05$.

#CHD group compared with CAVD group, $p < 0.05$.

CAVD, calcific aortic valve disease; CHD, cardiovascular disease; Lp(A), lipoprotein(a); TC, total cholesterol; TG, total glucose; LDL, low-density lipoprotein; HDL, high-density lipoprotein; APOA, apolipoprotein A; APOB, apolipoprotein B.

HDL concentrations were significantly lower in CAVD and CHD groups than controls ($P < 0.05$), but the TG concentration was significantly higher in the CHD group than in the control group ($P < 0.05$) (Table 2).

SNP genotype distribution. rs3798221, rs7770628, and rs6415084 were prevalent in our study, but rs10455872 was not. Of 219 participants, only two had AG minor alleles at rs10455872, and both were CAVD patients. The other 217 participants had AA minor alleles. Allelic frequencies of variants rs10455872(G), rs7770628(C), rs6415084(T), and rs3798221(T) were 0.005, 0.146, 0.126, and 0.422, respectively. There were no significant differences in genotypic or allelic frequencies among CAVD, CHD, and control groups (Table 3).

SNP genotypes, Lp(a) levels, and their effects on CAVD and CHD. Logistic regression analysis showed that there were no significant correlations between *LPA* genetic variants (rs7770628, rs6415084, and rs3798221) and CAVD or CHD risk. However, there were significant positive associations between Lp (a) levels and the risks of CAVD and CHD, compared with the control group. Patients with hypertension had a significantly greater risk of CHD compared with controls ($P < 0.05$) (Table 4).

Effects of LPA SNPs on Lp(a) concentrations in Han Chinese. We next used linear regression analysis to test the association between Lp (a) levels and *LPA* SNPs rs7770628, rs6415084, and rs3798221, adjusting for APOB levels. Positive associations between rs7770628, rs6415084, rs3798221, and Lp(a)

Table 3. *LPA* SNP genotype and allele frequency distribution.

Subjects		All participants N=219	Control group N=71	CAVD group N=71	CHD group N=77	P value
SNP ID		No. (%)	No. (%)	No. (%)	No. (%)	
rs7770628						
Genotype	TT	161 (73.5)	54 (76.1)	53 (74.6)	54 (70.1)	0.931
	CT	52 (23.7)	15 (21.1)	16 (22.5)	21 (27.3)	
	CC	6 (2.7)	2 (2.8)	2 (2.8)	2 (2.6)	
Allele	T	374 (85.4)	123 (86.6)	122 (85.9)	129 (83.8)	
	C	64 (14.6)	19 (13.4)	20 (14.1)	25 (16.2)	
rs6415084						
Genotype	CC	169 (77.2)	55 (77.5)	56 (78.9)	58 (75.3)	0.968
	CT	45 (20.5)	14 (19.7)	14 (19.7)	17 (22.1)	
	TT	5 (2.3)	2 (2.8)	1 (1.4)	2 (2.6)	
Allele	C	383 (87.4)	124 (87.3)	126 (88.7)	133 (86.4)	
	T	55 (12.6)	18 (12.7)	16 (11.3)	21 (13.6)	
rs10455872						
Genotype	AA	217 (99.1)	71 (100)	69 (97.2)	77 (100)	0.122
	AG	2 (0.9)	0 (0.0)	2 (2.8)	0 (0.0)	
Allele	A	436 (99.5)	142 (100)	140 (98.6)	154 (100)	
	G	2 (0.5)	0 (0.0)	2 (1.4)	0 (0.0)	
rs3798221						
Genotype	GT	107 (48.9)	35 (49.3)	35 (49.3)	37 (48.1)	0.934
	GG	73 (33.3)	25 (35.2)	24 (33.8)	24 (31.2)	
	TT	39 (17.8)	11 (15.5)	12 (16.9)	16 (20.8)	
Allele	G	253 (57.8)	85 (59.9)	83 (58.5)	85 (55.2)	
	T	185 (42.2)	57 (40.1)	59 (41.5)	69 (44.8)	

SNP, single nucleotide polymorphism; CAVD, calcific aortic valve disease; CHD, cardiovascular disease.

levels were observed. Participants with the rs7770628 CT/CC genotype had significantly higher Lp(a) concentrations than rs7770628 TT carriers, rs6415084 CT/TT carriers had significantly higher Lp(a) levels than those with the rs6415084 CC genotype, while participants with the rs3798221 GG/GT genotype had significantly higher Lp(a) levels than rs3798221 TT carriers (Table 5).

Discussion

CVD is the leading cause of death worldwide,[19] and morbidity and mortality associated with CAVD and CHD are rising as a result of aging populations. CAVD affects 25% of individuals aged over 65 years and 50% of those above 85.[20] There are currently no effective treatments for CAVD. Although there are many strategies for CHD prevention and treatment, cardiovascular events can still occur in individuals with well-controlled total cholesterol and LDL levels. Recently, an increasing body of evidence has identified high Lp(a) levels (>125 nmol/l or >50 mg/dl) as a causal and independent risk factor for CVD.[21,22] A wide variation in Lp(a) levels among different ethnicities was observed by multiple studies. Similarly, we observed Lp(a) levels ranging from 2.0 to 504.2 nmol/L among

Table 4. *LPA* SNP genotype, Lp(a) level and their effects.

Variant	Valuation	CAVD group and Control group OR (95% CI)					CHD group and Control group OR (95% CI)				
			P value		P value	P value		P value		P value	P value
Age (years)	40–88	0.996 (0.956, 1.036)	0.829	0.997 (0.958,1.038)	0.899	0.851	1.045 (0.997,1.095)	0.065	1.045 (0.997, 1.095)	0.064	1.050 (1.001, 1.102)	0.045
Hypertension	0 = No Hypertension 1 = Hypertension	1.839 (0.879, 3.845)	0.106	1.826 (0.872, 3.822)	0.110	0.112	2.688 (1.258, 5.740)	0.011	2.760 (1.286, 5.927)	0.009	2.735 (1.273, 5.880)	0.010
LP(a) ln	0.69–6.22	1.733 (1.212, 2.480)	0.003	1.800 (1.249, 2.595)	0.002	0.003	1.686 (1.211, 2.348)	0.002	1.733 (1.241, 2.422)	0.001	1.777 (1.275, 2.475)	0.001
rs7770628	0 = TT; 1= CT/ CC	0.685 (0.292, 1.608)	0.385	–			0.843 (0.363, 1.960)	0.691	–		–	
rs6415084	0 = CT/TT; 1 = CC	–		1.862 (0.754, 4.598)	0.178	–	–		1.507 (0.632, 3.596)	0.355	–	
rs3798221	0 = TT; 1 = GG/GT	–	–			0.519 (0.378, 1.634)	0.519	–			0.506 (0.230, 1.112)	0.090

CAVD, calcific aortic valve disease; CHD, cardiovascular disease; OR, odds ratio; CI, confidence interval.

219 participants. Our data also suggest that the risks of CAVD and CHD increase with rising Lp(a) concentrations, and that Lp(a) is an independent and strong risk factor for CVD in the Han Chinese population.

To date, most studies on rs10455872 have been conducted in European Caucasian, African-American, Hispanic-American, and South Asian populations,[16,17,23,24] with few studies in Han Chinese. We observed only two carriers of rs10455872 AG minor alleles out of 219 participants; both were CAVD patients. In contrast, 217 participants carried AA minor alleles. The allelic frequency of rs10455872 (G) was 0.005, which suggests a notable difference between non-Chinese and Han Chinese populations that is also supported by the rs10455872G genotype having high homology and a low mutation rate in Han Chinese, unlike in other ethnicities. [25] Because of the low frequency of minor alleles at this locus, we could not determine whether this SNP is associated with CHD/ CAVD development in Han Chinese.

Lp(a) contributes to CVD risk through multiple mechanisms[26] including its ability to enter and accumulate in the intima of arteries and aortic valve leaflets.[27] The unique structure of Lp(a) may explain its role in lesion development. It is composed of two parts, Apo B (an LDL-like particle) and Apo A, which are covalently bound by a single disulfide bond.[28] The heterogeneous size of Apo A is determined by *LPA* on chromosome 6q27. Apo A contains 10 KIV subtypes, of which KIV-2 is the most important and has a wide variation in copy number. Large Apo A isoforms, which have high KIV-2 copy numbers, are inefficiently secreted. rs3798221 has been associated with KIV-2 copy number in different ethnicities,[12] while rs7770628 was closely related to both KIV-2 copy number and Lp(a) levels in a post-hoc analysis of a Chinese population.[18] Moreover, rs7770628 was suggested to have similar associations with Lp(a) levels and

Table 5. The effect of *LPA* SNPs on serum Lp(a) levels in the Chinese Han population.

Variant	Valuation	β (95% CI)	P value	β (95% CI)	P value	β (95% CI)	P value
APOB	0.37–5.75	0.368 (0.036, 0.700)	0.030	0.333 (–0.006, 0.672)	0.054	0.403 (0.061, 0.744)	0.021
rs7770628	0 = TT; 1 = CT/CC	0.738 (0.413, 1.064)	0.000	–		–	
rs6415084	0 = CT/TT; 1 = CC	–		–0.686 (–1.035, –0.336)	0.000	–	
rs3798221	0 = TT; 1=GG/GT	–		–		0.427 (0.113, 0.740)	0.008

SNP, single nucleotide polymorphism; APOB, apolipoprotein B; CI, confidence interval.

KIV copy numbers in several ethnicities.[29] An inverse relationship between Lp(a) levels and KIV-2 copy number has been reported,[30,31] and a low KIV-2 copy number was shown to be associated with CVD.[32]

LPA has approximately 50 genotyped SNPs that vary among different ethnicities. Zewinger et al. found that an increased plasma concentration of Lp(a) and a carrier of minor alleles at rs10455872 were pertinent to elevated CHD severity,[33] while the CHARGE consortium detected a significant association between rs10455872 and the appearance of aortic valve calcification through a genome-wide association study. A similar result was observed in white European, African-American, Hispanic-American, and independent Danish cohorts.[34] Lp(a) concentrations are thought to be determined by *LPA* genotype, which would allow the prediction of CAVD development, and this was borne out by a prospective analysis in a large Swedish cohort.[10] The frequency of the G allele at rs10455872 was shown to be 7% in Caucasians[35] and 2% in Iranian CAD patients; this latter study found no correlation between *LPA* variants and CAD.[36]*LPA* rs3798221 was previously shown to be closely related to the occurrence of myocardial infarctions[37] and Lp(a) levels in different sexes.[38] Our data also suggest that rs3798221 and rs7770628 are pertinent to Lp(a) levels in Han Chinese, but there were no clear correlations with the risk of CAVD or CHD.

Genetic variation at *LPA* rs6415084 was previously observed in 517 Han Chinese CHD patients in 2013.[25] Compared with rs6415084 CC carriers, CT/TT carriers were shown to have significantly higher Lp(a) concentrations. We similarly found that the minor alleles of rs6415084 were associated with a higher Lp(a) plasma concentration, but there was no clear correlation between minor alleles and the risk of CAVD or CHD.

Although rs6415084, rs3798221, and rs7770628 were linked to increased Lp(a) levels, we detected no significant associations between the three SNPs and the risk of CAVD or CHD in the Han Chinese population. When compared with the control group, the risk of CAVD or CHD increased with increasing Lp(a) concentrations, suggesting that Lp(a) is an independent risk factor for CVD in Han Chinese. We were, however, unable to confirm whether rs10455872 is linked to the risk of CAVD or CHD.

Our study has a number of limitations. First, we had a relatively small cohort and variation was only detected in four SNPs. A larger sample size is therefore needed to identify more *LPA* SNPs in the Han Chinese population. Further exploration of *LPA* SNP variants, KIV2 copy number, and the risk of CAVD and CHD is also warranted. Additionally, we plan to

establish cell models to explore the functions of the abovementioned loci. Second, our study was limited to a cross-sectional analysis of the Han Chinese population, so our findings should be confirmed in other ethnic groups. Finally, PCR is not currently available in many clinical contexts. However, its increased accessibility in many hospitals will favor mid- and low-level risk patient groups which can be closely followed-up and treated in a timely manner via minimally invasive procedures such as transcatheter aortic valve implantation.

Conclusions

Our data suggest differences between the Han Chinese population and Caucasians and other ethnicities with respect to *LPA* SNPs and the risk of CAVD and CHD. We found that CAVD and CHD risks are not linked to genetic variation in *LPA* at SNPs rs6415084, rs3798221, and rs7770628 despite the observed association between the three SNPs and an increased level of Lp (a). However, the association between CAVD/CHD risk and increased Lp(a) levels indicates that Lp(a) is an independent risk factor for CVD in the Han Chinese population.

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Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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