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ORIGINAL RESEARCH

Effect of Triglyceride-Glucose Indices and Circulating PCSK9-Associated Cardiovascular Risk in STEMI Patients with Primary Percutaneous Coronary Artery Disease: A Prospective Cohort Study

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Background and Aims: This study aimed to determine whether convertase subtilisin/kexin type 9 (PCSK9)-associated cardiovascular risk is modulated by triglyceride-glucose (TyG) in ST-segment elevation myocardial infarction (STEMI) patients with primary percutaneous coronary disease (PCI).

Methods: A total of 1541 patients with STEMI (aged ≥18 years) undergoing primary PCI were consecutively enrolled between March 2017 and March 2019.

Outcomes: When stratifying the overall population according to TyG indices less than or greater than the median (TyG median = 9.07) as well as according to quartiles of PCSK9 levels, higher TyG index levels were significantly associated with all-cause mortality only when TyG levels were 9.07 or higher (ie, relative to quartile 1 [Q1], the adjusted HR for all-cause mortality was 3.20 [95% CI, 0.54–18.80] for Q2, $p = 0.199$; 7.89 [95% CI, 1.56–40.89] for Q3, $p = 0.013$; and 5.61 [95% CI, 1.04–30.30] for Q4, $p = 0.045$. During a median follow-up period of 1.96 years, the HR for all-cause mortality was higher in the subset of patients with TyG ≥median and PCSK9 ≥median (p for trend = 0.023) among those with type 2 diabetes mellitus (T2DM). However, there were no statistically significant differences among the subgroups. Among T2DM patients with a TyG index greater than the median, the Kaplan-Meier curve showed that patients with the highest PCSK9 levels had an increased risk of all-cause mortality (log-rank $p = 0.017$) and cardiac-cause mortality (log-rank $p = 0.037$) compared with lower PCSK9 quartile levels.

Conclusion: Elevated PCSK9 levels are related to all-cause mortality and cardiac-related mortality when TyG levels are greater than the median, but not when levels are less than the median. This suggests a potential benefit of lowering circulating PCSK9 levels in STEMI patients with insulin resistance.

Keywords: triglyceride glucose index, proprotein convertase subtilisin/kexin type 9, mortality, type 2 diabetes, ST-segment elevation myocardial infarction, primary percutaneous coronary intervention

Introduction

Pro-protein convertase subtilisin/kexin type 9 (PCSK9) produced in the liver and intestines plays a critical role in cholesterol metabolism by mediating the degradation of hepatic low-density lipoprotein cholesterol receptors (LDLR) in lysosomes, thereby resulting in decreased LDL-C clearance from circulation and elevated plasma levels.[1,2] PCSK9 levels are genetically mediated and are considered causal in the development of atherosclerotic cardiovascular disease (ASCVD).[3–5] Recent studies have demonstrated that PCSK9 can directly or indirectly contribute to atherosclerosis,

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from initiation to progression, by leading to endothelial cell apoptosis, secretion of inflammatory cytokines, and inhibition of platelet activation, thereby inducing an increase in the migratory capacity of monocytes and platelet count/fibrinogen levels[6,7] (Figure 1). Notably, PCSK9 has emerged as a therapeutic target for ASCVD, with PCSK9 monoclonal antibodies (mAbs) including evolocumab and alirocumab (which are clinically used to decrease circulating PCSK9 levels), providing substantial and proven benefits for those at very high risk of cardiovascular events.[8,9]

Despite these recent advances, the degree to which insulin resistance patient populations may benefit most from PCSK9 reduction remains unclear. It is likewise unclear whether there is an association between PCSK9 reduction and the incidence of cardiovascular endpoints among patients with insulin resistance. The triglyceride-glucose (TyG) index, derived from fasting blood glucose and fasting triglyceride (TG) levels, has been proposed as a surrogate biomarker for insulin resistance.[11–14] Accordingly, in this study, we tested the hypothesis that PCSK-associated ASCVD risk would be statistically significantly modulated by levels of systemic insulin resistance in a large, contemporary STEMI population undergoing primary PCI. This study aimed to determine whether PCSK9-associated cardiovascular risk is modulated by TyG levels in ST-segment elevation myocardial infarction (STEMI) patients with primary percutaneous coronary disease (PCI). To our knowledge, the present study is the first study within a large cohort of STEMI patients undergoing primary PCI to demonstrate that PCSK9-associated ASCVD risk may be significantly mediated by concomitant levels of TyG.

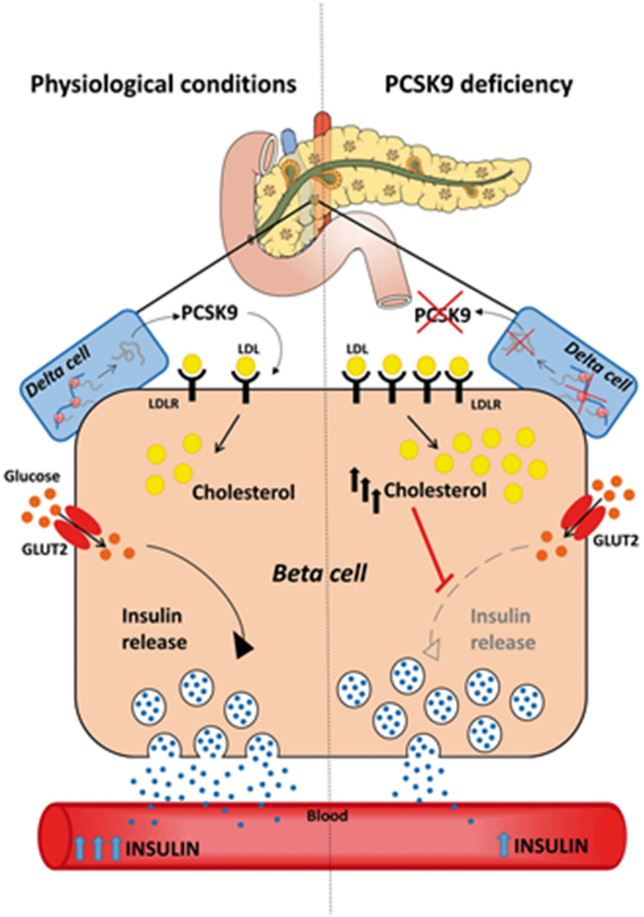

Figure 1 Influence of PCSK9 deficiency on β-cell function. Expression of low-density lipoprotein receptor (LDL-R) in β cells was controlled by the production of PCSK9. The deficiency of PCSK9 contribute to raised expression of LDL-R which result in increased accumulation of cholesterol esters and impact the glucose-stimulated insulin secretion and impaired glucose tolerance observed (referred from).[6]

Notes: Reproduced from Da Dalt L, Ruscica M, Bonacina F, et al. PCSK9 deficiency reduces insulin secretion and promotes glucose intolerance: the role of the low-density lipoprotein receptor. *Eur Heart J*. 2019;40(4):357–368, by permission of Oxford University Press.[10]

Methods

Study Population

A total 1541 patients with STEMI aged≥18 years who undergoing primary PCI were consecutively enrolled between March 2017 and March 2019 and their baseline plasma PCSK9 levels were determined by ELISA (Flow chart was shown in Supplement Figure 1). The definition of STEMI followed the established criteria.[15] The study protocol, approved by the Ethics Committee of Fuwai Hospital, Peking Union Medical College & Chinese Academy of Medical Sciences, was conducted in accordance with the principles outlined in the Declaration of Helsinki. Personal information related to the identities of the patients was concealed. Every patient signed the informed written consent before enrolled in the study.

Definition

Hypertension was defined as blood pressure (BP) ≥140/90 mmHg at rest over three measurements or a previous diagnosis of hypertension and current use of antihypertensive drugs.[16] Patients were diagnosed with DM if they met one of the following criteria: (i) fasting plasma glucose level ≥7.0 mmol/L, (ii) 2-h plasma glucose value ≥11.1 mmol/L in the 75-g oral glucose tolerance test (OGTT), and (iii) casual plasma glucose level ≥11.1 mmol/L.[17] Dyslipidaemia was defined by any of the following parameters:[18] total cholesterol level ≥5.0 mmol/L, low-density lipoprotein cholesterol (LDL-C) level ≥3.0 mmol/L, triglyceride level ≥1.7 mmol/L, high-density lipoprotein cholesterol (HDL-C) level <1.2 mmol/L (in women) or <1.0 mmol/L (in men). Patients who did not meet the standards for never smokers (never smoked in their lifetime) or former light smokers (stopped smoking at least 15 years ago, with ≤10 total pack-years of smoking) were considered current smokers.[19] Chronic kidney disease (CKD) was defined as abnormal kidney structure or function for more than 3 months, and end-stage renal disease (ESRD) was the final common pathway for CKD.[20] MACEs were defined as a composite of all-cause death, myocardial infarction (MI) recurrence, and ischaemic stroke.

Laboratory Tests

Measurement of circulating PCSK9 levels Circulating levels of PCSK9 were measured using enzyme-linked immunosorbent assay (DY3888; R&D Systems, Catalog) according to the manufacturer's instructions and compared with purified human PCSK9 standards.

Measurements

Baseline data, including patient clinical demographics such as age, sex, smoking status, history of disease (including hypertension, diabetes, hyperlipidemia and chronic kidney disease) and PCI, laboratory results, primary PCI procedures, and medical treatments, were obtained from hospital records. Serum levels of fasting plasma glucose and lipid profiles, including TG, total cholesterol (TC), lipase activator (LPA) and HDL-C, were determined by standard laboratory techniques at Fuwai Hospital. The TyG index was computed using the following formula: ln[fasting TG (mg/dL)×FPG (mg/dL)/2].[12]

Endpoints and Follow-Up

Recurrence MI was diagnosed as the symptom of typical chest pain or changes in typical serial electrocardiograms combined with positive cardiac troponins. Follow-up was performed by well-trained physicians routinely at 1, 6, and 12 months after the discharge via direct interviews, telephone calls and hospital discharge records or clinical notes, and well-trained physicians and nurses performed the clinical follow-up with the patients. The follow-up protocol was approved by obtaining permission from the Institutional Review Board of Chinese Academy of Medical Sciences Fuwai Hospital. Well-trained physicians in charge of the follow-up primary endpoints, including all-cause, death, cardiac death, recurrence MI, ischemic stroke, identified and extracted the primary endpoints from medical and hospital records, laboratory reports, clinical notes, and emergency records which required to be sent to our centers). There are two professional physicians blinded to the clinical and laboratory tests data confirmed the follow up endpoints.

Statistical Analysis

Continuous variables with normal or skewed distribution are shown as medians (interquartile range, IQR). Categorical variables are presented as frequencies (%). The subjects were divided into two groups according to the median level of TyG. For comparisons between groups, two-tailed independent-samples Student's *t*-test for normally distributed variables and a Mann–Whitney *U*-test for variables with highly skewed distributions were performed. The chi-squared test was applied to compare the categorical variables. Univariate and multivariate Cox regression was carried out to determine the potential association between PCSK9 levels and the risk of endpoints events including all-cause mortality, cardiac death, recurrence MI and ischemic stroke. Hazard ratios (HRs) and 95% confidence intervals (CIs) for the relationship between PCSK9 combined with TyG and the incidence of endpoints events were generated with Cox regression models. Adjusted for the variables in model including age, gender, history of hypertension, history of diabetes mellitus, history of hyperlipidemia, history of coronary artery bypass graft, history of PCI, history of chronic kidney disease, current smoking, high-sensitivity C-reactive protein (hs-CRP), LDL-C, height, weight, heart rate at admission, systolic blood pressure, diastolic blood pressure, the use of statin, the TIMI flow prior and post-PCI, creatinine, lipase activator (lpa) and the use of intra-aortic balloon pump. PCSK9 categories depended on quartiles using the lowest quartile group as the reference. Subgroups were divided according to age (<65 years versus≥65 years), LDL-C (<70 mg/dL versus≥70 mg/dL)], and the status of T2DM (present versus absent). The areas under the receiver operating characteristic curve (ROC), sensitivity, specificity, Youden index and 95% CIs were calculated to evaluate the predictive ability of TyG combined with PCSK9 for mortality. Kaplan-Meier survival curves were constructed to evaluate the incidence rate of mortality according to the optimal quartile point of the PCSK9 among T2DM the groups divided by the median of TyG, and discrepancy rates of cumulative events were compared using the Log rank test.

All analyses were performed using SPSS (version 20.0; IBM Corp., Armonk, NY, USA), R Programming Language X64 4.0.4 (R Foundation for Statistical Computing, Vienna, Austria), and MedCalc version 18.2.1 (MedCalc Software, Ostend, Belgium). P values of<0.05 were considered statistically significant in all of the analyses, and all P values were two-tailed.

Results

Baseline Participant Characteristics

Table 1 describes the baseline clinical and biochemical characteristics of 1541 participants stratified according to achieved TyG levels at baseline (<median vs ≥median). The mean age of the participants was 61.0 years. Notably, 80.3% of the participants were men and 64.8% had hypertension. However, when baseline characteristics were evaluated according to median TyG levels, those with TyG levels of 9.07 or more (versus levels less than 9.07) had higher mean PCSK9 levels (means and interquartile ranges [IQRs]; 44.1 [23.7, 83.8] versus 49.0 [27.1, 92.7]) and a more frequent cluster of common cardiovascular risk factors (diabetes mellitus [DM], higher active greater low-density lipoprotein cholesterol levels, higher triglyceride levels, higher hs-CRP levels, and higher systolic and diastolic blood pressure levels). These participants were also more likely to be receiving beta-blockers. The prevalence of individual medical and demographic characteristics and the intersections of cardiovascular risk factors and endpoints are shown in Figure 2. The most frequently observed risk factor among participants with a TyG index less than the median was hyperlipidemia, followed by current smoking status, hypertension, and type 2 diabetes mellitus (T2DM). The prevalence of the risk factors mentioned above was similar among participants with TyG indices greater than the median. The most frequently observed intersection pattern was the simultaneous presence of all eight features.

Association Between Circulating PCSK9 Levels Combined with TyG and Incident of Endpoints

Table 2 describes univariable and fully adjusted multivariable associations between endpoints (including all-cause mortality, cardiac-cause mortality, recurrent myocardial infarction [MI], and ischemic stroke) stratified according to TyG and PCSK9 levels at baseline. When stratifying the overall population according to TyG indices (stratified at the median TyG level, 9.07) and quintiles of PCSK9 levels, higher TyG index levels were statistically significantly associated with all-cause mortality only when TyG levels were 9.07 or greater (relative to Q1, the adjusted HR for all-cause mortality was 3.20 [95% CI, 0.54–18.80] for Q2,

Table 1 Baseline Clinical and Optical Coherence Tomography Characteristics of the Study Population

Variables	Whole Cohort (N = 1541)	No. (%)		*P* value
		TyG Level During Treatment		
		TyG ≤ Median (N = 770)	TyG>Median (N = 771)	
Age (years)	61.0 (52.0, 68.0)	63.0 (54.0, 69.0)	59.0 (50.2, 67.0)	< 0.001*
Male [%(n)]	1238 (80.3)	628 (81.6)	610 (79.1)	0.254
Heart rate (beats/min)	75.0 (65.0, 85.0)	72.0 (64.0, 83.0)	76.0 (66.0, 87.0)	< 0.001*
SBP (mmHg)	125.0 (111.0, 138.0)	124.0 (110.0, 136.0)	126.0 (112.0, 140.0)	0.045*
DBP (mmHg)	78.0 (70.0, 88.0)	77.0 (69.0, 87.0)	80.0 (70.0, 89.0)	0.017*
EF at admission	55.0 (50.0, 60.0)	55.0 (50.0, 60.0)	55.0 (49.0, 59.0)	0.125
Risk factors				
Hypertension[%(n)]	999 (64.8)	490 (63.6)	509 (66)	0.355
Hyperlipidemia[%(n)]	1387 (90.0)	682 (88.6)	705 (91.4)	0.073
Smoking[%(n)]	1084 (70.7)	546 (71.4)	538 (70)	0.582
Diabetes mellitus	531 (34.5)	157 (20.4)	374 (48.5)	< 0.001*
CKD[%(n)]	106 (6.9)	46 (6)	60 (7.8)	0.188
Laboratory examinations				
HDL-C (mg/dl)	1.0 (0.9, 1.2)	1.1 (0.9, 1.3)	1.0 (0.9, 1.2)	< 0.001*
LDL-C (mg/dl)	100.0 (76.9, 125.4)	93.8 (73.1, 118.0)	105.8 (81.3, 130.8)	< 0.001*
Triglycerides (mmol/L)	1.5 (1.0, 2.0)	1.1 (0.8, 1.4)	2.0 (1.6, 2.8)	< 0.001*
LPA (g/L)	170.0 (73.0, 355.0)	179.0 (79.0, 368.0)	159.0 (70.0, 341.5)	0.160
hs-CRP (mg/L)	5.6 (1.9, 10.9)	4.4 (1.5, 10.6)	6.8 (2.6, 11.1)	< 0.001*
Creatinine (mmol/L)	83.8 (71.9, 96.9)	82.8 (71.4, 94.6)	84.5 (72.7, 98.5)	0.014*
Fasting glucose (mmol/L)	7.2 (5.9, 9.5)	6.2 (5.4, 7.5)	8.8 (7.0, 12.0)	< 0.001*
PCSK9	46.8 (25.3, 88.7)	44.1 (23.7, 83.8)	49.0 (27.1, 92.7)	0.010*
Discharge medication regimen				
Statin[%(n)]	1453 (95.3)	723 (94.6)	730 (96.1)	0.233
Aspirin[%(n)]	1462 (95.9)	733 (95.9)	729 (95.9)	1.000
Clopidogrel[%(n)]	770 (50.5)	388 (50.8)	382 (50.3)	0.879
Ticagrelor[%(n)]	728 (47.8)	356 (46.6)	372 (48.9)	0.386
ACEI/ARB[%(n)]	1078 (70.7)	536 (70.2)	542 (71.3)	0.659
Beta-Blockers[%(n)]	1306 (85.7)	635 (83.1)	671 (88.3)	0.005*
Procedure of PCI				
Type of ACC				
A	29 (2.2)	16 (2.4)	13 (1.9)	0.601
B1	143 (10.7)	64 (9.7)	79 (11.6)	-
B2	277 (20.6)	141 (21.3)	136 (20)	-
C	893 (66.5)	440 (66.6)	453 (66.5)	-
Use of IABP	77 (5.6)	31 (4.5)	46 (6.6)	0.098
Endpoints				
MACEs[%(n)]	183 (11.9)	89 (11.6)	94 (12.2)	0.760
All caused mortality[%(n)]	83 (6.2)	40 (6.1)	43 (6.4)	0.887
Cardiac death[%(n)]	50 (3.8)	24 (3.6)	26 (3.9)	0.941
Recurrence MI[%(n)]	63 (7.0)	30 (6.8)	33 (7.1)	0.958
Ischemic stroke[%(n)]	45 (3.7)	22 (3.7)	23 (3.7)	1.000

Notes: Continuous data are presented as median (IQR). Categorical data are presented as number (%). *P<0.05.

Abbreviations: DM, diabetes mellitus; SBP, systolic blood pressure; DBP, diabetes blood pressure; PCI, percutaneous coronary intervention; CKD, chronic kidney disease; HDL, high density lipoprotein; LDL, low density lipoprotein; LPA, lipse activator; hs-CRP, high sensitive C-reactive protein; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; MACE, major adverse cardiovascular events; MI, myocardial infarction. TyG, triglyceride glucose, PCSK9, proprotein convertase subtilisin/kexin type 9.

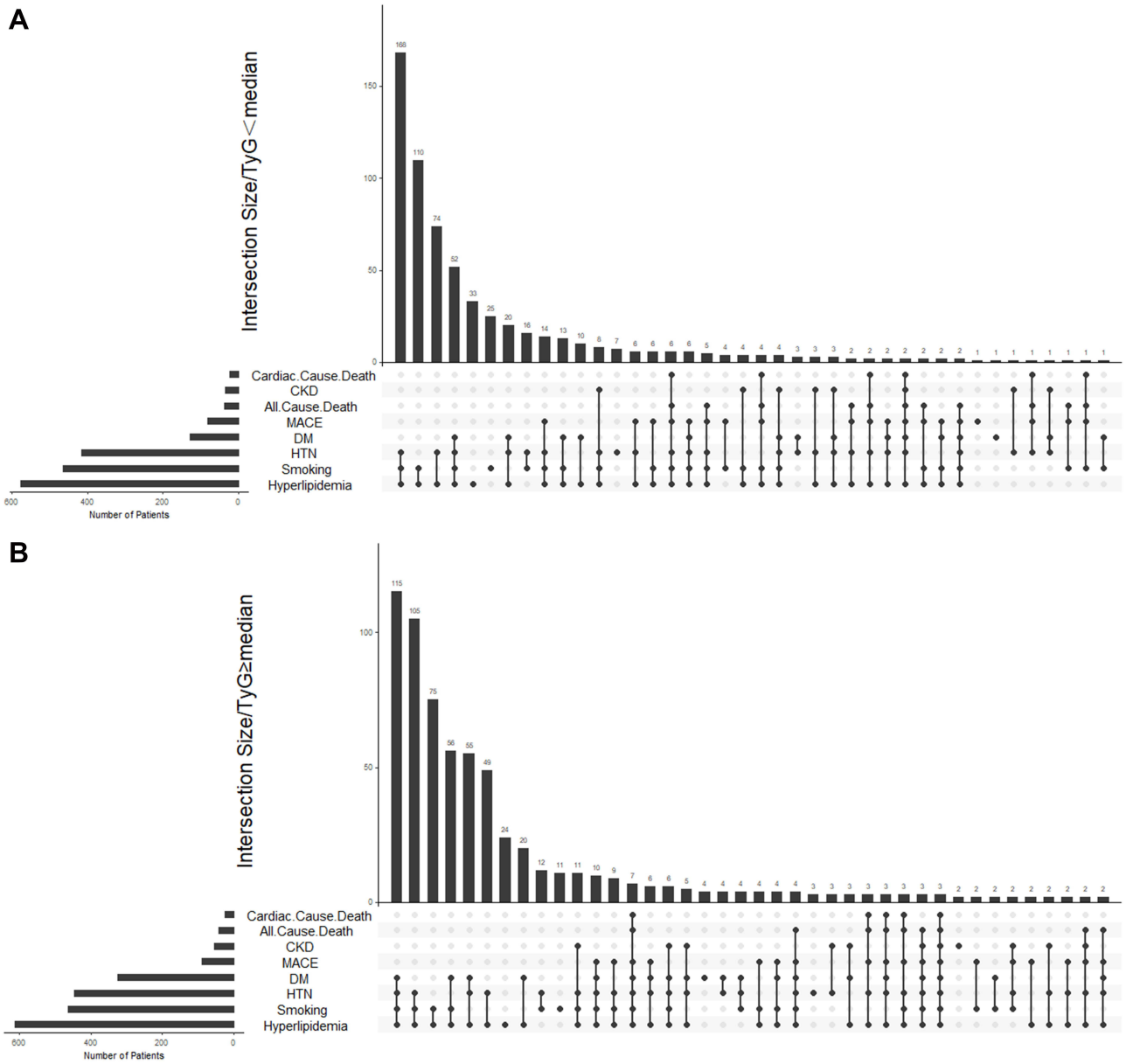

Figure 2 Upset plot of factors and intersections of cardiovascular risk characteristics (hyperlipidemia, status of smoking, hypertension, diabetes mellitus and chronic kidney disease) and endpoints during follow-up period (all cause death, cardiac cause death, major adverse cardiovascular events) of TyG <median (**A**) and TyG ≥median (**B**).
Abbreviations: CKD, chronic kidney disease; HTN, hypertension, DM, diabetes mellitus; MACE, major adverse cardiovascular events; TyG, triglyceride glucose.

$p = 0.199$; 7.89 [95% CI, 1.56–40.89] for Q3, $p = 0.013$; and 5.61 [95% CI, 1.04–30.30] for Q4, $p = 0.045$. The effect estimate for the trend test was 1.62 [95% CI, 1.07–2.45], p for trend = 0.023. In the setting of lower TyG levels (ie, less than the median), higher PCSK9 levels were not associated with a greater risk of clinical events. We observed a statistically significant interaction between PCSK9 and PCSK9 dichotomies in terms of all-cause mortality (p for interaction (crude) = 0.008, p for interaction (adjusted) = 0.033). Figure 3 shows the continuous hazard ratio across logarithmic PCSK9 levels for all-cause mortality according to crude and adjusted restricted cubic spline analyses among participants in all cohorts, as well as among diabetes mellitus (DM) and non-DM patients, stratified according to median levels of TyG. The RCS analysis showed that an increase in PCSK9 was consistently associated with a higher risk of all-cause mortality.

Table 2 Univariate and Multivariate Analyses of the Impact of TyG and PCSK9 on Endpoints

Subgroups	Variable	N	N Events %	Crude HR (95% CI)	Crude *p* value	Adjusted[#] HR (95% CI)	Adjusted *p* value	*P* for Interaction[a]	*P* for Interaction[b]
All-cause Mortality									
TyG < median	Q1	213	15 (7)	1 (Reference)	-	1 (Reference)	-	0.008*	0.033*
	Q2	192	6 (3.1)	0.50 (0.19–1.28)	0.149	0.92 (0.24–3.56)	0.908		
	Q3	185	9 (4.9)	0.91 (0.40–2.08)	0.820	1.28 (0.35–4.72)	0.709		
	Q4	180	10 (5.6)	0.87 (0.39–1.96)	0.741	2.67 (0.73–9.74)	0.138		
	Trend. test	770	40 (5.2)	0.99 (0.75–1.30)	0.925	1.35 (0.88–2.07)	0.175		
	Per SD PCSK9	770	40 (5.2)	1.14 (0.89–1.46)	0.299	1.60 (1.06–2.42)	0.026*		
TyG ≥ median	Q1	172		1 (Reference)	-	1 (Reference)	-		
	Q2	193	9 (4.7)	1.78 (0.60–5.33)	0.299	3.20 (0.54–18.80)	0.199		
	Q3	200	14 (7)	3.06 (1.10–8.51)	0.032*	7.98 (1.56–40.89)	0.013*		
	Q4	206	15 (7.3)	3.02 (1.10–8.32)	0.033*	5.61 (1.04–30.30)	0.045*		
	Trend. test	771	43 (5.6)	1.41 (1.07–1.86)	0.016*	1.62 (1.07–2.45)	0.023*		
	Per SD PCSK9	771	43 (5.6)	1.06 (0.81–1.37)	0.672	1.23 (0.85–1.79)	0.270		
Cardiac-cause Mortality									
TyG < median	Q1	213	12 (5.6)	1 (Reference)	-	1 (Reference)	-	0.011*	0.038*
	Q2	192	2 (1)	0.21 (0.05–0.96)	0.043*	0.22 (0.02–2.37)	0.211		
	Q3	185	8 (4.3)	1.06 (0.43–2.60)	0.903	1.08 (0.22–5.19)	0.925		
	Q4	180	2 (1.1)	0.24 (0.05–1.06)	0.060	0.70 (0.10–4.88)	0.721		
	Trend. test	770	24 (3.1)	0.77 (0.53–1.11)	0.163	0.95 (0.53–1.69)	0.864		
	Per SD PCSK9	770	24 (3.1)	0.73 (0.42–1.27)	0.264	1.05 (0.51–2.13)	0.902		
TyG ≥ median	Q1	172	4 (2.3)	1 (Reference)	-	1 (Reference)	-		
	Q2	193	4 (2.1)	1.01 (0.25–4.04)	0.991	1.44 (0.13–15.6)	0.765		
	Q3	200	9 (4.5)	2.57 (0.79–8.36)	0.118	13.31 (2.06–86.07)	0.007*		
	Q4	206	9 (4.4)	2.33 (0.71–7.57)	0.161	3.12 (0.43–22.73)	0.261		
	Trend. test	771	26 (3.4)	1.39 (0.98–1.98)	0.068	1.55 (0.93–2.57)	0.089		
	Per SD PCSK9	771	26 (3.4)	1.02 (0.72–1.44)	0.912	1.13 (0.65–1.97)	0.675		

(*Continued*)

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Table 2 (Continued).

Subgroups	Variable	N	N Events %	Crude HR (95% CI)	Crude *p* value	Adjusted[#] HR (95% CI)	Adjusted *p* value	*P* for Interaction[a]	*P* for Interaction[b]
Recurrence myocardial infarction									
TyG < median	Q1	213	8 (3.8)	1 (Reference)	-	1 (Reference)		0.727	0.629
	Q2	192	8 (4.2)	1.38 (0.52–3.69)	0.516	1.27 (0.42–3.86)	0.675		
	Q3	185	8 (4.3)	1.91 (0.72–5.11)	0.196	1.58 (0.52–4.83)	0.424		
	Q4	180	6 (3.3)	1.12 (0.39–3.26)	0.832	0.66 (0.18–2.47)	0.541		
	Trend. test	770	30 (3.9)	1.08 (0.79–1.47)	0.624	0.94 (0.65–1.36)	0.751		
	Per SD PCSK9			0.90 (0.61–1.32)	0.587	0.70 (0.39–1.26)	0.233		
TyG ≥ median	Q1	172	9 (5.2)	1 (Reference)	-	1 (Reference)			
	Q2	193	10 (5.2)	1.21 (0.49–2.97)	0.685	1.01 (0.34–2.99)	0.981		
	Q3	200	8 (4)	1.11 (0.42–2.88)	0.837	0.82 (0.25–2.71)	0.750		
	Q4	206	6 (2.9)	0.67 (0.24–1.90)	0.457	0.40 (0.10–1.51)	0.175		
	Trend. test	771	33 (4.3)	0.89 (0.66–1.21)	0.467	0.76 (0.52–1.11)	0.159		
	Per SD PCSK9	771	33 (4.3)	0.71 (0.43–1.16)	0.166	0.56 (0.26–1.18)	0.128		
Ischemic stroke									
TyG < median	Q1	213	7 (3.3)	1 (Reference)	-	1 (Reference)		0.601	0.216
	Q2	192	3 (1.6)	0.57 (0.15–2.21)	0.415	0.88 (0.19–4.04)	0.869		
	Q3	185	3 (1.6)	0.77 (0.20–2.98)	0.704	1.23 (0.23–6.68)	0.810		
	Q4	180	9 (5)	1.98 (0.73–5.4)	0.181	2.83 (0.74–10.86)	0.130		
	Trend. test	770	22 (2.9)	1.29 (0.90–1.86)	0.166	1.44 (0.91–2.27)	0.118		
	Per SD PCSK9	770	22 (2.9)	1.35 (1.03–1.76)	0.029*	1.30 (0.88–1.92)	0.187		
TyG ≥ median	Q1	172	8 (4.7)	1 (Reference)	-	1 (Reference)			
	Q2	193	4 (2.1)	0.52 (0.16–1.72)	0.282	0.40 (0.08–1.89)	0.248		
	Q3	200	5 (2.5)	0.73 (0.24–2.24)	0.581	0.98 (0.25–3.83)	0.980		
	Q4	206	6 (2.9)	0.78 (0.27–2.24)	0.640	0.91 (0.26–3.23)	0.887		
	Trend. test	771	23 (3)	0.94 (0.65–1.35)	0.734	1.04 (0.67–1.59)	0.875		
	Per SD PCSK9	771	23 (3)	0.99 (0.68–1.45)	0.961	1.06 (0.67–1.68)	0.798		

Notes: [#]Adjusted for sex, age, hypertension, hyperlipidemia, diabetes mellitus, creatinine, pre and post thrombolysis in myocardial infarction flow, lipse activator, the use of intra aortic balloon pump, history of CABG, history of PCI, history of chronic kidney disease, low density lipoprotein, high sensitive C-reactive protein, height, weight, status of smoking, diastolic blood pressure, systolic blood pressure, heart rate, the use of statin at discharge. Subsequent quartile is compared with the first quartile. Q, quartile; Median (range) PCSK9 values per PCSK9 quartile: Q1 (n = 385), 15.0 (7.4, 20.7); Q2 (n = 385), 34.0 (29.8, 39.5); Q3 (n = 385), 63.6 (53.8, 74.0); Q4 (n = 386), 139.3 (107.2, 208.5). *P<0.05. a, crude p for interaction; b, adjusted p for interaction.

Abbreviations: TyG, triglyceride glucose; PCSK9, proprotein convertase subtilisin/kexin type; HR, hazard ratio; CI, confidence interval; Q, quartile.

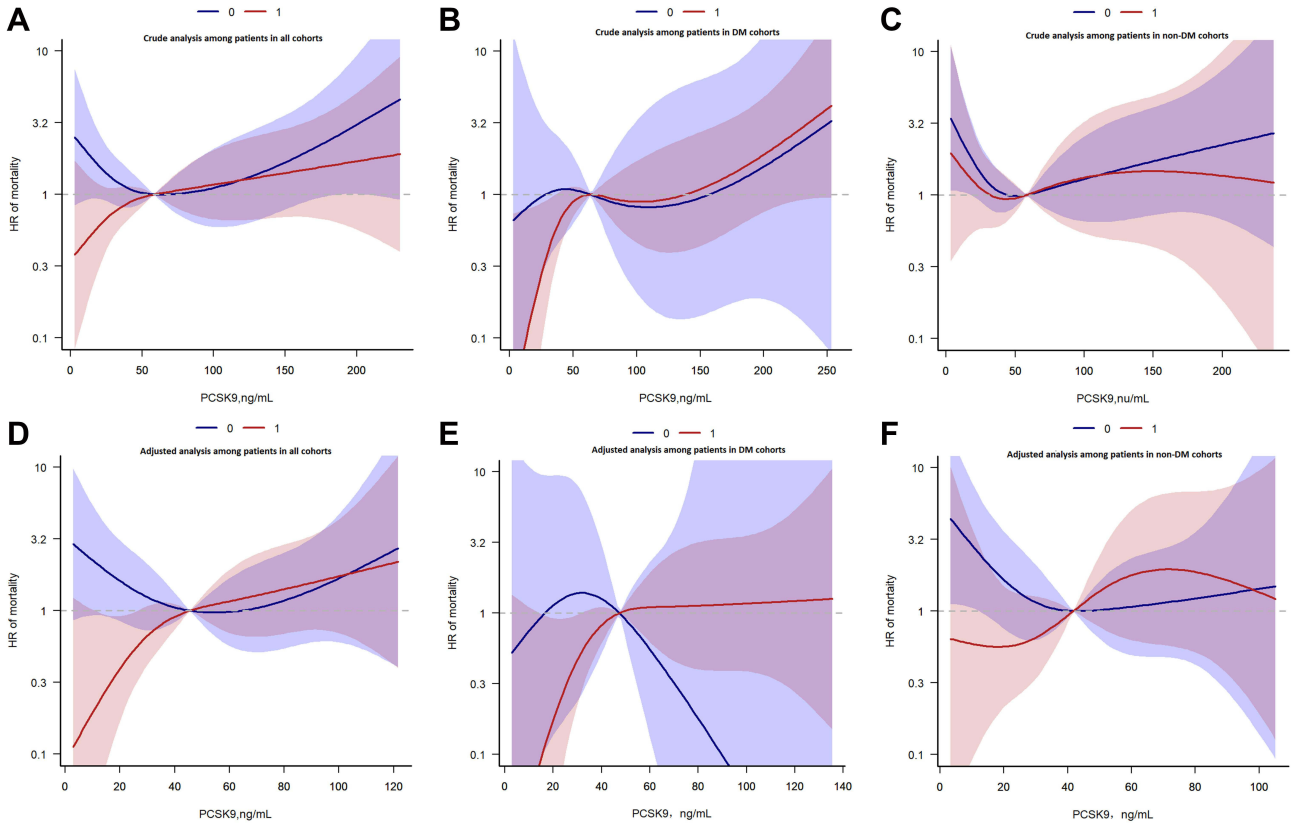

Figure 3 Continuous hazard ratio across logarithmic PCSK9 for all-cause mortality according to restricted cubic spline crude and adjusted analysis among patients in all cohorts, DM patients and non-DM patients stratified by TyG according to median. (**A**), Crude analysis among patients in all cohorts; (**B**), Crude analysis among patients in DM cohorts; (**C**), Crude analysis among patients in non-DM cohorts; (**D**), Adjusted analysis among patients in all cohorts; (**E**), Adjusted analysis among patients in DM cohorts; (**F**), Adjusted analysis among patients in non-DM cohorts. 0=TyG <median; 1=TyG≥median; HR, hazard ratio, line, predicted HR, dashed area, 95% confidence interval. Adjusted for age, gender, hypertension, hyperlipidemia, lipse activator, triglycerides, the use of intra-aortic balloon pump, history of CABG, history of PCI, high sensitive C-reactive protein, height, weight, low density lipoprotein, fasting glucose, diastolic blood pressure, systolic blood pressure, heart rate, the use of statin at discharge.

Subgroup Analyses

During a median of 1.96 days of follow-up of, the hazard ratio for all-cause mortality was higher in the subset of participants with TyG ≥median and PCSK9 ≥median (p for trend = 0.023) among those with T2DM (Table 3). However, there were no statistically significant differences in the trend tests among subgroups divided by age (<65 vs ≥65 years) or LDL-C levels (<70 vs ≥70 mg/L).

Figure 4 shows the Kaplan-Meier curves for the cumulative incidence of all-cause mortality up to a median of 1.96 years, stratified by quartiles of PCSK9 index levels among the T2DM subgroups, and divided by the median TyG level. Among T2DM participants with a TyG index greater than the median, the K-M curve showed that participants in the group with the highest PCSK9 levels had an increased risk of all-cause mortality (log-rank p = 0.017) (Figure 4A) and cardiac-cause mortality (log-rank p = 0.037) (Figure 4B) compared with the other quartiles of PCSK9 levels. However, there were no statistically significant differences between the four groups among T2DM patients with TyG indices less than the median (Figure 4C and 4D).

Discussion

We demonstrate for the first time, to our knowledge, that circulating PCSK9 level mediated ASCVD risk appears to be mediated by concomitant TyG levels in a large, contemporary, cohort with STEMI who undergoing the primary PCI. In fully adjusted analyses, we elucidated a stepwise association between PCSK9 levels and all-cause mortality only in individuals with greater degrees of systemic insulin resistance (ie, baseline TyG levels ≥median). No association between rising PCSK9 levels and all-cause mortality was noted when TyG levels were less than the median. These data shed new light on the apparent interdependence of two known mediators of primary and residual ASCVD risk: PCSK9 and systemic insulin resistance.

Table 3 Stratified Analyses of the Associations Between Risk for Incident All-Cause Mortality and the Groups According to PCSK9&TyG

Subgroup	Crude HR (95% CI)	Crude *P* value	Adjusted[a] (HR 95% CI)	Adjusted *P* value	*P* for Interaction
DM					0.332
TyG<medianandPCSK9<median	1		1		
TyG<medianandPCSK9≥median	1.94 (0.43–8.68)	0.387	0.43 (0.03–5.90)	0.524	
TyG≥medianandPCSK9<median	1.17 (0.31–4.41)	0.816	0.60 (0.09–4.08)	0.606	
TyG≥medianandPCSK9≥median	2.87 (0.84–9.75)	0.092	4.07 (0.71–23.4)	0.116	
Trend.test	1.42 (0.97–2.08)	0.068	2.22 (1.12–4.42)	0.023*	
Non-DM					
TyG<medianandPCSK9<median	1		1		
TyG<medianandPCSK9≥median	1.05 (0.53–2.09)	0.884	1.00 (0.40~2.52)	0.997	
TyG≥medianandPCSK9<median	0.56 (0.22–1.42)	0.221	0.37 (0.09~1.59)	0.181	
TyG≥medianandPCSK9≥median	1.04 (0.49–2.20)	0.920	0.78 (0.24~2.52)	0.681	
Trend.test	0.95 (0.74–1.22)	0.709	0.93 (0.62~1.39)	0.707	
Age<65 yr					0.754
TyG<medianandPCSK9<median	1		1		
TyG<medianandPCSK9≥median	1.04 (0.32–3.41)	0.949	0.91 (0.2–4.07)	0.903	
TyG≥medianandPCSK9<median	0.88 (0.28–2.71)	0.818	0.47 (0.1–2.19)	0.334	
TyG≥medianandPCSK9≥median	2.12 (0.81–5.59)	0.127	1.59 (0.41–6.15)	0.502	
Trend.test	1.29 (0.93–1.81)	0.128	1.23 (0.75–2.03)	0.409	
Age≥65 yr					
TyG<medianandPCSK9<median	1		1		
TyG<medianandPCSK9≥median	1.31 (0.63–2.73)	0.464	0.95 (0.33–2.73)	0.929	
TyG≥medianandPCSK9<median	0.83 (0.35–1.95)	0.664	0.55 (0.14–2.2)	0.400	
TyG≥medianandPCSK9≥median	1.54 (0.76–3.13)	0.228	2.24 (0.61–8.22)	0.223	
Trend.test	1.10 (0.88–1.39)	0.400	1.25 (0.8–1.98)	0.329	
LDL-C≥70					0.787
TyG<medianandPCSK9<median	1		1		
TyG<medianandPCSK9≥median	1.50 (0.70–3.20)	0.292	1.04 (0.41–2.67)	0.934	
TyG≥medianandPCSK9<median	0.86 (0.38–1.92)	0.711	0.49 (0.15–1.62)	0.243	
TyG≥medianandPCSK9≥median	1.78 (0.89–3.55)	0.104	1.63 (0.57–4.64)	0.363	
Trend.test	1.15 (0.92–1.44)	0.233	1.22 (0.84–1.77)	0.293	
LDL-C<70					
TyG<medianandPCSK9<median	1		1		
TyG<medianandPCSK9≥median	0.63 (0.20–1.94)	0.422	0.79 (0.08–7.35)	0.835	
TyG≥medianandPCSK9<median	0.57 (0.15–2.14)	0.402	1.17 (0.09–15.08)	0.903	
TyG≥medianandPCSK9≥median	1.32 (0.49–3.52)	0.582	5.9 (0.64–54.3)	0.117	
Trend.test	1.09 (0.77–1.56)	0.622	1.74 (0.78–3.88)	0.180	

Notes: [a]Adjusted for age, hypertension, lipse activator, triglycerides, the use of intra aortic balloon pump, history of CABG, history of PCI, high sensitive C-reactive protein, height, weight, low density lipoprotein, fasting glucose, diastolic blood pressure, systolic blood pressure, heart rate, the use of statin at discharge. Median of TyG=9.07; Median of PCSK9= 46.84 ng/mL. * P<0.05.

Abbreviations: TyG, triglyceride glucose; PCSK9, proprotein convertase subtilisin/kexin type; DM, diabetes mellitus; LDL-C, low density lipoprotein-cholesterol; HR, hazard ratio; CI, confidence interval.

TyG Levels and PCSK9-Associated Cardiovascular Risk

The most striking finding in the current study was the positive association between circulating PCSK9 levels and an increased risk of all-cause mortality in patients with TyG index levels greater than the median (after both crude and full adjustment). Previous studies have demonstrated that individuals with lower plasma PCSK9 levels had a higher

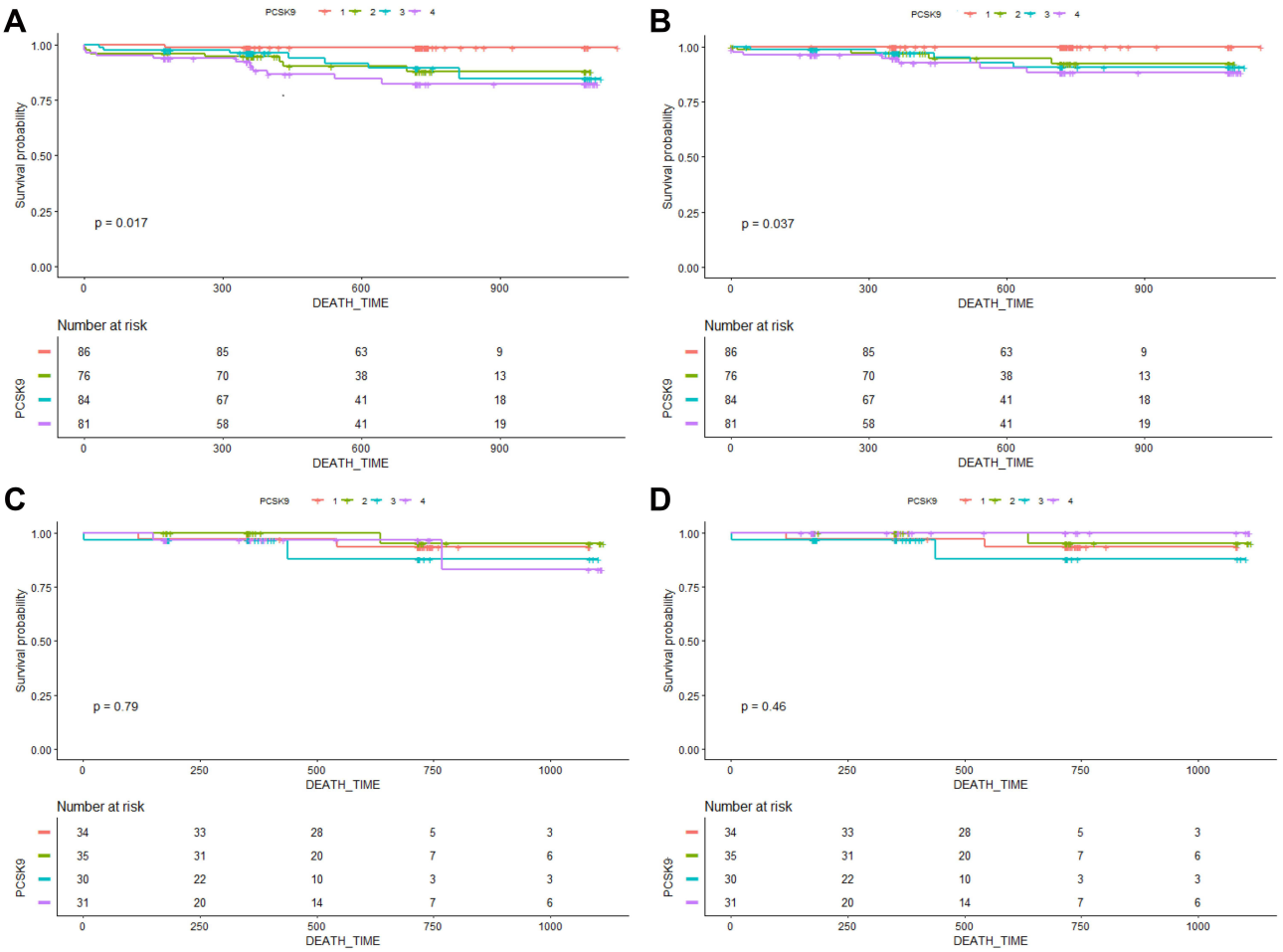

Figure 4 Kaplan-Meier Curves of all-cause mortality and cardiac cause mortality stratified by achieved proprotein convertase subtilisin/kexin type (PCSK9) quartiles in the setting of achieved triglyceride glucose (TyG) levels of less than median vs median or more among patients with diabetes mellitus. (**A**), Kaplan-Meier Curves of all-cause mortality stratified by PCSK9 quartiles in the setting of achieved TyG levels of median or more among patients with DM. Test of trend across quartiles: P = 0.017. (**B**), Kaplan-Meier Curves of cardiac-cause mortality stratified by PCSK9 quartiles in the setting of achieved TyG levels of median or more among patients with DM. Test of trend across quartiles: P = 0.037. (**C**), Kaplan-Meier Curves of all-cause mortality stratified by PCSK9 quartiles in the setting of achieved TyG levels of less than median among patients with DM. Test of trend across quartiles: P = 0.790. (**D**), Kaplan-Meier Curves of cardiac cause mortality stratified by PCSK9 quartiles in the setting of achieved TyG levels of less than median among patients with DM. Test of trend across quartiles: P = 0.460. Subsequent quartile is compared with the first quartile. Q, quartile; Median (range) PCSK9 values per PCSK9 quartile: Q1 (n = 385), 15.0 (7.4, 20.7); Q2 (n = 385), 34.0 (29.8, 39.5); Q3 (n = 385), 63.6 (53.8, 74.0); Q4 (n = 386), 139.3 (107.2, 208.5). *P<0.05.
Abbreviations: PCSK9, proprotein convertase subtilisin/kexin type; TyG, triglyceride glucose; DM, diabetes mellitus.

prevalence of DM, based on 539 stable coronary artery disease cohorts.[21] Furthermore, a study at the cellular level[22] found that liraglutide, a glucagon-like peptide-1 receptor agonist, can suppress blood glucose and decrease PCSK9 expression through an HNF1α-dependent mechanism. A population-based prospective study enrolling 4205 subjects with pre-diabetes concluded that elevated circulating PCSK9 levels were correlated with an increased incidence of type 2 diabetes during a median follow-up period of 3.1 years.[23] Moreover, evidence from animal studies and at the cellular level has shown that downregulation of PCSK9 and inhibition of PCSK9 and the LDL receptor by polydatin ameliorates glucose metabolism.[24] Higher levels of PCSK9 have been correlated with type 1[25] and type 2 diabetes,[26] and this correlation increased substantially with worsening glycemic control.[27] Longitudinal analyses by Ramin-Mangata et al[28] showed that the presence of T2DM may modify the association between plasma PCSK9 and non-HDL cholesterol among DM patients. These findings suggest a possible role of PCSK9 in glucose metabolism.

We observed that elevated PCSK9 concentrations were positively related to all-cause mortality and cardiac-related mortality when TyG levels were higher than the median. Nevertheless, the exact molecular mechanisms of PCSK9 and insulin resistance remain unclear, and it is necessary to investigate the precise mechanisms underlying these associations in future studies. PCSK9 monoclonal antibodies (mAbs), including evolocumab and alirocumab, have been clinically

used to decrease circulating PCSK9 levels, and are proven to provide substantial benefits for patients at very high risk of cardiovascular events.[29] Although the present analysis outlines the utility of measuring TyG levels among a STEMI cohort undergoing primary PCI to better assess PCSK9-associated ASCVD risk, the data are also noteworthy for a lack of Lp(a)-associated ASCVD risk given TyG levels less than 9.07 (ie, the median level), irrespective of the magnitude of PCSK9 elevation. The present data are supported by the clinical prospective observations of Shi et al[30] in a Chinese study, which demonstrated an association between circulating PCSK9 levels and the risk of T2DM. Similarly, a closer analysis of Table 2 within the present study suggests that, for individuals with TyG levels at the median or higher, each SD unit increase in PCSK9 level confers a 23% increased risk of all-cause mortality.

Mechanisms of PCSK9 Combined with TyG in Atherosclerosis and Cardiovascular Risk

Within the current literature, Mendelian randomization studies have recently proven that genetic variants of PCSK9 are associated with higher circulating fasting glucose concentrations as well as an increased risk of T2DM.[31,32] Lorenzo et al have shown that PCSK9 critically controls LDLR expression in the pancreas and may contribute to the maintenance of proper physiological balance in order to limit cholesterol overload in beta cells, as described in experimental models; a possible mechanism underlying this causal association may be the identification of LDLR in the pancreatic islets as a potential target for locally produced PCSK9.[10] Furthermore, these observations suggest that PCSK9 decreases plasma insulin levels, which is paralleled by cholesterol ester accumulation and increased insulin content. More interestingly, insulin secretion and cellular toxicity have been associated with the accumulation of cholesterol in pancreatic islets, indicating a critical role of cholesterol metabolism in the pancreas.[33,34] Consequently, genetic and acquired that increase LDL-R expression should be closely related to altered glucose metabolism. Notably, the deficiency of selective β cells favors cholesterol accumulation in β cells, which is closely related to impaired insulin secretion under hypercholesterolemic conditions.[6,35] An increase in cholesterol esters reduced by PCSK9 may weaken the intracellular response, which is designed to mitigate free cholesterol cell toxicity and could result in long-term cell apoptosis.[34,36,37] However, it is important to stress that the differences in lipid and lipoprotein metabolism in mice as compared with humans suggest the need to further address the crosstalk among these pathways. Based on our findings, this observation could be a result of the synergistic effects of systemic insulin resistance and PCSK9-related pro-atherosclerotic or pro-thrombotic mechanisms, lowering the PCSK9 cut-off point for mediating secondary ASCVD-related events.

Limitation

This study has some limitations that must be given consideration. First, the use of a single baseline PCSK9 measurement (ie, the absence of evaluating effects of longitudinal changes in the incidence of cardiovascular events during the follow-up period to predict outcomes) did not allow us to assess the causal risk for incident cardiovascular endpoints. Thus, further studies are necessary to clarify the mechanisms underlying the association between PCSK9 levels and insulin resistance. Third, it is unclear whether our findings in STEMI individuals in China can be generalized to other diseases or other ethnicities. Therefore, further prospective investigations among diverse populations, larger sample sizes, and studies with long-term follow-up should be undertaken. Lastly, loss or gain of function PCSK9 gene mutations may have had an impact on the statistical results of the current study.[38] However, due to the study design, we did not analyze PCSK9 mutations.

Conclusions

To our knowledge, the present study is the first study within a large cohort of STEMI patients undergoing primary PCI to demonstrate that PCSK9-associated ASCVD risk may be significantly mediated by concomitant levels of insulin resistance which represented by TyG. Elevated PCSK9 levels are related to all-cause mortality and cardiac-related mortality when TyG levels are greater than the median, but not when these levels are less than the median. These findings shed further insight into the mechanisms underlying PCSK9-associated ASCVD risk and might prove useful for identifying individuals who would benefit the most from novel PCSK9-lowering therapies. These findings likewise suggest a potential benefit of lowering the circulation levels of PCSK9 in STEMI patients with insulin resistance.

Data Sharing Statement

The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

Ethics Approval

It is from the ethics committee of the department of Cardiology, Fuwai Hospital, National Center for Cardiovascular Diseases, Peking Union Medical College, China.

Consent for Publication

Written informed consent for publication was obtained from all participants.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

No conflicts of interest, financial or otherwise, are declared by the authors.

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