SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of the full PEGASUS-TIMI 54 trial vs. the TMAO Nested Case-Control Cohort.

Variable	Entire Trial (N=21,162)	TMAO Nested Case-Control Cohort (N=1,803)
Matched variables		
Age (median, IQR)	65 (59, 71)	67.9 (59.5, 74)
Female (n, %)	5,050 (23.9)	462 (25.6)
eGFR (median, IQR)	72.4 (61.0, 84.0)	68.2 (55.0, 79.8)
Other variables		
BMI (median, IQR)	27.8 (25.2, 31.2)	28.7 (25.7, 32.0)
White race (n, %)	18,327 (86.6)	1,736 (96.3)
Region		
Eastern Europe (n, %)	6,290 (29.7)	584 (32.4)
North America (n, %)	3,907 (18.5)	654 (36.3)
Western Europe (n, %)	6,138 (29.0)	565 (31.3)
Asia/Pacific	2,369 (11.2)	0 (0)
South America	2,458 (11.6)	0 (0)
Current smoking (n, %)	3,536 (16.7)	353 (19.6)
Hypertension (n, %)	16,407 (77.5)	1,480 (80.2)
Dyslipidemia (n, %)	16,241 (76.8)	1,480 (82.1)
Diabetes mellitus (n, %)	6,806 (32.2)	612 (33.9)
Multivessel CAD (n, %)	12,558 (59.4)	1,154 (64.0)
> 1 Prior MI (n, %)	3,499 (16.5)	366 (20.3)
History of PAD (n, %)	1,143 (5.4)	170 (9.4)
Qualifying event		
STEMI (n, %)	11,329 (53.6)	846 (47.0)
NSTEMI (n, %)	8583 (40.6)	899 (49.9)

Years from event (median, IQR)	1.7 (1.2, 2.3)	1.7 (1.2, 2.3)
Medications		
Aspirin (n, %)	21,132 (99.9)	1800 (99.8)
Statin (n, %)	19,604 (92.6)	1,685 (93.5)
Beta-blocker (n, %)	17,486 (82.6)	1,547 (85.8)
ACEI or ARB (n, %)	17,030 (80.5)	1424 (79.0)

ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; CAD, coronary artery disease; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MI, myocardial infarction; NSTEMI, Non-ST-elevation myocardial infarction; PAD, peripheral artery disease; STEMI, ST-elevation myocardial infarction; TMAO, Trimethylamine N-oxide

Table S2. Spearman Correlation Between TMAO and Cardiovascular Markers.

	TMAO	Betaine	Choline
TMAO (μM)	1.00	-0.03 (P=0.29)	0.26 (P<0.001)
Betaine (μM)	-0.03 (P=0.29)	1.00	0.35 (P<0.001)
Choline (μM)	0.26 (P<0.001)	0.35 (P<0.001)	1.00
Age (years)	0.21 (P<0.001)	0.07 (P=0.004)	0.19 (P<0.001)
Weight (kgs)	0.05 (P=0.044)	-0.16 (P<0.001)	0.02 (P=0.47)
eGFR(ml/min/1.73m²)	-0.41 (P<0.001)	0.04 (P=0.11)	-0.30 (P<0.001)
BMI (mg/m²)	0.11 (P<0.001)	-0.19 (P<0.001)	0.07 (P=0.002)
Hs-TnT (ng/L)	0.29 (P<0.001)	0.04 (P=0.061)	0.25 (P<0.001)
NT-pro BNP (ng/mL)	0.20 (P<0.001)	0.12 (P<0.001)	0.18 (P<0.001)
Hs-CRP (mg/dL)	0.08 (P<0.001)	-0.06 (P=0.020)	0.10 (P<0.001)

BMI, body mass index; eGFR, estimates glomerular filtration; Hs-CRP, high sensitive C-reactive protein; Hs-TnT, high sensitive Troponin T; MI, myocardial infarction: NT-pro BNP, N-terminal pro B-type natriuretic peptide; TMAO, trimethylamine N-oxide. All biomarkers were log-transformed for this analysis.

Table S3. Risk of Cardiovascular Events by 1-SD Log-Transformed TMAO Increase.

Odds Ratios for Primary Endpoint (CV death, MI and Stroke) by 1-SD Log-Transformed TMAO Increase	
	OR (95% CI)
Matched *	1.09 (0.98-1.21, P=0.12)
Matched with clinical adjustment #	1.05 (0.93-1.18, P=0.43)
Matched with clinical and biomarker adjustment ‖	1.05 (0.92-1.19, P=0.47)
Odds Ratios for CV Death by 1-SD Log-Transformed TMAO Increase	
Matching variable adjustment ¶	1.22 (1.01-1.48, P=0.035)
Multivariate clinical adjustment §	1.13 (0.92-1.39, P=0.24)
Multivariate with clinical and biomarker adjustment †	1.07 (0.85-1.34, P=0.58)
Odds Ratios for MI by 1-SD Log-Transformed TMAO Increase	
Matching variable adjustment ¶	1.04 (0.92-1.18, P=0.51)
Multivariate clinical adjustment §	0.99 (0.87-1.12, P=0.86)
Multivariate with clinical and biomarker adjustment †	0.94 (0.83-1.08, P=0.38)
Odds Ratios for Stroke by 1-SD Log-Transformed TMAO Increase	
Matching variable adjustment ¶	1.25 (1.01-1.53, P=0.038)
Multivariate clinical adjustment §	1.11 (0.88-1.39, P=0.38)
Multivariate with clinical and biomarker adjustment †	1.10 (0.87-1.39, P=0.44)

* Conditional (matched) logistic regression was used for the primary endpoint with matching variables (age, sex, eGFR). # In addition to matching variables (age, sex and eGFR), further adjustment was performed for the following clinical variables: hypertension, hypercholesterolemia, diabetes mellitus, peripheral artery disease, qualifying index event, smoking, region, body mass index, aspirin dosage and treatment arm. ‖ In addition to matching and clinical variables, further adjustment was done for log-transformed biomarkers (hs-TnT, NT-proBNP and hs-CRP).
¶ Unconditional (unmatched) logistic regression was used for each individual component CV death, MI and stroke events. § Multivariable adjustment (unmatched) was performed for the following clinical variables: age, sex, eGFR, hypertension, hypercholesterolemia, diabetes mellitus, peripheral artery disease, qualifying index event, smoking, region, body mass index aspirin dosage and treatment arm. † In addition to multivariate clinical variables adjustment, further adjustment was done for log-transformed biomarkers (hs-TnT, NT-proBNP and hs-CRP).
CV, Cardiovascular; eGFR, estimated glomerular filtration rate; MI, myocardial infarction: TMAO, Trimethylamine N-oxide.

Table S4. Risk of Cardiovascular Events by TMAO Quartiles and eGFR Status (< 60 vs. ≥ 60 ml/min/1.73m²).

Adjusted Odds Ratios for Primary Endpoint (CV death, MI and Stroke) by TMAO Quartiles *			
	2nd Quartile	3rd Quartile	4th Quartile
eGFR ≥ 60	1.77 (0.81-3.84)	2.03(0.96-4.28)	2.14 (1.04-4.40)
eGFR < 60	0.94 (0.68-1.30)	1.07 (0.77-1.50)	0.99 (0.68-1.46)
P for overall interaction = 0.29			
Adjusted Odds Ratios for CV Death by TMAO Quartiles ¶			
eGFR ≥ 60	3.22 (0.67-15.39)	1.83 (0.38-8.81)	3.17 (0.71-14.05)
eGFR < 60	0.99 (0.50-1.96)	1.95 (1.05-3.64)	1.31 (0.63-2.73)
P for overall interaction = 0.097			
Adjusted Odds Ratios for MI by TMAO Quartiles ¶			
eGFR ≥ 60	1.61 (0.66-3.96)	1.93 (0.83-4.52)	1.52 (0.66-3.48)
eGFR < 60	0.87 (0.61-1.25)	0.93 (0.64-1.36)	0.95 (0.63-1.46)
P for overall interaction = 0.44			
Adjusted Odds Ratios for Stroke by TMAO Quartiles ¶			
eGFR ≥ 60	0.97 (0.17-5.57)	2.39 (0.52-11.09)	2.23 (0.50-10.04)
eGFR < 60	1.32 (0.61-2.85)	1.76 (0.82-3.77)	2.22 (1.01-4.87)
P for overall interaction = 0.84			

CV, Cardiovascular disease; eGFR, estimated glomerular filtration rate; MI, myocardial infarction: TMAO, Trimethylamine N-oxide.

* Conditional (matched) logistic regression was used for the primary endpoint with matching variables (age, sex, eGFR). In addition to matching variables (age, sex and eGFR), further adjustment was performed for the following clinical variables: hypertension, hypercholesterolemia, diabetes mellitus, peripheral artery disease, qualifying index event, smoking, region, body mass index, aspirin dosage and treatment arm.

¶Multivariable adjustment (unmatched) was performed for the following clinical variables: age, sex, eGFR, hypertension, hypercholesterolemia, diabetes mellitus, peripheral artery disease, qualifying index event, smoking, region, body mass index, aspirin dosage and treatment arm.

Table S5. Risk of Cardiovascular Events by TMAO Quartiles and Antiplatelet Regimen.			
Odds Ratios for Primary Endpoint (CV death, MI and Stroke) by TMAO Quartiles			
	2nd Quartile	3rd Quartile	4th Quartile
Placebo	1.05 (0.47-2.37)	1.23 (0.49-3.09)	1.56 (0.60-4.02)
Pooled Ticagrelor	0.92 (0.59-1.44)	1.09 (0.71-1.68)	1.18 (0.75-1.87)
P for overall interaction = 0.952			
Odds Ratios for CV Death by TMAO Quartiles §			
Placebo	1.06 (0.41-2.72)	1.22(0.49-3.02)	1.31 (0.51-3.36)
Pooled Ticagrelor	1.55 (0.73-3.28)	2.17 (1.04-453)	2.34 (1.12-4.89)
P for overall interaction = 0.742			
Odds Ratios for MI by TMAO Quartiles §			
Placebo	1.06 (0.62-1.79)	0.83 (0.48-1.45)	0.99 (0.56-1.74)
Pooled Ticagrelor	0.88 (0.58-1.34)	1.23 (0.82-1.86)	0.99 (0.65-1.52)
P for overall interaction = 0.399			
Odds Ratios for Stroke by TMAO Quartiles §			
Placebo	1.12 (0.32-3.85)	2.36 (0.79-7.04)	2.28 (0.74-7.00)
Pooled Ticagrelor	1.24 (0.538-2.93)	1.65 (0.72--3.74)	1.91 (0.85-4.32)
P for overall interaction = 0.895			

In addition to matching variables (age, sex and eGFR), further adjustment was performed for the following clinical variables: hypertension, hypercholesterolemia, diabetes mellitus, peripheral artery disease, qualifying index event, smoking, region, body mass index, aspirin dosage and treatment arm.
§ Multivariable adjustment (unmatched) was performed for the following clinical variables: age, sex, eGFR, hypertension, hypercholesterolemia, diabetes mellitus, peripheral artery disease, qualifying index event, smoking, region, body mass index, aspirin dosage and treatment arm.
CV, Cardiovascular disease; MI, myocardial infarction: TMAO, Trimethylamine N-oxide.

Table S6. Risk of Cardiovascular Events by Choline and Betaine Quartiles.

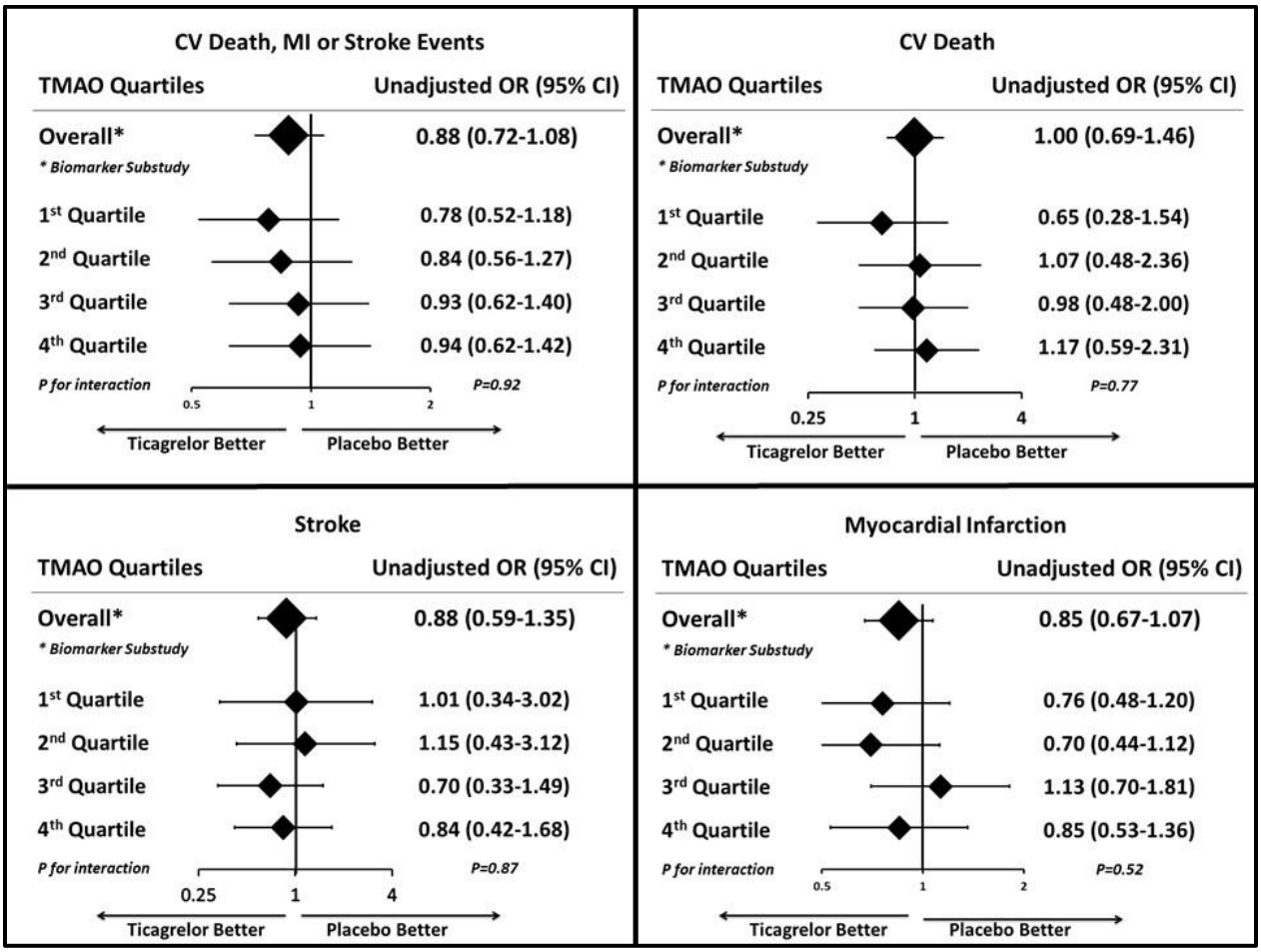
Odds Ratios for Primary Endpoint (CV death, MI and Stroke) #				
	2nd Quartile	3rd Quartile	4th Quartile	P for trend
Choline	1.03 (0.77-1.39)	1.28 (0.95-1.72)	1.06 (0.78-1.44)	P=0.43
Betaine	0.78 (0.58-1.04)	0.75 (0.56-1.01)	0.81 (0.60-1.09)	P=0.16
Odds Ratios for CV Death §				P for trend
Choline	0.81 (0.47-1.39)	1.04 (0.61-1.76)	0.64 (0.37-1.13)	P=0.24
Betaine	0.78 (0.47-1.31)	0.76 (0.44-1.32)	1.24 (0.75-2.06)	P=0.41
Odds Ratios for MI §				P for trend
Choline	1.07 (0.77-1.49)	1.33 (0.95-1.85)	1.15 (0.81-1.62)	P=0.25
Betaine	0.82 (0.60-1.13)	0.80 (0.58-1.10)	0.77 (0.55-1.08)	P=0.13
Odds Ratios for Stroke §				P for trend
Choline	0.98 (0.53-1.81)	1.22 (0.67-2.20)	0.82 (0.44-1.56)	P=0.71
Betaine	0.71 (0.40-1.26)	0.83 (0.47-1.47)	0.89 (0.50-1.59)	P=0.79

In addition to matching variables (age, sex and eGFR), further adjustment was performed for the following clinical variables: hypertension, hypercholesterolemia, diabetes mellitus, peripheral artery disease, qualifying index event, smoking, region, body mass index, aspirin dosage and treatment arm.

§ Multivariable adjustment (unmatched) was performed for the following clinical variables: age, sex, eGFR, hypertension, hypercholesterolemia, diabetes mellitus, peripheral artery disease, qualifying index event, smoking, region, body mass index, aspirin dosage and treatment arm.

CV, Cardiovascular disease; MI, myocardial infarction.

Figure S1. Treatment Effect of Ticagrelor Versus Placebo on the Composite and Individual Endpoints of CV Death, MI and Stroke By TMAO Quartiles.



CI, confidence intervals; CV, Cardiovascular disease; MI, myocardial infarction; OR, odds ratio; TMAO, Trimethylamine N-oxide.