



Increased Angiopoietin like protein 4 (ANGPTL4) is associated with higher concentration of LDL-triglycerides in type 2 diabetes

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Background / Aims

- Diabetic dyslipidemia is typically characterized by an increase in plasma triglycerides (TG), small dense (sd) LDL-C and a concomitant increase in LDL-TG.
- LDL-TG, a sub-fraction of TG has been reported elevated and to be a more powerful risk factor than LDL-cholesterol (LDL-C).¹⁾²⁾
- Angiopoietin-like protein (ANGPTL) 3 and ANGPTL4 are associated with increased plasma TG levels due to their role in regulating the activity of lipoprotein lipase in circulation.³⁾⁴⁾
- However, the association between ANGPTL3, 4 and LDL-TG are unclear. Therefore, to explore the role of ANGPTL3, 4, we examined the association between the plasma level of ANGPTL3, ANGPTL4 and levels of LDL-TG.

Methods

- 126 diabetic patients (male/female 102/24) were enrolled into the study.
- Blood samples were taken after overnight fasting.
- Sd LDL-C concentrations were measured using the homogeneous methods we established (Denka Seiken, Tokyo, Japan). Concentrations of large, buoyant (lb) LDL-C were estimated by subtracting the sd LDL-C from the LDL-C.
- Serum LDL-TG concentrations were measured by the LDL-TG-EX newly developed homogeneous methods (Denka Seiken, Co., Ltd., Tokyo, Japan).
- Serum ANGPTL3 and ANGPTL4 levels were measured by sandwich enzyme immunoassays using commercial ELISA kits [Immuno-Biological Laboratories (IBL), Gunma, Japan].
- Preheparin lipoprotein lipase (LPL) mass were measured by sandwich ELISA.
- Measurements of waist circumference, visceral fat and subcutaneous fat CT scans were performed to measure the areas of visceral fat and subcutaneous fat, as well as waist circumference at the level of the umbilicus with a workstation on a Ziostation (Ziosoft Inc., Tokyo).

Results

In univariate analysis, LDL-TG was positively correlated with sd LDL-C and ANGPTL4, and negatively correlated with preheparin LPL mass. However LDL-TG was not correlated with ANGPTL3. Multiple linear regression analysis showed that ANGPTL4 and preheparin LPL mass were independent predictors of LDL-TG concentrations.

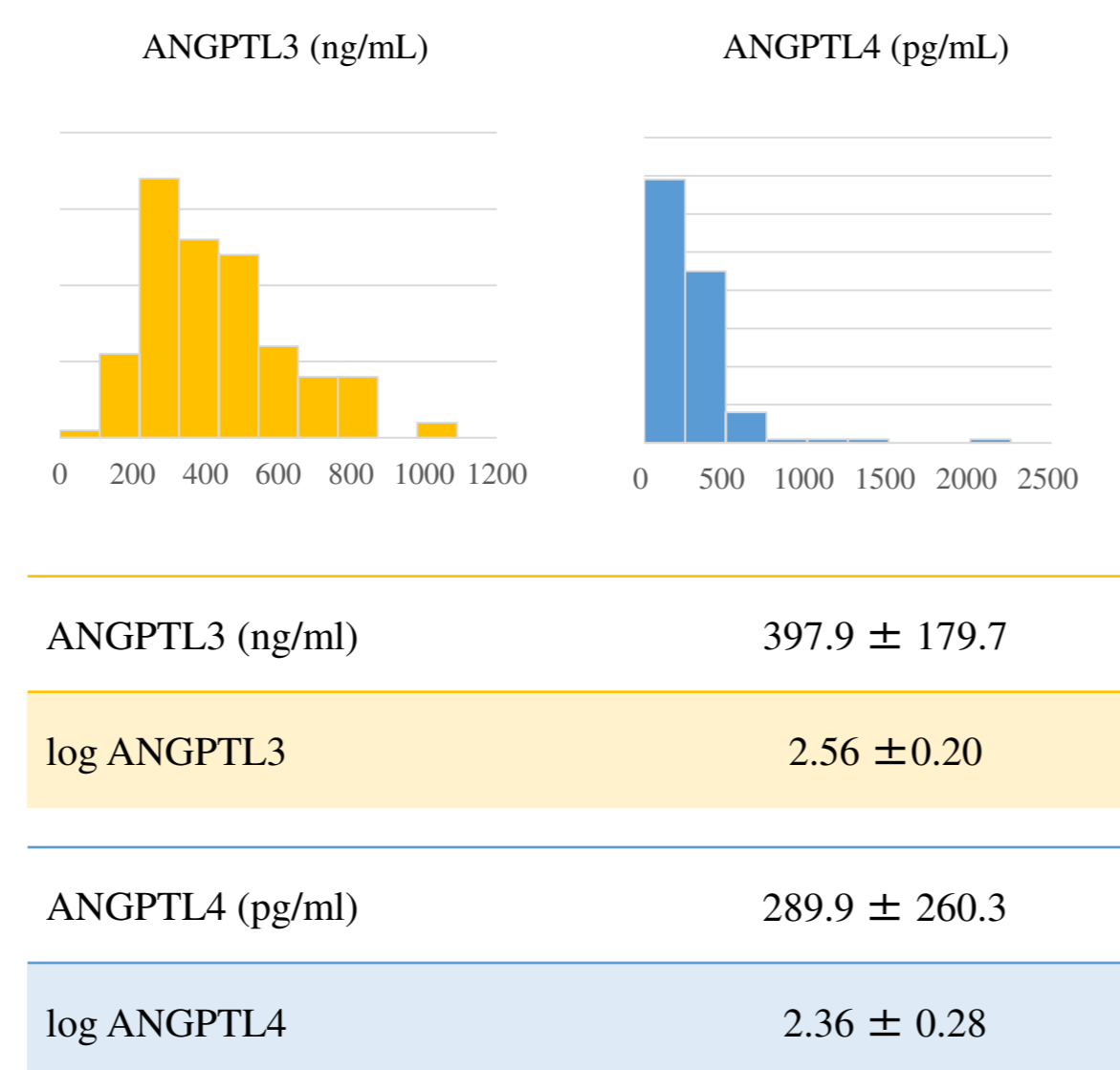
General characteristics of T2DM

	Mean ± SD, n (%)		Mean ± SD, n (%)
n (Male/Female)	126(102/24) (81%/19%)	Retinopathy (NDR/SDR/PPDR/PDR)	94/15/5/10 (75/13/4/8)
Age (years)	61 ± 10	Nephropathy stage (1/2/3/4/5)	75/32/19/0/0 (60/25/15/0/0)
Duration (years)	12 ± 8	Antidiabetic drug	110/28/8/13 (87/22/6/33)
Body Weight (kg)	74.6 ± 14.7	(OADs/insulin/GLP-1i/none)	
BMI (kg/m ²)	26.7 ± 4.3	Prior use of oral OADs	82/86/9/26/9 (64/45/3/20/7)
Waist Circumference (cm)	93.4 ± 11.9	(BG/DP4-i/TZD/α-GI/SGLT2-i)	
AST (IU/L)	25.5 ± 13.6	Prior use of lipid-lowering agent	56/15/9/15/49
ALT (IU/L)	27.6 ± 21.3	(statin/fibrate/ezetimibe/EPA-DHA/none)	(44/12/7/12/39)
γ-GTP (IU/L)	45.7 ± 45.1	Visceral fat (cm ²)	170.1 ± 66.1
BUN (mg/dL)	15.4 ± 4.9	Subcutaneous fat (cm ²)	194.3 ± 96.2
Cr (mg/dL)	0.79 ± 0.23	History of coronary artery disease (%)	11(10)
eGFR (mL/min/1.73 m ²)	79.5 ± 21.0		
FPG (mg/dL)	142.4 ± 48.2		
HbA1c (%)	7.4 ± 1.3		
log UACR	1.5 ± 0.7		

Serum lipid parameters in T2DM

	Mean ± SD
Total Cholesterol (mg/mL)	184.3 ± 43.7
TG (mg/dL)	141.5 ± 98.3
logTG	2.07 ± 0.26
HDL-C (mg/dL)	50.8 ± 13.1
LDL-C (mg/dL)	108.2 ± 37.0
non HDL-C (mg/dL)	133.6 ± 43.0
apoAI (mg/dL)	130.7 ± 23.3
apoAII (mg/dL)	29.2 ± 5.1
apoB (mg/dL)	91.3 ± 26.2
apoCII (mg/dL)	4.6 ± 2.0
apoCIII (mg/dL)	10.7 ± 5.1
apoE (mg/dL)	4.2 ± 1.2
RPL-C (mg/dL)	8.2 ± 7.9
Preheparin LPL mass (ng/mL)	57.6 ± 20.5
sd LDL-C (mg/dL)	36.6 ± 20.7
lb LDL-C (mg/dL)	71.5 ± 31.0
LDL-TG (mg/dL)	25.1 ± 6.8

Distribution of circulating levels of ANGPTL3, ANGPTL4



Multivariable linear regression analyses show independent relationship of LDL-TG with serum parameters

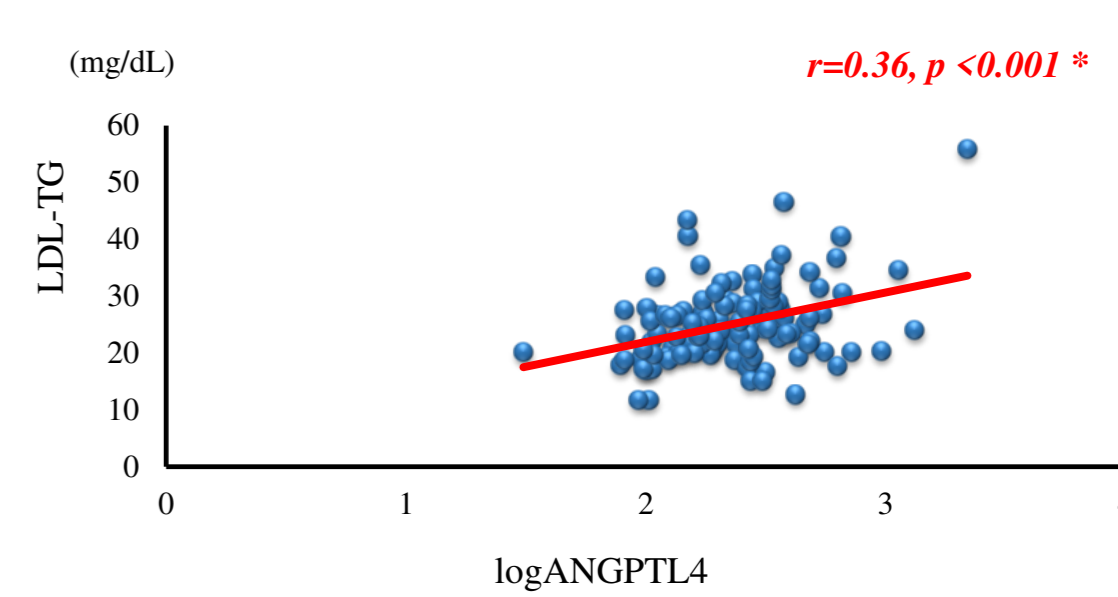
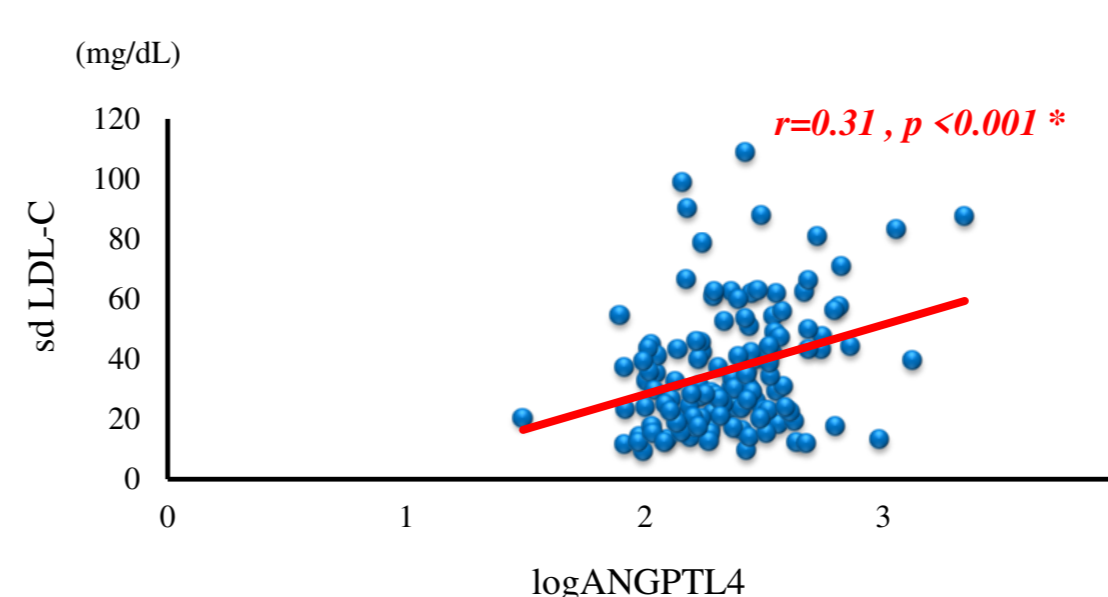
Model	β coefficient	t value	p value	Full-model R ²
Model 1				
			<0.0001*	0.27
	apoCIII	0.37	4.59	<0.0001*
	log ANGPTL4	0.29	3.69	<0.05*
Model 2				
			<0.0001*	0.30
	apoCIII	0.36	4.53	<0.0001*
	Preheparin LPL mass	-0.19	-2.39	<0.05*
	log ANGPTL4	0.23	2.81	<0.05*
Model 3				
			<0.001*	0.32
	apoCII	0.30	1.89	0.06
	apoCIII	0.11	0.72	0.48
	Preheparin LPL mass	0.20	2.40	<0.05*
	log ANGPTL4	-0.18	-2.24	<0.05*

Data were expressed as mean ± SD.

Differences were considered statistically significant at values of p<0.05; *

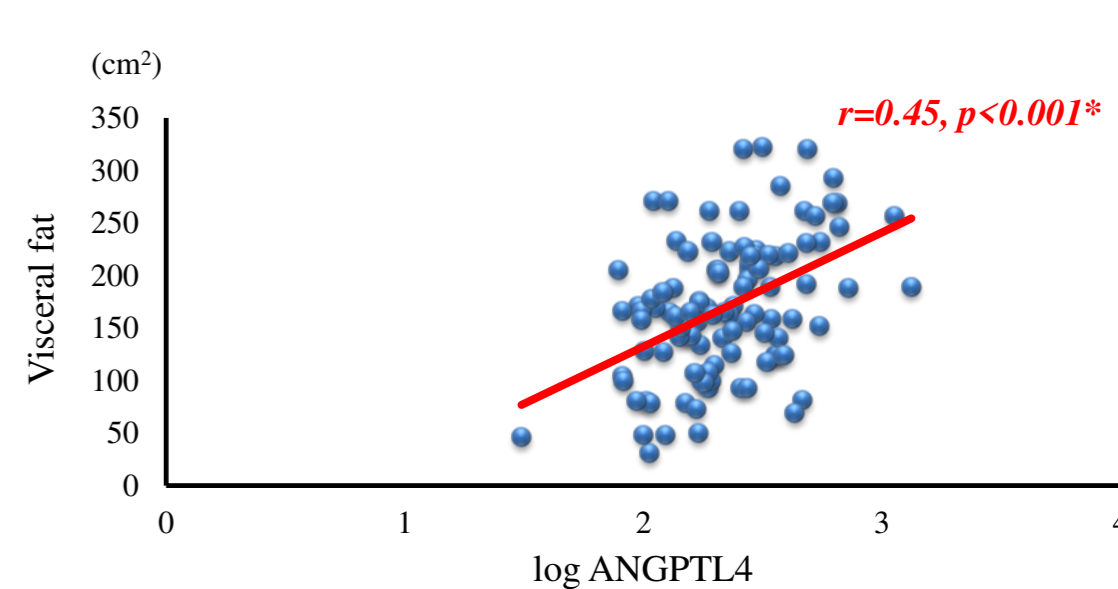
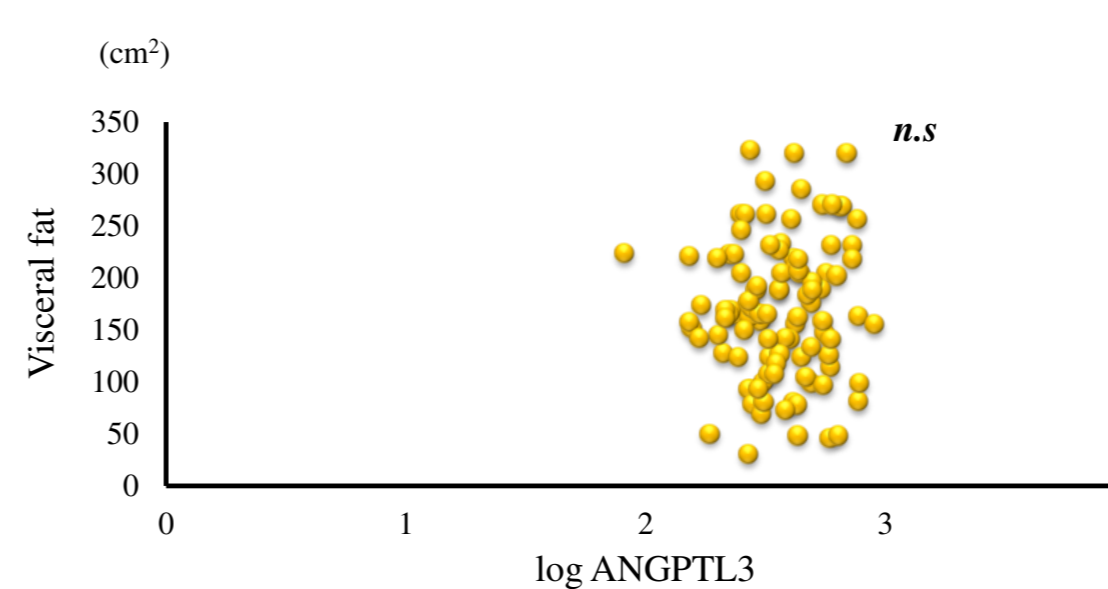
Univariate relationships of serum ANGPTL3 and ANGPTL4 with various lipid parameters

	log ANGPTL3		log ANGPTL4	
	r	p	r	p
Total Cholesterol (mg/mL)	0.31	<0.001*	0.25	<0.05*
TG (mg/dL)	0.04	0.68	0.25	<0.001*
logTG	0.06	0.52	0.28	<0.001*
HDL-C (mg/dL)	0.28	<0.001*	-0.01	0.91
LDL-C (mg/dL)	0.23	<0.05*	0.23	<0.05*
non HDL-C (mg/dL)	0.22	<0.05*	0.26	<0.001*
apoB (mg/dL)	0.21	<0.05*	0.3	<0.001*
apoCII (mg/dL)	0.23	<0.05*	0.31	<0.001*
apoCIII (mg/dL)	0.23	<0.05*	0.23	<0.05*
apoE (mg/dL)	0.26	<0.001*	0.24	<0.05*
RPL-C (mg/dL)	0.05	0.58	0.20	<0.05*
Preheparin LPL mass (ng/mL)	0.21	<0.05*	-0.33	<0.001*
sd LDL-C (mg/dL)	0.15	0.10	0.31	<0.001*
lb LDL-C (mg/dL)	-0.1	0.29	0.01	0.89
LDL-TG (mg/dL)	0.12	0.17	0.36	<0.001*



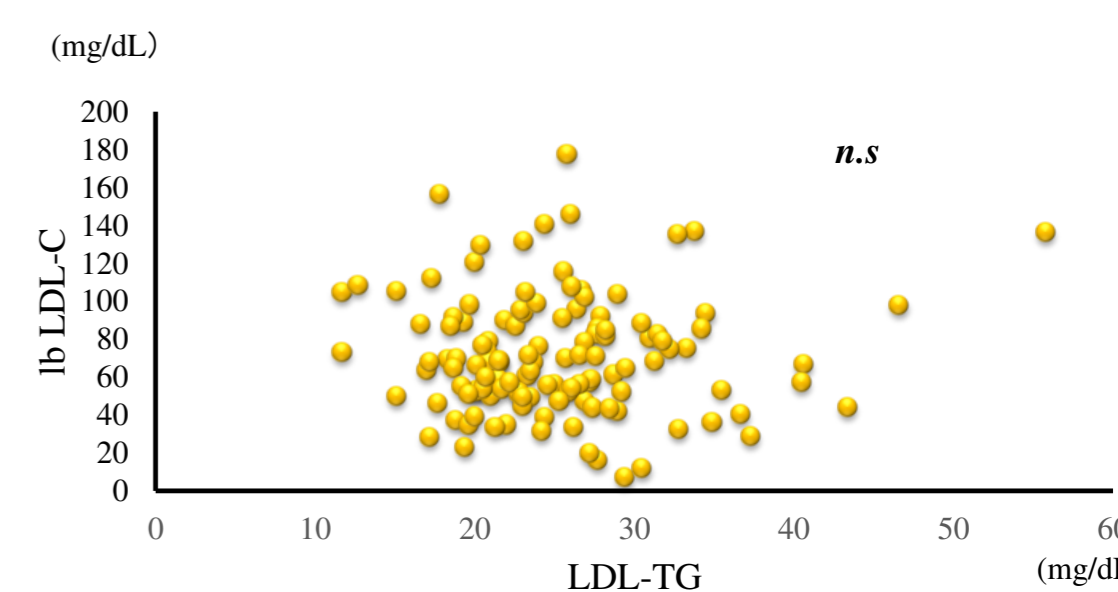
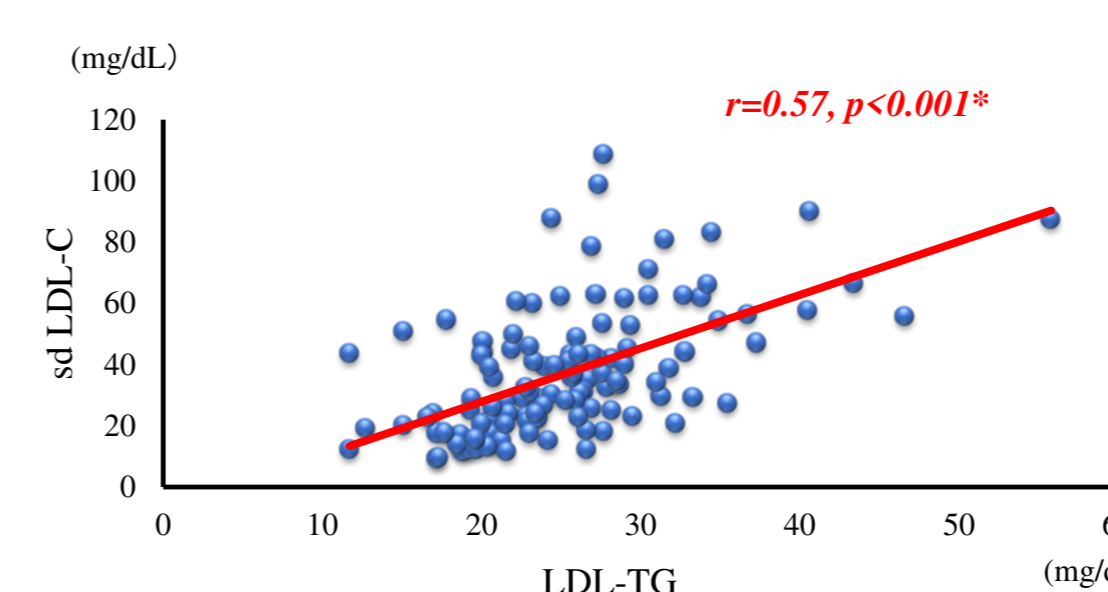
Univariate relationships of serum ANGPTL3 and ANGPTL4 with various parameters

	log ANGPTL3		log ANGPTL4	
	r	p	r	p
Age (years)	0.14	0.13	0.001	0.99
Duration (years)	0.07	0.41	-0.05	0.58
Body Weight (kg)	0.02	0.85	0.14	0.12
BMI (kg/m ²)	0.02	0.80	0.18	<0.05*
Waist Circumference (cm)	0.06	0.54	0.28	<0.001*
Visceral fat (cm ²)	0.04	0.66	0.45	<0.001*
Subcutaneous fat (cm ²)	0.00	0.97	0.19	0.06
AST (IU/L)	-0.01	0.93	0.17	0.06
ALT (IU/L)	-0.10	0.28	0.07	0.43
γ-GTP (IU/L)	-0.07	0.48	0.07	0.48
Cr (mg/dL)	0.04	0.69	0.10	0.27
eGFR (mL/min/1.73 m ²)	-0.17	0.06	-0.09	0.31
FPG (mg/dL)	0.03	0.78	0.18	<0.05*
HbA1c (%)	0.09	0.31	0.12	0.17
log UACR	0.06	0.49	0.05	0.61



Univariate relationships of serum LDL-TG level with various parameters

	LDL-TG		LDL-TG	
	r	p	r	p
Age (years)	-0.07	0.43		
Duration (years)	-0.18	<0.05*		
Body Weight (kg)	0.01	0.88		
BMI (kg/m ²)	0.06	0.48		
Waist Circumference (cm)	0.16	0.1		
Visceral fat (cm ²)	0.31	<0.05*		
Subcutaneous fat (cm ²)	0.12	0.21		
AST (IU/L)	0.18	<0.05*		
ALT (IU/L)	0.16	0.08		
γ-GTP (IU/L)	0.22	<0.05*		
BUN (mg/dL)	0.04	0.69		
Cr (mg/dL)	0.13	0.16		
eGFR (mL/min/1.73 m ²)	-0.14	0.13		
FPG (mg/dL)	0.15	0.10		
HbA1c (%)	0.03	0.76		
log UACR			0.04	0.69
Total Cholesterol (mg/mL)			0.42	<0.001*
TG (mg/dL)			0.53	<0.001*
logTG			0.53	<0.001*
HDL-C (mg/dL)			-0.24	<0.001*
LDL-C (mg/dL)			0.33	<0.001*
non HDL-C (mg/dL)			0.51	<0.001*
apoB (mg/dL)			0.53	<0.001*
apoCII (mg/dL)			0.49	<0.001*
apoCIII (mg/dL)			0.43	<0.001*
apoE (mg/dL)			0.50	<0.001*
RPL-C (mg/dL)			0.58	<0.001*
Preheparin LPL mass (ng/mL)			-0.31	<0.001*
sd LDL-C (mg/dL)			0.57	<0.001*
lb LDL-C (mg/dL)			0.01	0.88



Conclusions

Higher plasma levels of ANGPTL4 but not ANGPTL3 was closely associated with higher levels of LDL-TG. The findings suggest that ANGPTL4 was related to LDL-TG-enrichment as a powerful cardiovascular disease risk marker in T2DM.

References

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Conflicts of Interest: Research Support Denka Seiken Co., Ltd.