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Circulating PCSK7 Level is Independently Associated with Obesity, Triglycerides Level and Fatty Liver Index in a General Population without Medication

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Aim: Dyslipidemia and altered iron metabolism are typical features of non-alcoholic fatty liver disease (NAFLD). Proprotein convertase subtilisin/kexin type 7 (PCSK7), a transmembrane-anchored endonuclease, is associated with triglycerides level and processing of transferrin receptor 1. However, the significance of circulating PCSK7 has not been fully addressed, though prosegment PCSK7 is secreted from cells. We investigated the associations of plasma PCSK7 level with several parameters.

Methods: Plasma PCSK7 concentration was measured in 282 subjects (male/female: 126/156) without medication of the Tanno-Sobetsu Study, a population-based cohort study.

Results: There was no significant sex difference in PCSK7 level. Current smoking habit, but not alcohol drinking habit, was associated with increased PCSK7 level. PCSK7 concentration was negatively correlated with age and blood urea nitrogen and was positively correlated with body mass index (BMI) and levels of γ-glutamyl transpeptidase (γGTP), triglycerides and fatty liver index (FLI), which is calculated by BMI, waist circumference and levels of γGTP and triglycerides, as a noninvasive and simple predictor of NAFLD. There were no significant correlations of PCSK7 level with levels of iron and plasma PCSK9, a secreted PCSK family member and a regulator of low-density lipoprotein cholesterol level. Multivariable regression analyses after adjustment of age, sex and current smoking habit showed that PCSK7 concentration was independently associated with BMI ($\beta = 0.130$, $P = 0.035$), triglycerides ($\beta = 0.141$, $P = 0.027$) or FLI ($\beta = 0.139$, $P = 0.030$).

Conclusions: Plasma PCSK7 concentration is independently associated with chronic liver disease including obesity and elevated triglycerides level in a general population of individuals who had not regularly taken any medications.

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Key words: Proprotein convertase subtilisin/kexin type 7, Triglycerides, Obesity, Fatty liver

Introduction

The proprotein convertase subtilisin/kexin (PCSK) family constitutes a group of nine calcium-dependent serine proteases for the cleavage and subsequent activation of protein substrates including growth factors, growth factor receptors, metalloproteinases, clotting factors and viral proteins[1-3]. PCSKs promote physiological and pathological processes by cell proliferation,

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degradation of the extracellular matrix, activation of the immune response, and activation of viral proteins that allow for efficient entry into the host cells, resulting in the development of several diseases[1-3]. Among PCSKs, PCSK type 9 (PCSK9) is synthesized primarily in the liver[4], and secreted PCSK9 from the liver directly binds to the low-density lipoprotein (LDL) receptor, subsequently promoting degradation of the LDL receptor[2, 5, 6] through an endosomal/lysosomal pathway[7]. It has recently been reported that PCSK9 inhibitors, evolocumub and alirocumab, significantly decrease LDL cholesterol level and reduce cardiovascular events[8, 9]. Furthermore, circulating PCSK9 concentration is associated with several aspects of lipid and inflammation pathways and severity of coronary artery disease[10-12].

On the other hand, PCSK type 7 (PCSK7) is ubiquitously expressed as a membrane-anchored protease[13]. PCSK7 is concentrated in the trans-Golgi network and shuttles between the plasma membrane and the trans-Golgi network[2, 14]. Variants in the PCSK7 gene have been reported to be associated with circulating lipids[15-17] and liver damage during iron overload[18, 19]. It has also been reported that transferrin receptor 1 (TfR1) is cleaved and activated only by PCSK7[20, 21]. In relation to these PCSK7-related phenotypes, dyslipidemia and altered iron metabolism are typical features of non-alcoholic fatty liver disease (NAFLD), which has been highlighted as a lifestyle-related disease that is associated with obesity, metabolic syndrome, type 2 diabetes, insulin resistance, dyslipidemia and cardiovascular disease[22, 23]. However, the impact of PCSK7 on hepatic fat accumulation and liver damage during iron overload in NAFLD has not been fully evaluated.

It has recently been reported that PCSK7 was detected in serum and that serum PCSK7 level was positively correlated with triglycerides level in a small number of patients at risk for NAFLD (n=72)[24]. However, little is known about the significance of circulating PCSK7, especially in a general population. In the present study, we investigated the cross-sectional associations of PCSK7 level with several parameters including PCSK9, a secreted PCSK family member, and NAFLD-related markers including obesity, dyslipidemia, iron and fatty liver index (FLI)[25], a noninvasive and simple biomarker for diagnosis of NAFLD, in a general population who had not regularly taken any medications.

Methods

Study Subjects

In a population-based cohort, the Tanno-Sobetsu Study, a total of 627 Japanese subjects (male/female: 292/335) were recruited from residents of Sobetsu Town in 2016. Subjects treated with any medications were excluded for elimination of drug effects on PCSK7, and subjects with no medication (n=282, male/female: 126/156) were enrolled. Medical checkups, including measurement of blood pressure and calculation of body mass index (BMI), and collection of blood samples were performed as previously described[26, 27]. This study was approved by the Ethical Committee of Sapporo Medical University and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all of the study subjects.

Measurements

Blood samples were corrected after an overnight fast. Plasma concentrations of PCSK7 and PCSK9 were measured using enzyme-linked immunosorbent assay (ELISA) kits for PCSK7 (MyBioSource, San Diego, CA) and PCSK9 (R&D Systems, Minneapolis, MN), respectively. The intra- and inter-assay coefficients of variation in the kits were <10%. Variables of liver function, renal function, glucose and lipid metabolism were measured as previously described[26, 27]. FLI was calculated using the following formula including BMI, waist circumference (WC) and levels of γ-glutamyl transpeptidase (γGTP) and triglycerides[25]: $FLI = [e^{(0.953 \times \ln(\text{triglycerides}) + 0.139 \times BMI + 0.718 \times \ln(\gamma GTP) + 0.053 \times WC - 15.745)}] / [1 + e^{(0.953 \times \ln(\text{triglycerides}) + 0.139 \times BMI + 0.718 \times \ln(\gamma GTP) + 0.053 \times WC - 15.745)}] \times 100$. Estimated glomerular filtration rate (eGFR) was calculated by an equation for Japanese[28]. Homeostasis model assessment of insulin resistance (HOMA-R) was calculated as insulin (μU/mL) × glucose (mg/dL)/405[29]. Hemoglobin A1c (HbA1c) was expressed in National Glycohemoglobin Standardization Program (NGSP) scale.

A self-administered questionnaire survey was performed to obtain information on habits of current smoking and alcohol drinking. Hypertension was defined as systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg. Diabetes mellitus was defined as a combination of fasting plasma glucose ≥ 126 mg/dL and HbA1c ≥ 6.5%. Dyslipidemia was defined as LDL cholesterol ≥ 140 mg/dL, high-density lipoprotein (HDL) cholesterol <40 mg/dL or triglycerides ≥ 150 mg/dL. Hyperuricemia was defined as uric acid >7 mg/dL.

Statistical Analysis

Variables are presented as means ± standard deviations (SD) for normal distributions or medians (interquartile ranges) for skewed variables. Normality

Table 1. Characteristics of the recruited subjects without medication

	Total (n=282)	Male (n=126)	Female (n=156)	P
Age (years)	56±16	55±17	57±16	0.425
Body mass index	22.8±3.8	23.7±3.6	22.0±3.8	<0.001
Waist circumference (cm)	83.2±11.4	85.7±10.9	81.3±11.4	0.001
Systolic blood pressure (mmHg)	127±20	131±17	124±21	0.009
Diastolic blood pressure (mmHg)	75±11	76±10	73±11	0.031
Current smoking habit	68 (24.1)	40 (31.7)	28 (17.9)	0.007
Alcohol drinking habit	118 (41.8)	68 (54.0)	50 (32.1)	<0.001
Disease				
Hypertension	76 (27.0)	35 (27.8)	41 (26.3)	0.789
Diabetes mellitus	3 (1.1)	3 (2.4)	0 (0)	0.088
Dyslipidemia	128 (45.3)	53 (42.1)	75 (48.1)	0.337
Hyperuricemia	25 (8.9)	22 (17.5)	3 (1.9)	<0.001
Biochemical data				
AST (IU/L)	22 (19-26)	22 (20-27)	21 (18-25)	0.008
ALT (IU/L)	18 (14-24)	21 (16-29)	16 (13-20)	<0.001
γGTP (IU/L)	21 (15-32)	26 (20-39)	17 (14-27)	<0.001
FLI	13 (6-35)	25 (10-43)	9 (4-22)	<0.001
Iron (μg/dL)	107±41	116±42	100±39	0.002
Blood urea nitrogen (mg/dL)	15±4	15±4	15±4	0.091
Creatinine (mg/dL)	0.8 (0.7-0.9)	0.9 (0.8-0.9)	0.7 (0.6-0.8)	<0.001
eGFR (mL/min/1.73m^2)	73±15	76±16	71±14	0.004
Uric acid (mg/dL)	5.2±1.3	6.0±1.1	4.6±1.0	<0.001
Total cholesterol (mg/dL)	213±38	201±35	223±38	<0.001
LDL cholesterol (mg/dL)	125±34	118±31	132±35	0.001
HDL cholesterol (mg/dL)	63±17	56±15	70±16	<0.001
non-HDL cholesterol (mg/dL)	150±39	145±39	153±38	0.087
Triglycerides (mg/dL)	83 (60-116)	92 (65-148)	76 (54-107)	0.001
Fasting glucose (mg/dL)	89 (85-95)	92 (86-98)	89 (83-93)	0.001
Insulin (μU/mL)	8.4 (3.9-17.6)	9.6 (4.4-20.0)	7.3 (3.4-14.3)	0.036
HOMA-R	1.80 (0.88-4.03)	2.23 (1.05-4.49)	1.55 (0.84-3.32)	0.015
Hemoglobin A1c (%)	5.4 (5.1-5.6)	5.4 (5.2-5.6)	5.3 (5.1-5.6)	0.103
PCSK9 (ng/mL)	200±84	204±84	196±7	0.463
PCSK7 (ng/mL)	9.3±2.3	9.6±2.4	9.1±8.7	0.099

Variables are expressed as number (%), means±SD or medians (interquartile ranges).
AST, aspartate transaminase; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; FLI, fatty liver index; γGTP, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; PCSK7, proprotein convertase subtilisin/kexin type 7; PCSK9, proprotein convertase subtilisin/kexin type 9.

of each variable was tested by the Shapiro-Wilk W test. Comparisons between two groups for parametric and nonparametric parameters were performed by Student's t-test and the Mann-Whitney U test, respectively. The chi-square test was performed for intergroup differences in percentages of parameters. Pearson's correlation analysis was performed for the correlation between two variables. Non-normally distributed variables were logarithmically transformed for regression analyses. Multivariable regression analyses were performed to identify independent links between PCSK7 and the variables with a significant correlation after adjustment of age, sex and current smoking habit, showing the standardized regression coefficient (β), adjusted R^2 and Akaike's information criterion (AIC). Statistical significance was determined as a p value <0.05. All data were analyzed by using JMP15.2.1 for Macintosh (SAS Institute, Cary, NC).

Results

Basal Characteristics of the Studied Subjects

Basal characteristics of the 282 recruited subjects without medication (male/female: 126/156) are shown in **Table 1**. The numbers of subjects with habits of current smoking and alcohol drinking were

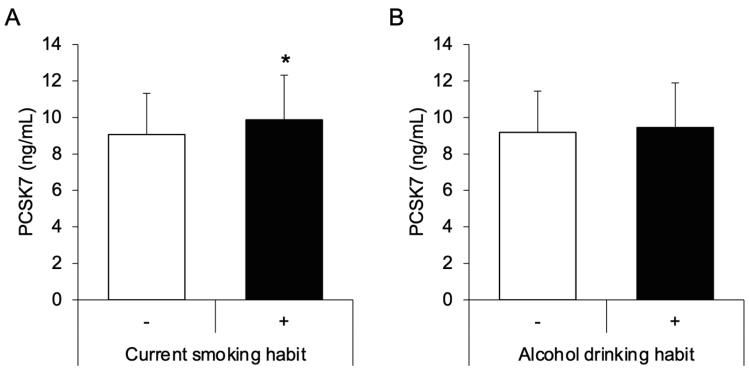

Fig. 1. Comparisons of PCSK7 level by habits of smoking and alcohol drinking

A, B. Comparisons of proprotein convertase subtilisin/kexin type 7 (PCSK7) in subjects with and those without habits of current smoking (-/+: 214/68) (A) and alcohol drinking (-/+: 164/118) (B) shown by box plots. Variables are expressed as means ± SD. $^{*}P<0.05$.

68 (24.1%) and 118 (41.8%), respectively. Hypertension, diabetes mellitus, dyslipidemia and hyperuricemia were found in 76, 3, 128, and 25 subjects, respectively. Male subjects had significantly larger BMI and WC, significantly higher frequencies of habits of current smoking and alcohol drinking, and higher levels of systolic and diastolic blood pressures, iron, liver enzymes, levels of uric acid triglycerides and parameters of renal function and glucose metabolism except for HbA1c than did female subjects. Levels of total cholesterol, LDL cholesterol and HDL cholesterol were significantly lower in male subjects than in female subjects. No significant difference in PCSK7 or PCSK9 was found between male and female subjects.

Plasma PCSK7 level was significantly higher in subjects with a current smoking habit than in those without the habit **(Fig. 1A)**. There was no significant difference in PCSK7 level between subjects with and those without an alcohol drinking habit **(Fig. 1B)**.

Correlation and Multivariable Regression Analyses for PCSK7 Level

As shown in **Table 2**, plasma PCSK7 concentration was negatively correlated with age **(Fig. 2A)** and blood urea nitrogen and was positively correlated with BMI **(Fig. 2B)** and levels of alanine transaminase, γGTP, triglycerides **(Fig. 2C)** and FLI **(Fig. 2D)**. When male and female subjects were separately analyzed, similar correlations between PCSK7 level and the parameters except for age were found in males but not in females **(Table 2)**. There were no significant correlations of PCSK7 level with levels of iron and PCSK9.

Multivariable regression analyses after adjustment of age, sex and current smoking habit showed that plasma PCSK7 concentration was independently associated with BMI ($\beta=0.130$, $P=0.035$) (Model 1, $R^2=0.091$, AIC: 1175.1), triglycerides ($\beta=0.141$, $P=0.027$) (Model 2, $R^2=0.093$, AIC: 1174.7) or FLI ($\beta=0.139$, $P=0.030$) (Model 3, $R^2=0.092$, AIC: 1174.8) **(Table 3)**.

Discussion

The present study showed for the first time that circulating PCSK7 level was independently associated with FLI as a noninvasive and simple predictor of NAFLD including BMI and triglycerides level after adjustment of age, sex and current smoking habit in a general population who had not taken any medication. PCSK7 is a transmembrane-anchored enzyme and is not shed or secreted from the cell[30]. However, prosegment PCSK7, an inhibitor of full PCSK7, is secreted from the cell[13]. The catalytic site of PCSKs is masked by the prosegment, which is dissociated by cleavage when PCSKs reach the trans-Golgi network[1, 2]. The dissociation of the prosegment to the PCSK core enables the enzyme to exert its catalytic activities[1, 2, 14]. The ELISA kit for PCSK7 used in present study may be able to detect prosegment PCSK7, though there is no information about the epitope of PCSK7 for the antibody. Circulating PCSK7 concentration has recently been reported to be positively correlated with triglycerides level in a small number of patients at risk for NAFLD ($n=72$)[24]. Although extracellular effects of the prosegment of PCSK7 remain unclear, the circulating level of PCSK7, a possibly prosegment form, may reflect PCSK7 activity in one part of or the whole body. The relatively modest correlations of circulating PCSK7 level with BMI, triglycerides level and FLI in the present study suggest that circulating PCSK7 level provides a limited indication of intracellular PCSK7 activity. Therefore, the findings in the present study may indicate a link between

Table 2. Correlation analyses for PCSK7

	Total (n=282)		Male (n=126)		Female (n=156)	
	r	P	r	P	r	P
Age	-0.233	<0.001	-0.260	0.003	-0.203	0.011
Body mass index	0.150	0.012	0.243	0.006	0.044	0.588
Waist circumference	0.101	0.091	0.136	0.128	0.041	0.609
Systolic blood pressure	0.002	0.973	0.174	0.052	-0.143	0.076
Diastolic blood pressure	0.091	0.130	0.143	0.112	0.030	0.714
log AST	-0.013	0.823	0.044	0.626	-0.118	0.143
log ALT	0.133	0.026	0.209	0.019	-0.014	0.859
log γGTP	0.130	0.029	0.127	0.156	0.086	0.284
log FLI	0.150	0.012	0.176	0.049	0.090	0.264
Iron	0.053	0.410	0.097	0.310	-0.032	0.711
Blood urea nitrogen	-0.132	0.026	-0.077	0.393	-0.204	0.011
log Creatinine	0.092	0.122	0.133	0.139	-0.048	0.553
eGFR	0.086	0.148	0.016	0.862	0.125	0.121
Uric acid	0.094	0.114	0.140	0.117	-0.036	0.659
Total cholesterol	-0.011	0.851	0.138	0.123	-0.075	0.352
LDL cholesterol	-0.031	0.603	0.060	0.507	-0.064	0.425
HDL cholesterol	-0.100	0.094	-0.026	0.771	-0.098	0.223
non-HDL cholesterol	0.033	0.578	0.135	0.132	-0.034	0.673
log Triglycerides	0.162	0.006	0.201	0.024	0.081	0.316
log Fasting glucose	-0.002	0.732	-0.042	0.642	-0.043	0.595
log Insulin	0.106	0.083	0.184	0.044	0.020	0.814
log HOMA-R	0.100	0.101	0.171	0.061	0.015	0.857
log Hemoglobin A1c	0.024	0.692	-0.005	0.960	0.036	0.657
PCSK9	0.008	0.890	-0.025	0.785	0.028	0.728

AST, aspartate transaminase; ALT, alanine transaminase; eGFR, estimated glomerular filtration rate; FLI, fatty liver index; γGTP, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; HOMA-R, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein; PCSK7, proprotein convertase subtilisin/kexin type 7; PCSK9, proprotein convertase subtilisin/kexin type 9.

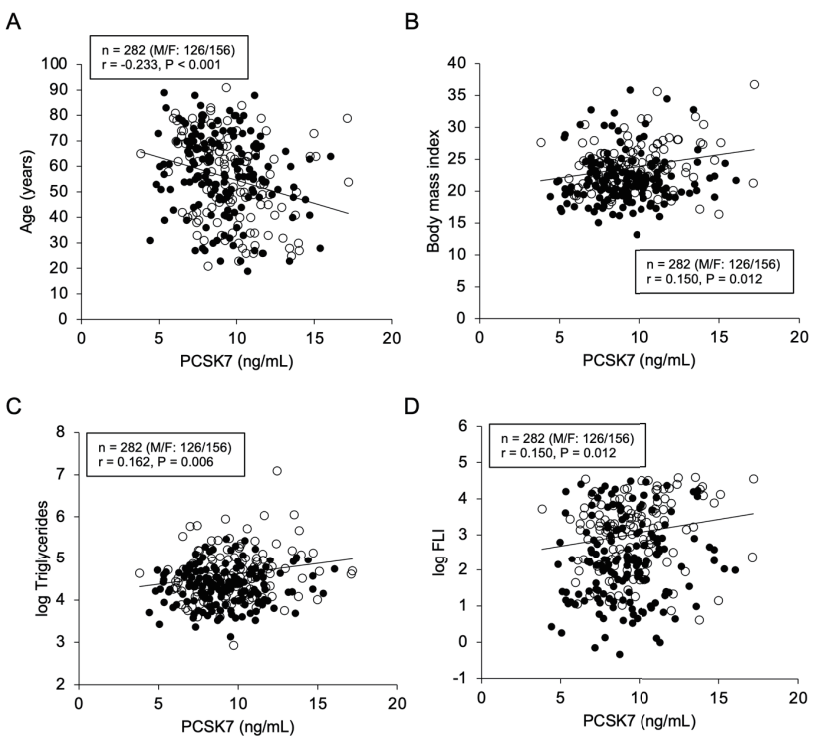

Fig. 2. Correlations of PCSK7 level with parameters

A-F. Age (A), body mass index (B), logarithmically transformed (log) triglycerides (C) and log fatty liver index (FLI) (D) were plotted against PCSK7 level in each subject (n=282). Open circles: males (n=126), closed circles: females (n=156).

Table 3. Multivariable regression analyses for PCSK7

	Model 1		Model 2		Model 3	
	β	*P*	*β*	*P*	*β*	*P*
Age	-0.192	0.002	-0.224	<0.001	-0.212	<0.001
Sex (Male)	0.067	0.279	0.064	0.299	0.051	0.424
Current smoking habit	0.091	0.148	0.057	0.375	0.076	0.226
Body mass index	0.130	0.035	-	-	-	-
log Triglycerides	-	-	0.141	0.027	-	-
log FLI	-	-	-	-	0.139	0.030
R^2	0.091		0.093		0.092	
AIC	1175.1		1174.7		1174.8	

AIC, Akaike's information criterion; FLI, fatty liver index; PCSK7, proprotein convertase subtilisin/kexin type 7.

PCSK7 level, possibly reflecting the activity of PCSK7, and the phenotype of NAFLD including obesity and hypertriglyceridemia.

The locus of the human PCSK7 gene is on chromosome 11q23.3 [1, 2] close to the gene cluster APOA5/APOA4/APOC3/APOA1, a region implicated in the regulation of lipoprotein metabolism[31, 32]. Multiple susceptibility loci at chromosome 11q23.3 have been reported to be associated with plasma triglyceride level in East Asians[15]. Furthermore, variants in the human PCSK7 gene were found to be associated with lipid parameters including levels of triglycerides, HDL cholesterol and small dense LDL cholesterol[16, 17, 33] as well as the risk of cardiovascular disease[34]. It has been reported that PCSK7 promotes degradation of apoprotein A-V, an indirect activator of lipoprotein lipase, by endoplasmic reticulum-lysosomal communication, resulting in an elevated level of triglycerides[35, 36]. In addition, PCSK7 can redundantly cleave and activate angiopoietin-like protein 4 (AGPTL4), a lipoprotein lipase inhibitor[37]. PCSK7-mediated cleavage of AGPTL4, which then acquires the ability to inhibit lipoprotein lipase, may impair the clearance of triglycerides. Furthermore, hepatic expression of PCSK7 was reported to be correlated with the expression of de novo lipogenesis genes, sterol regulatory element-binding transcription factor 1c and fatty acid synthase[24]. There might be several mechanisms of the involvement of PCSK7 with triglycerides level, though we could not address those factors including apolipoprotein A-V, ANGPTL4, lipoprotein lipase, and de novo lipogenesis genes in the present study.

NAFLD has been highlighted as a lifestyle-related disease that is associated with obesity, metabolic syndrome, type 2 diabetes mellitus, insulin resistance, dyslipidemia and cardiovascular disease[22, 23]. Diagnosis of NAFLD has required liver biopsy[38], but several noninvasive procedures have recently been established using imaging tools and biochemical indices including FLI[39]. FLI is a noninvasive and simple biomarker for diagnosis of NAFLD and is calculated by using BMI, WC and levels of γGTP and triglycerides[25]. It has been reported that FLI can predict not only NAFLD but also other metabolic-related diseases including diabetes mellitus[40-43], hypertension[44-47] and chronic kidney disease[48-51]. Dysregulation of iron metabolism represents another typical feature of NAFLD associated with more severe hepatic and cardio-metabolic damage[52]. "Dysmetabolic hyperferritinemia" is most frequently observed in patients with risk factors for iron accumulation and tracks with increased expression of iron transporters induced by excess fatty acids, but the underlying mechanism remains largely unexplained[52, 53]. It has been reported that PCSK7 is implicated in iron metabolism including TfR1 processing[21] and that variants in the PCSK7 gene are associated with soluble TfR1 level[20]. Although we could not investigate the association of PCSK7 with levels of soluble TfR1 and ferritin, there was no significant correlation between levels of PCSK7 and iron in the present study. Further investigations are needed to clarify the association between circulating PCSK7 level and iron metabolism including soluble TfR1 level in patients with NAFLD at several grades of severity.

It has been reported that PCSK7 knockout mice had no apparent pathological phenotype[54-56], though they exhibited anxiolytic and novelty-seeking behavior in a later study[54-56]. However, there has been no study focusing on lipid metabolism using a PCSK7-deficient model[56]. On the other hand, PCSK7 transgenic mice were reported to have a significantly higher level of triglycerides than that in control mice[17]. Additional *in vitro* and animal studies using PCSK7 transgenic and deficient models are needed to investigate the potential role of PCSK7 in obesity, dyslipidemia and chronic liver disease.

Drug development and clinical trials for treatment of NAFLD are currently being conducted worldwide[57]. Modulation of PCSK7 is a possibly novel therapeutic strategy for NAFLD. Several strategies aimed at inhibiting the activity of PCSKs, including the design of competitive inhibitors and interference with mRNA transcription, have been extensively investigated to abolish or minimize the proteolytic activation of these substrates[2, 58]. Not only modification of conventional risk factors of NAFLD but also intervention for NAFLD by direct and indirect regulation of PCSK7 might contribute to the prevention of NAFLD. Reduction of PCSK7 level by treatment for NAFLD might be beneficial for pathogenesis of the NAFLD-related phenotype including lipid metabolism and liver damage during iron overload. Further investigations are needed to determine whether PCSK7-guided prevention and/or treatment of NAFLD reduce the number of individuals with deterioration of NAFLD.

The present study has several limitations. First, the results of the present study do not prove causal relations between PCSK7 and correlated biomarkers because this was a cross-sectional study. A longitudinal study and interventional study are needed to clarify what underlies the relationship. Second, since only Japanese people were enrolled, the results of the present study might not correspond to other races. Lastly, circulating PCSK7 would be a prosegment form of PCSK7 but not a mature form of PCSK7, though information about the epitope of PCSK7 for the antibody in the ELISA kit for PCSK7 was not available from the company. This issue should be addressed in future studies. Furthermore, activity of PCSK7 in the cell was not investigated in the present study. Further investigation is needed to determine whether prosegment PCSK7 reflects activity of PCSK7 in the cell.

In conclusion, a high circulating plasma concentration of PCSK7 is independently associated with chronic liver disease including obesity and elevated triglycerides level in a general population of individuals who had not regularly taken any medications. A further understanding of the possible associations of PCSK7 with obesity, dyslipidemia and chronic liver disease may enable the development of novel therapies for NAFLD and its related metabolic and cardiovascular diseases.

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Disclosure

The authors declare that they have no competing interests.

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