in vivo *38*: 785-793 (2024)
doi:10.21873/invivo.13502

Serum CCDC25 Levels as a Potential Marker for Metabolic Syndrome

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Abstract. *Background/Aim: Metabolic syndrome (MetS) stands as a significant risk for developing various severe health problems. Therefore, the discovery of biomarkers capable of predicting the progression of metabolic conditions is crucial for improving overall health outcomes. Recently, we reported that coiled-coil domain containing 25 (CCDC25) might be associated with key proteins involved in metabolic pathways, by bioinformatics analysis. Thus, we assumed that serum CCDC25 levels might have an association with MetS status. Patients and Methods: In this study, based on the modified National Cholesterol Education Program-Adult Treatment Panel III (modified NCEP-ATP III) criteria, the participants who had three or more of abnormal criteria were defined as MetS, and those who had 1 or 2 abnormal criteria as pre-MetS groups; those who had no abnormal criteria were classified as the healthy control (HC) group. Serum CCDC25 levels were measured using the dot blot assay. Results: The results showed that serum CCDC25 levels of the MetS group (0.072±0.026 ng/μl) were significantly higher (p<0.001) than that of pre-MetS (0.031±0.011 ng/μl) or HC groups (0.018±0.007 ng/μl). We can discern a consistent trend indicating that serum CCDC25 level is well correlated with the number of abnormal criteria of MetS of each participant. Although serum CCDC25 levels correlated with the distribution of all 5 MetS criteria, the highest correlation was seen in serum CCDC25 levels and triglyceride (TG) levels, with r=0.563, followed by systolic blood pressure (SBP) levels (r=0.557) and high-density lipoprotein-cholesterol (HDL-C) levels (r=–0.545). Conclusion: CCDC25 showed correlations with all MetS parameters, particularly with TG, SBP, and HDL-C. This prompts speculation that heightened CCDC25 levels may indicate the development and/or progression of those MetS-associated diseases.*

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Key Words: CCDC25, coiled-coil domain containing 25, serum level, metabolic syndrome.

Metabolic syndrome (MetS) is a cluster of interconnected conditions that significantly raise the risk of developing severe health problems (1-4). MetS is defined based on the modified National Cholesterol Education Program-Adult Treatment Panel III (modified NCEP-ATP III) guidelines, and the individuals having three or more of the following five criteria are diagnosed as having MetS; abdominal obesity (BMI ≥27 kg/m^2 in males or ≥25 kg/m^2 in females) (5, 6), high blood pressure (BP ≥130/85 mmHg), elevated fasting blood glucose levels (FBG ≥100 mg/dl), increased triglyceride levels (TG ≥150 mg/dl), and decreased levels of high-density lipoprotein cholesterol (HDL-C <40 mg/dl in males or <50 mg/dl in females) (2). The prevalence of MetS ranges from 12% to 37% in the Asian population, although it varies depending on the criteria and regions (7). As mentioned above, MetS serves as a significant risk factor for non-communicable diseases (NCDs), including chronic disease (8), type 2 diabetes mellitus (9, 10), cardiovascular

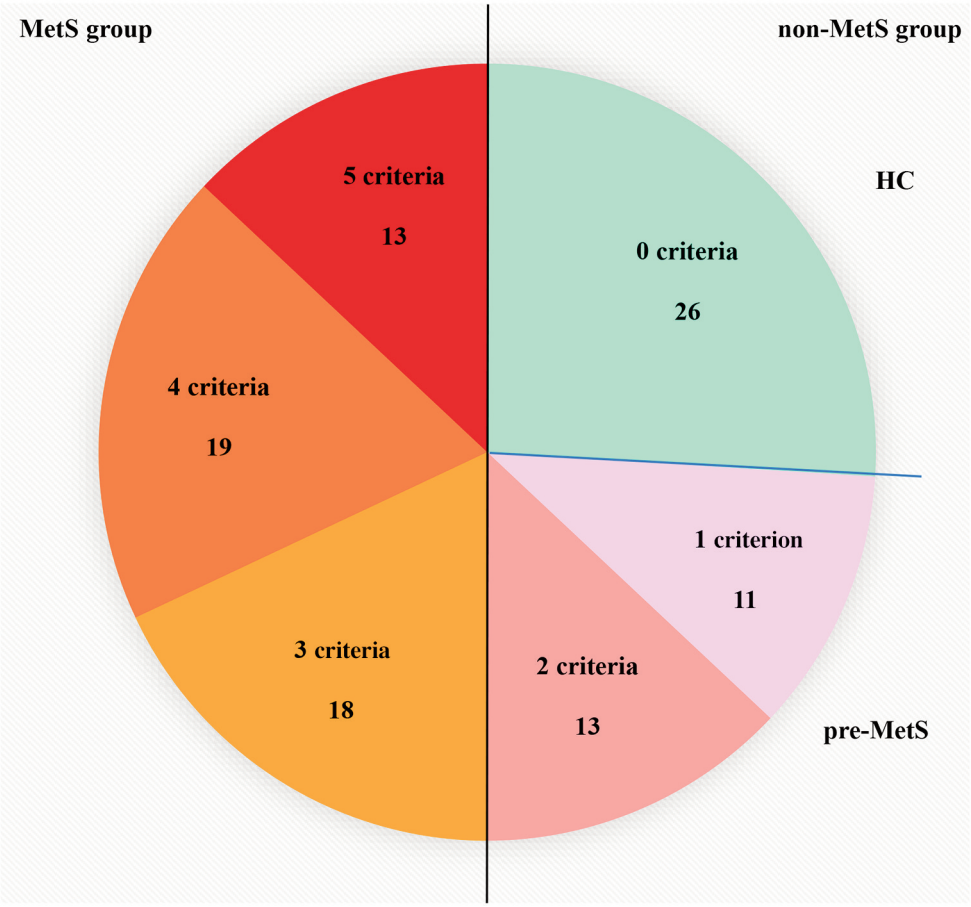

Figure 1. *Distribution of participants with various degrees of metabolic parameters. There were 100 participants in total: non-MetS (n=50); HC (n=26); pre-MetS (n=24); MetS (n=50). non-MetS, Non-metabolic syndrome; MetS, metabolic syndrome; HC, healthy control; pre-MetS, pre-metabolic syndrome.*

disease (9, 11), and stroke (12, 13). Some studies have also suggested a potential link between MetS and an increased risk of cancer (14, 15). Development and progression of MetS is associated with dysregulation of metabolic pathways and increases in the risk of complications (16, 17).

Coiled-coil domain containing 25 (CCDC25), a protein identified by genome analysis, is composed of 208 amino acids with a molecular weight of approximately 25 kDa, and its coding gene is located on human chromosome 8p21.1 (18). Although its precise biological function is not well understood, CCDC25 is predicted to localize in the nucleus. In contrast, CCDC25 is present on the cell surface membrane of breast and colon cancer cells as a target molecule of neutrophil extracellular traps (NETs) and is involved in tumor metastasis (19, 20). Apart from cellular CCDC25, our group showed significant overexpression of CCDC25 in cholangiocarcinoma (CCA) tissue (21), and in the sera of CCA patients (22), suggesting its potential as a biomarker for diagnosis of CCA. The diagnostic potential of CCDC25 for CCA was signified further by our discovery of significantly higher elevation of CCDC25 levels in the sera of CCA patients compared to that of the other prevalent cancer patients (23). Related to our study, using microarray analysis, potential diagnostic, and prognostic value of CCDC25 expression was reported for hepatocellular carcinoma (HCC) (24). During our series of study on the biological roles of CCDC25 in CCA, bioinformatic analysis of CCDC25 signaling pathway revealed its direct and/or indirect association with metabolic-related proteins, such as epidermal growth factor receptor (EGFR) (21, 25), mammalian target of rapamycin (mTOR), transforming growth factor beta 1 (TGFβ1), and vascular endothelial growth factor (VEGF) (25), all of which are key players in metabolic pathways (26, 27). Thus, in this study, we explored whether serum CCDC25 levels are related to MetS.

Table I. *Metabolic characteristics of the HC, pre-MetS, and MetS groups.*

Parameters	Criteria	HC (n=26)	pre-MetS (n=24)	MetS (n=50)	*p*-Value
BMI (kg/m^2)	≥27.00 (M)	20.75±1.25 (19.10-24.20)	22.00±2.38 (17.40-26.60)	27.25±2.35 (20.50-33.50)	**<0.001**
	≥25.00 (F)	20.95±1.65 (19.10-24.80)	23.50±1.85 (17.90-34.70)	26.95±2.10 (17.40-37.40)	
SBP (mmHg)	≥130.00	121.50±4.50 (100.00-129.00)	136.50±10.38 (113.00-189.00)	146.00±9.63 (110.00-188.00)	**<0.001**
DBP (mmHg)	≥85.00	72.50±4.63 (62.00-83.00)	78.00±6.63 (64.00-99.00)	81.50±6.38 (59.00-106.00)	**<0.001**
FBG (mg/dl)	≥100.00	83.00±3.00 (69.00-95.00)	94.50±12.88 (70.00-219.00)	127.50±43.13 (82.00-348.00)	**<0.001**
TG (mg/dl)	≥150.00	77.50±10.50 (41.00-147.00)	105.00±21.75 (59.00-344.00)	188.50±34.25 (69.00-314.00)	**<0.001**
HDL-C (mg/dl)	<40.00 (M)	56.50±6.00 (45.00-74.00)	57.00±14.00 (33.00-91.00)	38.50±5.50 (20.00-58.00)	**<0.001**
	<50.00 (F)	64.00±5.88 (51.00-83.00)	60.00±6.00 (43.00-74.00)	43.00±6.00 (26.00-75.00)	

Data are presented as the median with quartile deviation (QD) and min, minimum to max, maximum. Values in bold indicate statistical significance, calculated using the Kruskal-Wallis H test. HC, healthy control; pre-MetS, pre-metabolic syndrome; MetS, metabolic syndrome; M, male; F, female; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol.

Patients and Methods

Sample size calculation. In this study, G*Power software version 3.1.9.4 was used to calculate the necessary sample sizes (28). Fifty sera from non-metabolic syndrome (non-MetS) and metabolic syndrome (MetS) individuals ensured a statistical power of 100% within each group.

Sample collection. This study was approved by the Human Ethics Committee, Khon Kaen University, with the approval number of HE652170. Fifty sera from non-MetS and MetS individuals were randomly selected from the leftover sera from individuals that underwent annual health check-up at the Faculty of Associated Medical Sciences (AMS-KKU Excellence Laboratory), Khon Kaen University, Thailand. All sera were collected from participants on November 12, 2021. The samples were stored at –20˚C before being analyzed. The clinical and laboratory examination data extracted from the individual records were the following: body mass index (BMI) and systolic (SBP) and diastolic (DBP) blood pressures, fasting blood glucose (FBG), glycosylated hemoglobin (HbA1c), cholesterol, triglyceride (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen (BUN), creatinine, estimated glomerular filtration rate (eGFR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP).

Metabolic syndrome criteria. In this study, the participants were divided into non-MetS and MetS groups according to the modified NCEP-ATP III criteria, using BMI instead of abdominal obesity. The criteria used in this study were as follows: BMI: male ≥27 kg/m^2 or female ≥25 kg/m^2 (5, 6), SBP ≥130 mmHg and/or DBP ≥85 mmHg, FBG ≥100 mg/dl, TG ≥150 mg/dl, HDL-C: male <40 mg/dl or female <50 mg/dl (2). Individuals who met three or more of these criteria were categorized as having MetS, and those having less than three criteria were grouped as non-MetS (2). Then, the non-MetS group was further divided into two groups: those having no abnormalities in MetS criteria as healthy controls (HC), and those having one or two criteria as the pre-metabolic syndrome (pre-MetS) group.

Dot blot assay for serum CCDC25 levels. To create a standard curve, serum standard 0.193 ng/μl concentration was serially diluted two-fold at 0.096, 0.048, 0.024, 0.012, and 0.006 ng/μl, respectively. Pooled serum (*n*=40) was used as a positive control for intensity normalization (23). To calculate the relative intensity of each sample spot, we compared it to the positive control as described previously (29). A nitrocellulose membrane (GE Healthcare Life Sciences, Little Chalfont, UK) was placed on the Bio-Dot Microfiltration apparatus (Bio-Rad Laboratories, Inc., Hercules, CA, USA) at room temperature after being immersed in 1X Tris-buffered saline with 0.1% Tween-20 (1X TBS-T). Two microliters of positive control serum and each undiluted sera of the participants were spotted onto the membrane. To prevent non-specific binding, the membrane was incubated with 5% skimmed milk in 1X TBS-T at room temperature for 1 hr. The membrane was then exposed to 1:1000 dilution of rabbit polyclonal primary antibody against human CCDC25 (Cat. No. Orb2517; Biorbyt, Cambridge, UK) for overnight incubation at 4˚C. After washing with 1X TBS-T, 1:2000 dilution of horseradish peroxidase-conjugated goat anti-rabbit IgG secondary antibody (Cat. No. ab7083; Biorbyt) was applied to the membrane. The membrane was then incubated with this solution at room temperature for 1 hr before being rinsed with 1X TBS-T. The chemiluminescent pattern was identified and measured using the improved chemiluminescence plus reagent (GE Healthcare Life Sciences) and the Amersham imager 600 (GE Healthcare Life Sciences).

Table II. *Clinical parameters of the HC, pre-MetS, and MetS groups.*

Parameters	Normal range	HC (n=26)	pre-MetS (n=24)	MetS (n=50)	*p*-Value
Age (years)	–	52.00±8.25 (36.00-71.00)	57.50±4.25 (35.00-69.00)	58.50±5.00 (35.00-74.00)	**0.034**
Sex n (%)	Female Male	16 (16.00) 10 (10.00)	13 (13.00) 11 (11.00)	32 (32.00) 18 (18.00)	0.718
HbA1c (%)	<6.50	6.40±0.43 (4.80-7.20)	7.30±0.89 (6.20-12.50)	8.30±2.09 (4.80-17.70)	**<0.001**
TC (mg/dl)	<200.00	181.50±28.38 (116.00-333.00)	243.00±26.50 (134.00-341.00)	215.00±40.13 (93.00-352.00)	**<0.001**
LDL-C (mg/dl)	<100.00	106.50±11.75 (38.00-223.00)	151.00±31.38 (51.00-234.00)	129.50±28.50 (37.00-266.00)	**<0.001**
BUN (mg/dl)	5.80-19.10	13.00±2.00 (5.00-21.00)	13.50±1.75 (6.00-19.00)	14.00±2.38 (5.00-37.00)	0.053
Creatinine (mg/dl)	0.50-1.50	0.80±1.00 (0.50-1.00)	0.70±0.15 (0.30-1.00)	0.75±0.10 (0.40-1.40)	0.713
eGFR (ml/min/1.73 m^2)	≥90.00	99.24±9.12 (70.21-163.31)	99.94±10.81 (80.31-268.75)	99.00±16.14 (49.84-221.52)	0.555
AST (U/l)	12.00-32.00	27.00±5.75 (16.00-39.00)	26.50±5.75 (16.00-100.00)	26.00±6.00 (16.00-92.00)	0.788
ALT (U/l)	4.00-36.00	18.00±4.75 (7.00-45.00)	22.00±4.50 (10.00-61.00)	21.50±6.50 (8.00-86.00)	0.120
ALP (U/l)	37.00-147.00	55.50±9.75 (37.00-87.00)	69.00±7.88 (48.00-106.00)	76.00±17.50 (32.00-147.00)	**0.004**

Data are presented as the median with quartile deviation (QD) and min-max in brackets, except for sex. Values in bold indicate statistical significance, calculated using the Kruskal-Wallis H test (and chi-square for sex). HbA1c, glycosylated hemoglobin; TC, total cholesterol, LDL-C, low-density lipoprotein cholesterol; BUN, blood urea nitrogen; eGFR, estimated glomerular filtration rate; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

Statistical analysis. To assess the distribution of the data, we opted to utilize the Kolmogorov-Smirnov test. Due to the non-normal distribution of the acquired data, the information is presented as median±quartile deviation (QD). The Mann-Whitney *U*-test and the Kruskal-Wallis H test were used to compare the data sets of HC, pre-MetS, and MetS groups. The chi-square test was used to calculate the differences of proportion of age. The Spearman correlation coefficient was used to calculate the correlation between CCDC25 and metabolic parameters. All statistical analyses were carried out using GraphPad Prism software (ver. 8.0.2 GraphPad Software Inc., La Jolla, CA, USA) and SPSS software (ver. 28.0.1.0.; SPSS, Inc., IBM, Armonk, NY, USA). $p<0.05$ was considered a statistically significant difference.

Results

Baseline metabolic characteristics of the study groups. In this study, 50 participants in each of the non-MetS and MetS groups were categorized according to the modified NCEP-ATP III criteria. The non-MetS group consisted of 26 healthy controls (HC) did not match filter criteria and 24 pre-MetS participants who met one ($n=11$) or two ($n=13$) criteria. In the MetS group, 18 had three, 19 had four, and 13 had five criteria (Figure 1). Subsequently, data analyses were made based on the three groups: HC, pre-MetS and MetS groups. The baseline metabolic characteristics of HC, pre-MetS and MetS groups are given in Table I. Among the HC, pre-MetS and MetS groups, a significant difference ($p<0.001$) was observed in the median of the BMI, SBP, DBP, FBG, TG, and HDL-C levels.

Clinical parameters of the study groups. The clinical parameters of the HC, pre-MetS, and MetS groups are given in Table II. The median values of HbA1c, TC, and LDL-C in pre-MetS and MetS groups were much higher ($p<0.001$) than that of the HC group. Statistically significant differences among the groups were also observed in the median of the age ($p<0.05$) and the median of the ALP value ($p<0.01$).

Serum CCDC25 levels and MetS status. Serum CCDC25 levels of HC, pre-MetS, and MetS groups are given in Figure 2. Serum CCDC25 levels of the MetS group [0.072±0.026 (0.019-0.178) ng/μl] was significantly higher ($p<0.001$) than that of the pre-MetS [0.031±0.011 (0.013-0.072) ng/μl], and the HC group [0.018±0.007 (0.007-0.036) ng/μl], as shown in Figure 2A. When examining the comparison between serum CCDC25 levels and the degree of matching MetS criteria, a significant difference was observed for up to three matching criteria (Figure 2B).

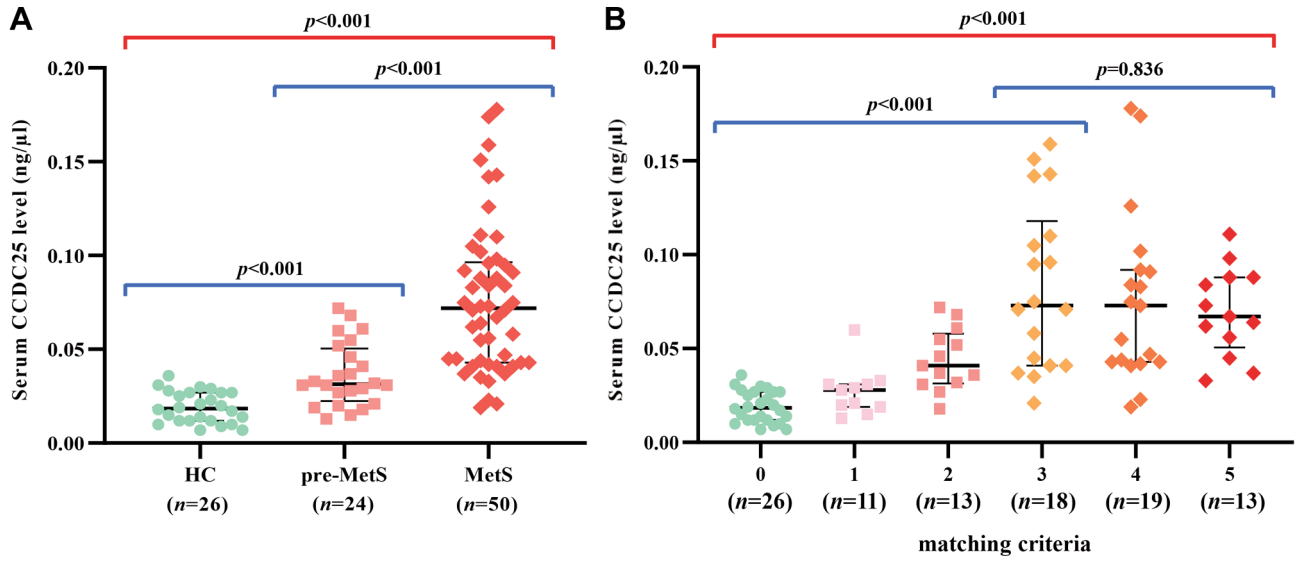

Figure 2. *(A) Serum CCDC25 levels of HC, pre-MetS and MetS. (B) Correlation between serum CCDC25 levels and MetS matching criteria. Statistical difference between the groups was examined using the Mann-Whitney U-test and Kruskal-Wallis H test. HC, healthy control; pre-MetS, pre-metabolic syndrome; MetS, metabolic syndrome groups; CCDC25, coiled-coil domain containing 25.*

However, in the MetS group, serum CCDC25 levels showed no significant variation, irrespective of the matching criteria for the degree of MetS.

Correlation between serum CCDC25 levels and each MetS criterion. The correlation between serum CCDC25 level and each MetS criterion of all samples (n=100) was analyzed using Spearman's correlation coefficient. The results are shown in Figure 3. Apparently, a significant positive correlation was observed between CCDC25 and BMI, SBP, DBP, FBG, and TG, while a significant negative correlation was observed between CCDC25 and HDL-C levels. Additionally, in the MetS group (shown as red circles in Figure 3) showed significant positive correlation between high CCDC25 with TG levels (r=0.502, p=0.029), while CCDC25 levels did not correlate with BMI, SBP, DBP, FBG, and HDL-C levels. Moreover, in 50 cases of the MetS groups, 42 samples (84%) had high levels of TG. In 42 samples of MetS with high TG levels, 23 samples (46%) had low CCDC25 levels, and 19 samples (38%) had high CCDC25 levels. Thus, CCDC25 in MetS requires further studies using a large sample size with a follow-up study.

Discussion

MetS is a condition that increases the risk of developing severe diseases (8-13). Therefore, efficient monitoring of MetS is critical in clinical supervision and in controlling severe conditions (30, 31). Since MetS is diagnosed by complicated criteria of multiple laboratory data sets, there exists a necessity to identify simple biomarkers capable of serving as indicators for individuals with MetS that put at high risk for cancer and other diseases (32). In the present study, we first demonstrate elevated serum CCDC25 levels in MetS compared to pre-MetS and HC groups. In our previous study, we found that high serum CCDC25 levels can be a biomarker for CCA (23). In this study, we demonstrated also that serum CCDC25 levels were elevated in MetS individuals. However, when serum CCDC25 levels in CCA and MetS were compared, its level in MetS was far lower than that in CCA (23). In the case of CCA, CCDC25 is produced by cancer cells (21, 25). In MetS, the source of CCDC25 remains unclear. Further study is required as to identify the source and mechanism of the elevation of serum CCDC25 in MetS. Furthermore, our findings revealed the observation of divergent trends across the groups categorized by CCDC25 levels and provided intriguing insights into the potential roles of CCDC25 in different metabolic processes. MetS is characterized by a constellation of metabolic abnormalities, including central obesity, insulin resistance, hypertension, and dyslipidemia (33). Elevated TG levels are a hallmark of dyslipidemia, often found in individuals with MetS (34) and increased risk of cardiovascular diseases (35). In this research, serum CCDC25 levels had positive correlation not only with TG but also with SBP. Moreover, it correlated negatively with HDL-C. Thus, it could be speculated that CCDC25 might play critical roles in lipid metabolism and associated vascular diseases. Since *de novo* lipogenesis in the liver contributes to the elevation of TG (36,

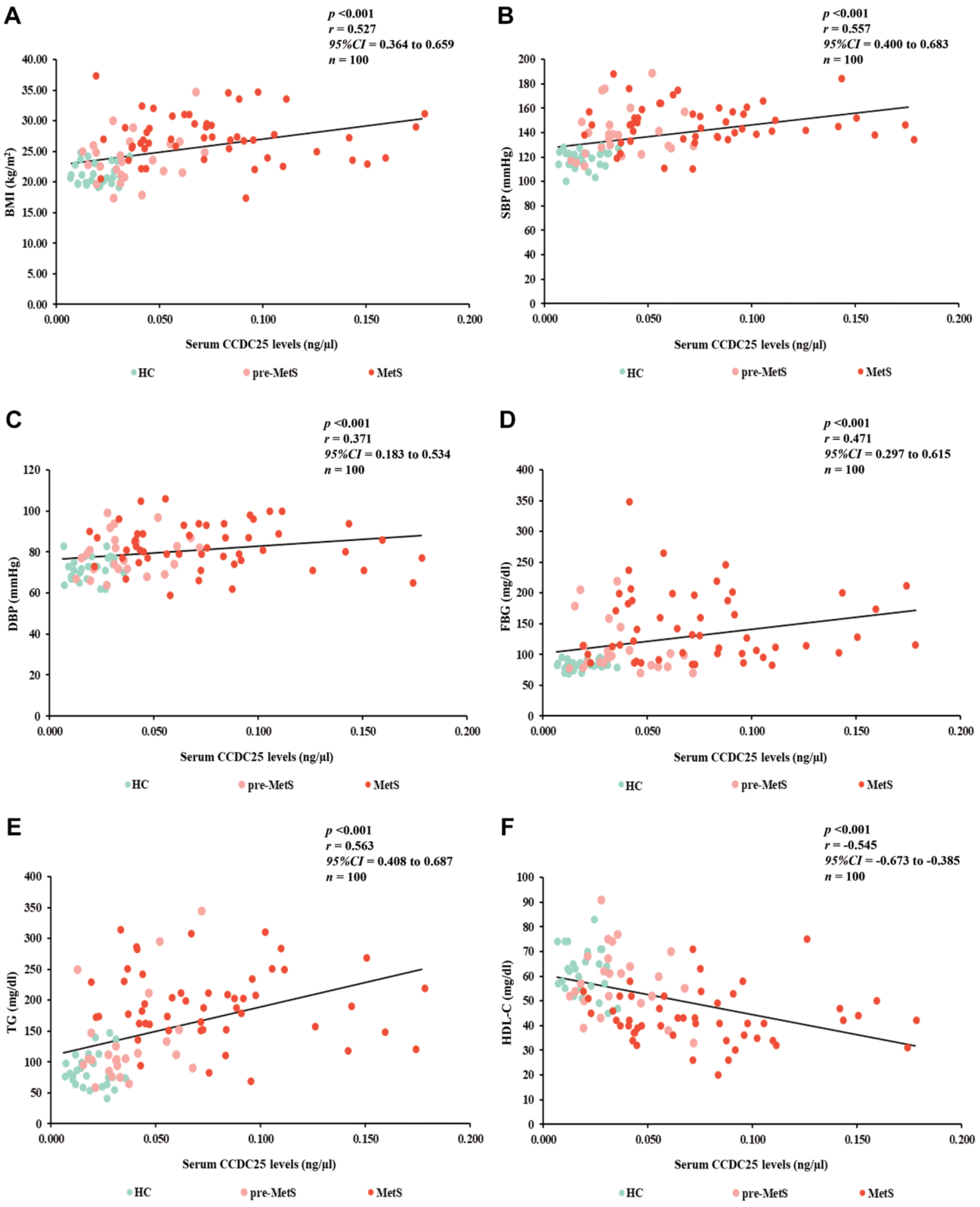

Figure 3. *Correlation between CCDC25 and clinical parameters. Spearman Correlation analysis between CCDC25, Coiled-coil domain containing 25 and (A) BMI, body mass index; (B) SBP, systolic blood pressure; (C) DBP, diastolic blood pressure; (D) FBG, fasting blood glucose; (E) TG, triglyceride and (F) HDL-C, high-density lipoprotein cholesterol.*

37) and cholesterol levels (37), and since CCDC25 expression on cancer cell surface is positively regulated by *de novo* cholesterol biosynthesis (38), we hypothesized that CCDC25 in circulation may also regulate lipid metabolism (39, 40). MetS is a risk factor for various diseases, such as Type 2 diabetes (1-4), cardiovascular diseases (41), chronic kidney diseases (1-4) or even cancer (42, 43). Besides, CCDC25 is regarded as a prominent indicator of CCA (21-23) and displays a substantial correlation with high TG levels in MetS, as shown in this study. Moreover, elevated TG levels, a constituent of MetS, may play a role in promoting oxidative stress, as suggested by Danciu *et al*. in 2023 (44). The dysregulation of cellular processes linked to MetS and oxidative stress could potentially impact CCDC25 levels. This prompts speculation that heightened CCDC25 levels may indeed indicate the development/ progression of those MetS-associated diseases. Moreover, our findings indicate a significant correlation between heightened CCDC25 levels and TG. Hence, individuals diagnosed with MetS and exhibiting elevated TG levels might consider evaluating serum CCDC25 levels as part of their annual health check-ups. Further studies are needed to confirm the potential integration of CCDC25 into health check-ups. This concept parallels with the report of Min-Oo *et al*. in 2023, using a biochemical parameter scoring system for predicting intrahepatic CCA survival and refining risk outcome assessment (45).

Conclusion

Our research revealed a significant correlation between serum CCDC25 and MetS. Our findings offer valuable insights for clinicians, particularly in patients with MetS. To substantiate this connection and uncover underlying mechanisms, long-term prospective cohort studies and mechanistic investigations will be required in the future.

Conflicts of Interest

There are no conflicts of interest pertaining to this study.

Authors' Contributions

Conceptualization, A.P. and S.P.; methodology, A.P. and S.P.; formal analysis, A.P., T.M.A. and S.P.; investigation, A.P.; data curation, A.P. and S.P.; writing – original draft preparation, A.P.; writing-review and editing, A.P., T.M.A., M.W., P.M., R.T., T.P., J.D. and S.P.; funding acquisition, S.P. All Authors have read and agreed to the published version of the manuscript.

Acknowledgements

The Authors gratefully acknowledge the support received from the Centre of Research and Development of Medical Diagnostic Laboratories (CMDL), Faculty of Associated Medical Sciences, which provided crucial backing for this study. We would like to extend our sincere thanks to Professor Yukifumi Nawa (Tropical Disease Research Center, Faculty of Medicine, Khon Kaen University), for his valuable editorial contributions to the manuscript. We are also grateful to the KKU Publication Clinic of Research Affairs for their assistance. Furthermore, we would like to express our gratitude for the generous funding provided by the Khon Kaen University Scholarship for ASEAN and GMS countries' personnel for the academic year 2021. The financial support from these organizations played a pivotal role in enabling the successful execution of this study.

References

1 Grundy SM, Brewer HB Jr, Cleeman JI, Smith SC Jr, Lenfant C, American Heart Association, National Heart, Lung, and Blood Institute: Definition of metabolic syndrome. Circulation 109(3): 433-438, 2004. DOI: 10.1161/01.CIR.0000111245.75752.C6

2 Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC Jr, Spertus JA, Costa F, American Heart Association, National Heart, Lung, and Blood Institute: Diagnosis and management of the metabolic syndrome. Circulation 112(17): 2735-2752, 2005. DOI: 10.1161/CIRCULATIONAHA.105.169404

3 McCullough AJ: Epidemiology of the metabolic syndrome in the USA. J Dig Dis 12(5): 333-340, 2011. DOI: 10.1111/j.1751-2980.2010.00469.x

4 Hayden MR: Overview and new insights into the metabolic syndrome: Risk factors and emerging variables in the development of type 2 diabetes and cerebrocardiovascular disease. Medicina (Kaunas) 59(3): 561, 2023. DOI: 10.3390/medicina59030561

5 Pongchaiyakul C, Nguyen TV, Kosulwat V, Rojroongwasinkul N, Charoenkiatkul S, Pongchaiyakul C, Sanchaisuriya P, Rajatanavin R: Defining obesity by body mass index in the Thai population: an epidemiologic study. Asia Pac J Clin Nutr 15(3): 293-299, 2006.

6 Pongchaiyakul C, Nguyen TV, Wanothayaroj E, Karusan N, Klungboonkrong V: Prevalence of metabolic syndrome and its relationship to weight in the Thai population. J Med Assoc Thai 90(3): 459-467, 2007.

7 Ranasinghe P, Mathangasinghe Y, Jayawardena R, Hills AP, Misra A: Prevalence and trends of metabolic syndrome among adults in the Asia-Pacific region: a systematic review. BMC Public Health 17(1): 101, 2017. DOI: 10.1186/s12889-017-4041-1

8 Borch-Johnsen K: The metabolic syndrome in a global perspective. The public health impact—secondary publication. Dan Med Bull 54(2): 157-159, 2007.

9 Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB: Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation 112(20): 3066-3072, 2005. DOI: 10.1161/CIRCULATIONAHA.105.539528

10 Shin JA, Lee JH, Lim SY, Ha HS, Kwon HS, Park YM, Lee WC, Kang MI, Yim HW, Yoon KH, Son HY: Metabolic syndrome as a predictor of type 2 diabetes, and its clinical interpretations and usefulness. J Diabetes Investig 4(4): 334-343, 2013. DOI: 10.1111/jdi.12075

11 Bonora E: The metabolic syndrome and cardiovascular disease. Ann Med 38(1): 64-80, 2006. DOI: 10.1080/07853890500401234

12 Kurl S, Laukkanen JA, Niskanen L, Laaksonen D, Sivenius J, Nyyssönen K, Salonen JT: Metabolic syndrome and the risk of

stroke in middle-aged men. Stroke 37(3): 806-811, 2006. DOI: 10.1161/01.STR.0000204354.06965.44
13 Arenillas JF, Moro MA, Dávalos A: The metabolic syndrome and stroke. Stroke 38(7): 2196-2203, 2007. DOI: 10.1161/STROKEAHA.106.480004
14 Russo A, Autelitano M, Bisanti L: Metabolic syndrome and cancer risk. Eur J Cancer 44(2): 293-297, 2008. DOI: 10.1016/j.ejca.2007.11.005
15 Esposito K, Chiodini P, Colao A, Lenzi A, Giugliano D: Metabolic syndrome and risk of cancer: a systematic review and meta-analysis. Diabetes Care 35(11): 2402-2411, 2012. DOI: 10.2337/dc12-0336
16 Hu F, Liu F: Targeting tissue-specific metabolic signaling pathways in aging: the promise and limitations. Protein Cell 5(1): 21-35, 2014. DOI: 10.1007/s13238-013-0002-3
17 Stefano GB, Challenger S, Kream RM: Hyperglycemia-associated alterations in cellular signaling and dysregulated mitochondrial bioenergetics in human metabolic disorders. Eur J Nutr 55(8): 2339-2345, 2016. DOI: 10.1007/s00394-016-1212-2
18 Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M, Martin MJ, Natale DA, O'Donovan C, Redaschi N, Yeh LS: UniProt: the Universal Protein knowledgebase. Nucleic Acids Res 32(Database issue): D115-D119, 2004. DOI: 10.1093/nar/gkh131
19 Yang L, Liu Q, Zhang X, Liu X, Zhou B, Chen J, Huang D, Li J, Li H, Chen F, Liu J, Xing Y, Chen X, Su S, Song E: DNA of neutrophil extracellular traps promotes cancer metastasis *via* CCDC25. Nature 583(7814): 133-138, 2020. DOI: 10.1038/s41586-020-2394-6
20 Liu R, Zhao E, Wang F, Cui H: CCDC25: precise navigator for neutrophil extracellular traps on the prometastatic road. Signal Transduct Target Ther 5(1): 162, 2020. DOI: 10.1038/s41392-020-00285-6
21 Proungvitaya S, Klinthong W, Proungvitaya T, Limpaiboon T, Jearanaikoon P, Roytrakul S, Wongkham C, Nimboriboonporn A, Wongkham S: High expression of CCDC25 in cholangiocarcinoma tissue samples. Oncol Lett 14(2): 2566-2572, 2017. DOI: 10.3892/ol.2017.6446
22 Chanakankun R, Proungvitaya T, Chua-On D, Limpaiboon T, Roytrakul S, Jusakul A, Titapun A, Jarearnrat A, Proungvitaya S: Serum coiled-coil domain containing 25 protein as a potential screening/diagnostic biomarker for cholangiocarcinoma. Oncol Lett 19(1): 930-942, 2020. DOI: 10.3892/ol.2019.11162
23 Jannawan A, Silsirivanit A, Seubwai W, Wongwattanakul M, Proungvitaya T, Aung TM, Roytrakul S, Chiawiriyabunya I, Proungvitaya S: High level of serum coiled-coil domain containing 25 (CCDC25) as a diagnostic marker for cholangiocarcinoma but not for other cancers. In Vivo 37(1): 294-303, 2023. DOI: 10.21873/invivo.13079
24 Deng H, Zhang J, Zheng Y, Li J, Xiao Q, Wei F, Han W, Xu X, Zhang Y: CCDC25 may be a potential diagnostic and prognostic marker of hepatocellular carcinoma: Results from microarray analysis. Front Surg 9: 878648, 2022. DOI: 10.3389/fsurg.2022.878648
25 Siriphak S, Chanakankun R, Proungvitaya T, Roytrakul S, Tummanatsakun D, Seubwai W, Wongwattanakul M, Proungvitaya S: Kallikrein-11, in association with coiled-coil domain containing 25, as a potential prognostic marker for cholangiocarcinoma with lymph node metastasis. Molecules 26(11): 3105, 2021. DOI: 10.3390/molecules26113105
26 Mirhafez SR, Pasdar A, Avan A, Esmaily H, Moezzi A, Mohebati M, Meshkat Z, Mehrad-Majd H, Eslami S, Rahimi HR, Ghazavi H, Ferns GA, Ghayour-Mobarhan M: Cytokine and growth factor profiling in patients with the metabolic syndrome. Br J Nutr 113(12): 1911-1919, 2015. DOI: 10.1017/S0007114515001038
27 Mao Z, Zhang W: Role of mTOR in glucose and lipid metabolism. Int J Mol Sci 19(7): 2043, 2018. DOI: 10.3390/ijms19072043
28 Kang H: Sample size determination and power analysis using the G*Power software. J Educ Eval Health Prof 18: 17, 2021. DOI: 10.3352/jeehp.2021.18.17
29 Taylor SC, Berkelman T, Yadav G, Hammond M: A defined methodology for reliable quantification of Western blot data. Mol Biotechnol 55(3): 217-226, 2013. DOI: 10.1007/s12033-013-9672-6
30 Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB: Prediction of coronary heart disease using risk factor categories. Circulation 97(18): 1837-1847, 1998. DOI: 10.1161/01.cir.97.18.1837
31 Lindstrom J, Tuomilehto J: The diabetes risk score: a practical tool to predict type 2 diabetes risk. Diabetes care 26(3): 725-731, 2003. DOI: 10.2337/diacare.26.3.725
32 Romano A, Del Vescovo E, Rivetti S, Triarico S, Attinà G, Mastrangelo S, Maurizi P, Ruggiero A: Biomarkers predictive of metabolic syndrome and cardiovascular disease in childhood cancer survivors. J Pers Med 12(6): 880, 2022. DOI: 10.3390/jpm12060880
33 Rochlani Y, Pothineni NV, Kovelamudi S, Mehta JL: Metabolic syndrome: pathophysiology, management, and modulation by natural compounds. Ther Adv Cardiovasc Dis 11(8): 215-225, 2017. DOI: 10.1177/1753944717711379
34 Haile K, Haile A, Timerga A: Predictors of lipid profile abnormalities among patients with metabolic syndrome in southwest Ethiopia: a cross-sectional study. Vasc Health Risk Manag 17: 461-469, 2021. DOI: 10.2147/VHRM.S319161
35 Wu H, Xiong L, Xu Q, Wu J, Huang R, Guo Q, Mao H, Yu X, Yang X: Higher serum triglyceride to high-density lipoprotein cholesterol ratio was associated with increased cardiovascular mortality in female patients on peritoneal dialysis. Nutr Metab Cardiovasc Dis 25(8): 749-755, 2015. DOI: 10.1016/j.numecd.2015.05.006
36 Hudgins LC, Hellerstein M, Seidman C, Neese R, Diakun J, Hirsch J: Human fatty acid synthesis is stimulated by a eucaloric low fat, high carbohydrate diet. J Clin Invest 97(9): 2081-2091, 1996. DOI: 10.1172/JCI118645
37 Park CY, Han SN: Lipid pathway in liver cells and its modulation by dietary extracts. In: The molecular Nutrition of Fats. Elsevier, pp 103-116, 2019.
38 Tang Q, Liang B, Zhang L, Li X, Li H, Jing W, Jiang Y, Zhou F, Zhang J, Meng Y, Yang X, Yang H, Huang G, Zhao J: Enhanced CHOLESTEROL biosynthesis promotes breast cancer metastasis *via* modulating CCDC25 expression and neutrophil extracellular traps formation. Sci Rep 12(1): 17350, 2022. DOI: 10.1038/s41598-022-22410-x
39 Bonomini F, Rodella LF, Rezzani R: Metabolic syndrome, aging and involvement of oxidative stress. Aging Dis 6(2): 109-120, 2015. DOI: 10.14336/AD.2014.0305
40 Cox RA, García-Palmieri MR: Cholesterol, triglycerides, and associated lipoproteins. In: Clinical Methods: The History,

Physical, and Laboratory Examinations. 3rd ed. Walker HK, Hall WD and Hurst JW (eds.). Boston, Butterworths, pp 153-160, 1990.

41 Li D, Wang L, Zhou Z, Song L, Chen S, Yang Y, Hu Y, Wang Y, Wu S, Tian Y: Lifetime risk of cardiovascular disease and life expectancy with and without cardiovascular disease according to changes in metabolic syndrome status. Nutr Metab Cardiovasc Dis 32(2): 373-381, 2022. DOI: 10.1016/j.numecd.2021.10.014

42 Yaturu S: Diabetes and Cancer. In: Type 2 Diabetes. Masuo K (ed.). InTech, pp 233-251, 2013.

43 Bleve A, Durante B, Sica A, Consonni FM: Lipid metabolism and cancer immunotherapy: immunosuppressive myeloid cells at the crossroad. Int J Mol Sci 21(16): 5845, 2020. DOI: 10.3390/ijms21165845

44 Danciu AM, Ghitea TC, Bungau AF, Vesa CM: The relationship between oxidative stress, selenium, and cumulative risk in metabolic syndrome. In Vivo 37(6): 2877-2887, 2023. DOI: 10.21873/invivo.13406

45 Min-Oo HH, Aung TM, Wongwattanakul M, Maraming P, Proungvitaya T, Proungvitaya S: Development of a prognostic score for cholangiocarcinoma patients using a combination of biochemical parameters. In Vivo 37(3): 1145-1155, 2023. DOI: 10.21873/invivo.13189

Received November 29, 2023
Revised January 13, 2024
Accepted January 24, 2024