Serum Dickkopf-3 Level Is Inversely Associated with Significant Coronary Stenosis in an Asymptomatic Chinese Cohort

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CLINICAL STUDY

Summary

Dickkopf-3 (DKK3) has been identified to play a protection role against atherosclerosis. However, little is known about the relationship between serum DKK3 levels and subclinical coronary atherosclerosis. We aimed to investigate the association of serum DKK3 with coronary stenosis in an asymptomatic Chinese population. A total of 550 Chinese adults aged 40-60 years and without symptoms or histories of cardiovascular diseases were randomly selected to undergo coronary computed tomography angiography. We defined ≥ 50% luminal narrowing as significant coronary stenosis and measured serum DKK3 levels by an enzyme-linked immunosorbent assay (ELISA). Fifty-nine participants had significant coronary stenosis and 223 had < 50% coronary stenosis. Proportions of significant coronary stenosis were 13.7%, 11.4%, and 7.1% in DKK3 tertiles 1-3, respectively (P_trend = 0.0427). In the univariable multinomial logistic regression model, a decreasing DKK3 tertile was associated with significant coronary stenosis with borderline significance (OR: 1.40; 95% confidence intervals (CI): 0.98-1.99, P = 0.0642). In the multivariable regression model, participants in the lowest DKK3 tertile were associated with a 1.42-fold increased risk of significant coronary stenosis than those in the highest DKK3 tertile (OR: 2.42; 95% CI: 1.10-5.33; P = 0.0279) after adjustment for conventional cardiovascular risk factors. In addition, associations between DKK3 and significant coronary stenosis were consistent among subgroups. However, no significant association was found between serum DKK3 levels and < 50% coronary stenosis. Therefore, we have added to the existing evidence that serum DKK3 is inversely associated with the risk of significant coronary stenosis in asymptomatic middle-aged Chinese.

Key words: Hormone, Atherosclerosis, Coronary artery disease, Computed tomography angiography

Coronary artery disease (CAD) is the leading cause of premature death and disabilities worldwide.1,2 There was an estimated 19% increase globally in CAD-related death from 2006 to 2016.3 Although the first clinical presentation of CAD often causes serious morbidity or even mortality, a prolonged asymptomatic and clinically silent phase always exists.4,5 Therefore, early and noninvasive detection of atherosclerotic cardiovascular diseases in asymptomatic individuals is of great importance.6

Dickkopf-3 (DKK3), a member of DKK family, is a secreted glycoprotein, which has been extensively studied in tumor research for its inverse correlation with a variety of carcinomas. It has been identified as a biomarker and a therapeutic target for the treatment of cancers.7,8,9 Recently, urinary DKK3 has been identified as an independent predictor for postoperative acute kidney injury and for subsequent loss of kidney function, highlighting its important role in clinical practice.10 Emerging evidence also indicates that DKK3 is involved in cardiac dysfunction and remodeling in animal studies.11,12 Moreover, a convincing pathological study in mice has led to the identification of DKK3 as a hormone that could induce endothelial cell migration, promote reendothelialization, and inhibit lesion formation in artery vessels.13 In addition, an inverse correlation between serum DKK3 and carotid atherosclerosis development was observed in a cohort of 684 Italians.14 Karamariti, et al.15 further reported that subjects with unstable angina had lower serum DKK3 levels than those...
with stable angina in a small sample of 88 patients. However, little is known about the relationship between DKK3 levels and subclinical coronary atherosclerosis in asymptomatic adults. Therefore, we did a cross-sectional analysis using a well-defined middle-aged Chinese cohort, of which all participants underwent coronary computed tomography (CT) angiography. Our primary hypothesis is that DKK3 levels are inversely associated with subclinical coronary atherosclerosis.

Methods

Study population: This is a community-based general population cohort with participants enrolled in two stages. The study design and standard procedures have been previously depicted. Briefly, 10,185 registered permanent residents aged ≥ 40 years living in the Songnian Community in Shanghai were screened for dysglycemia in June and July 2008 at the first stage. Participants were grouped according to fasting plasma glucose (FPG) levels: (1) normal glucose regulation (NGR), defined by an FPG < 5.6 mmol/L and without a history of diabetes; (2) impaired glucose regulation (IGR), defined by 5.6 mmol/L ≤ FPG < 7.0 mmol/L and without a history of diabetes; and (3) diabetes, defined by an FPG ≥ 7.0 mmol/L or with a history of diabetes. In the second stage, 4012 participants were randomly selected from the three groups in a ratio of 1.0 (diabetes) to 1.2 (IGR) to 1.44 (NGR). We oversampled those with lower glucose levels because they might have a lower participation rate than those with higher glucose levels. A standard questionnaire, anthropometric measurements, a 75-g oral glucose tolerance test (OGTT), and biochemical evaluations were conducted for each participant. Participants were reclassified as NGR, IGR, or diabetes according to their FPG and 2-hour post-load plasma glucose (2hPG) levels during OGTT and their history of diabetes using the 1999 World Health Organization criteria. No significant differences were observed in age and sex distributions between participants and nonparticipants.

For this study, we selected 50% of NGR subjects by simple random sampling using “proc surveypselect” in SAS and all IGR and diabetes subjects to undergo coronary CT angiography after excluding those with any of the following conditions: (1) age > 60 years, (2) with symptoms of cardiac ischemia such as chest pain or shortness of breath, (3) with a cardiovascular disease (CVD) history, (4) resting electrocardiogram (ECG) showing abnormal Q waves, (5) duration of diabetes > 5 years, (6) with impaired liver or renal function defined as alanine aminotransferase ≥ 2 fold of upper limit of normal range or serum creatinine level > 133 μmol/L (1.5 mg/dL) or estimated glomerular filtration rate < 60 mL/minute per 1.73 m², (7) being pregnant or having significant medical comorbidities, (8) undergoing X-ray examination or CT scan within 1 year, (9) with tachycardia (heart rate > 90 bpm) or arrhythmia such as atrial fibrillation that might cause artifacts in CT angiography examination, and (10) with a history of allergic reaction to iodine-containing contrast agent. Finally, 550 participants were included in this study, and more details of the selected population could also be found in the previous publications.

The Institutional Review Board of Ruijin Hospital, Shanghai Jiao Tong University School of Medicine approved the study protocol, which was conducted in accordance with the principle of Helsinki declaration. All participants signed the written informed consent before enrollment.

Data collection: Standard questionnaires were administered by trained medical staff through face-to-face interview with participants. Information collected using the questionnaire included (1) demographic characteristics including educational levels, (2) personal medical history and medication usage, and (3) lifestyles such as smoking, drinking, and physical activity. Current smoking or drinking was defined as regular cigarette smoking or alcohol drinking in the past 6 months. Physical activity at work and in leisure time was obtained and calculated into metabolic equivalent hours per week (METs-h/week) using the short form of the International Physical Activity Questionnaire. Body height, weight, and blood pressure (BP) were measured according to a standard protocol, and body mass index (BMI) was calculated as body weight divided by body height squared (kg/m²). BP was measured three times consecutively using a calibrated digital electronic device (OMRON Model HEM-752 FUZZY, Omron Company, Dalian, China) with 1-minute intervals between each measurement after at least 5-minute rest in a seated position. The last two measurements were averaged and used for analysis. Fasting blood samples were collected after an overnight fast for ≥ 10 hours. Biochemical parameters, such as blood glucose, total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and high-sensitivity C-reactive protein (hs-CRP), were measured by using an autoanalyzer (Beckman CX-7 Biochemical Autoanalyzer, Beckman Coulter, Brea, CA). Fasting serum insulin was measured via an electrochemiluminescence assay (Roche Diagnostics, Basel, Switzerland). The index of homeostasis model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: fasting insulin concentration (mIU/L) × fasting plasma glucose (mmol/L)/22.5.

Enzyme-linked immunosorbent assay (ELISA) for serum DKK3 level: Blood samples were centrifuged on site at 4°C, and serum were aliquoted and stored in −80°C before assay. We used a commercial DKK3 ELISA kit to measure serum DKK3 levels (R&D, DY1118), according to the manufacturer’s instructions. The inter- and intra-assay coefficients of variation for serum DKK3 were 12.5% and 8.9%, respectively.

CT angiography and image analysis: We used a dual-source CT scanner (SOMATOM Definition; Siemens Medical Solutions, Forchheim, Germany) for coronary CT angiography. A standard retrospectively ECG-gated scanning protocol was applied with 0.6-mm slice collimation, 330-ms gantry rotation time, 120-kV tube voltage, and a maximum tube current of 400 mAs/tube. All scans were performed using ECG-controlled tube current modulation. Each participant received a bolus of 70 mL (4 mL/second) iohexol injection (350 mg/mL iodine; Omnipaque; GE Healthcare Shanghai, Shanghai, China) via an 18-gauge catheter placed in the antecubital vein, and a 40-mL chaser was followed after.

Advance Publication2 W ANG, ET AL
plaque sizes were considered when structures > 1 mm² were not interpretable segments due to motion artifacts. Coronary artery stenosis > 1.5 mm and further excluded subjects with uninterpretable segments according to the American Heart Association criteria. Study participants. Coronary arteries were divided into 15 segments by an experienced senior radiologist who was masked to the clinical information of study participants. Online three-dimensional workstation (ADW 4.4; GE Healthcare, Waukesha, WI) was used to measure coronary stenosis. Participants were divided into three groups according to the characteristics of the study population, which had a mean age of 52.7 years and 42.6% men. Participants tended to be older and had lower BMI, serum hs-CRP levels, and HOMA-IR across serum DKK3 tertiles (all P values for trend < 0.05). There was no significant difference in sex composition; lifestyles such as physical activity, smoking, and drinking; chronic diseases such as diabetes, hypertension, and dyslipidemia; or usage of anti-hypertensive and anti-diabetic drugs among DKK3 tertiles. A total of 223 participants were identified as having < 50% coronary stenosis, and the proportions were 39.3%, 38.6%, and 43.7% in DKK3 tertiles 1-3, respectively (P value for trend = 0.3944; Figure 1B). A total of 59 participants had increased risks of significant coronary stenosis (OR: 1.74, 95% CI: 0.79-3.83, P = 0.1721 for DKK3 tertile 2; OR: 1.81, 95% CI: 0.84-3.90, P = 0.1335 for DKK3 tertile 2; OR: 2.69, 95% CI: 1.26-5.76, P = 0.0109 for DKK3 tertile 1) than those in DKK3 tertile 3 after adjustment for age and sex (Table III). The dose-response association between serum DKK3 levels and risks of having significant coronary stenosis changed little after further adjustment for BMI, physical activity, current smoking, current drinking, education level, family history of CAD, hs-CRP, HOMA-IR, diabetes, hypertension, and dyslipidemia (OR: 1.74, 95% CI: 0.79-3.83, P = 0.1721 for DKK3 tertile 2; OR:

CT angiography images were interpreted on an offline three-dimensional workstation (ADW 4.4; GE Healthcare, Waukesha, WI) by an experienced senior radiologist who was masked to the clinical information of study participants. Coronary arteries were divided into 15 segments according to the American Heart Association classification. We included those segments with a diameter > 1.5 mm and further excluded subjects with uninterpretable segments due to motion artifacts. coronary plaques were considered when structures > 1 mm² were detected within or adjacent to the coronary artery lumen that could be clearly distinguished from the vessel lumen and the surrounding pericardial tissue. Significant artery stenosis was defined as ≥ 50% narrowing of the vessel lumen.

Statistical analysis: Continuous variables were presented as means ± standard deviations or medians with interquartile ranges, and categorical variables were presented as numbers with percentages. Variables that did not meet normality assumptions such as triglycerides, HOMA-IR, and hs-CRP were log₁₀ transformed in regression analysis. Participants were divided into three groups according to tertiles of serum DKK3 levels because no definite threshold for a normal DKK3 was reported and our preliminary analysis using a locally weighted scatterplot smoothing model revealed that the association with risks of coronary stenosis was not linear across the range of DKK3 levels and that the dose-response relationship between DKK3 and significant coronary stenosis became obvious after the DKK3 level reached 7.8 ng/mL (data not shown), which coincided with the up limit of DKK3 tertile 1 (1.68-7.45 ng/mL). A univariable multinomial logistic regression analysis was performed to examine the association between DKK3 tertiles and the odds of having < 50% coronary stenosis and significant coronary stenosis. Multivariable multinomial logistic regression models were then used to assess risks of having < 50% coronary stenosis and significant coronary stenosis associated with serum DKK3 levels adjusted for conventional risk factors. Associations of one-tertile decrease in serum DKK3 with significant coronary stenosis were also examined in the overall study population and in subgroups of age, sex, BMI, with or without current smoking, current drinking, diabetes, hypertension, and dyslipidemia. A P value < 0.05 was considered statistically significant using SAS version 9.4 (SAS Institute Inc., Cary, NC).

Figure 1. Distribution of serum DKK3 levels in the study population (A), proportions of < 50% coronary stenosis (B), and proportions of significant coronary stenosis (C) according to DKK3 tertiles. Numbers in parenthesis under each bar were the number of participants with < 50% coronary stenosis (B) or numbers of participants with significant coronary stenosis (C) / numbers of participants in each tertile. The serum DKK3 level in the current population ranged from 1.68 ng/mL to 38.50 ng/mL, with the median value of 9.23 ng/mL (Figure 1A). Table I shows the characteristics of the study population, which had a mean age of 52.7 years and 42.6% men. Participants tended to be older and had lower BMI, serum hs-CRP levels, and HOMA-IR across serum DKK3 tertiles (all P values for trend < 0.05). There was no significant difference in sex composition; lifestyles such as physical activity, smoking, and drinking; chronic diseases such as diabetes, hypertension, and dyslipidemia; or usage of anti-hypertensive and anti-diabetic drugs among DKK3 tertiles. A total of 223 participants were identified as having < 50% coronary stenosis, and the proportions were 39.3%, 38.6%, and 43.7% in DKK3 tertiles 1-3, respectively (P value for trend = 0.3944; Figure 1B). A total of 59 participants had increased risks of significant coronary stenosis (OR: 1.74, 95% CI: 0.79-3.83, P = 0.1721 for DKK3 tertile 2; OR: 1.81, 95% CI: 0.84-3.90, P = 0.1335 for DKK3 tertile 2; OR: 2.69, 95% CI: 1.26-5.76, P = 0.0109 for DKK3 tertile 1) than those in DKK3 tertile 3 after adjustment for age and sex (Table III). The dose-response association between serum DKK3 levels and risks of significant coronary stenosis changed little after further adjustment for BMI, physical activity, current smoking, current drinking, education level, family history of CAD, hs-CRP, HOMA-IR, diabetes, hypertension, and dyslipidemia (OR: 1.74, 95% CI: 0.79-3.83, P = 0.1721 for DKK3 tertile 2; OR:

The multivariable multinomial logistic regression analysis showed that most conventional cardiovascular risk factors, such as age, sex, current smoking, current drinking, physical activity, BMI, HOMA-IR, hypertension, and dyslipidemia, were significantly associated with risks of having < 50% coronary stenosis, whereas age, sex, diabetes, and hypertension were significantly associated with risks of having significant coronary stenosis (Table II). A decreasing DKK3 tertile was associated with significant coronary stenosis with borderline significance (odds ratio, OR: 1.40; 95%, CI: 0.98-1.99, P value = 0.0642). The multivariable multinomial logistic regression analysis showed that participants in DKK3 tertiles 2 and 1 had increased risks of significant coronary stenosis (OR: 1.81, 95% CI: 0.84-3.90, P = 0.1335 for DKK3 tertile 2; OR: 2.69, 95% CI: 1.26-5.76, P = 0.0109 for DKK3 tertile 1) than those in DKK3 tertile 3 after adjustment for age and sex (Table III). The dose-response association between serum DKK3 levels and risks of significant coronary stenosis changed little after further adjustment for BMI, physical activity, current smoking, current drinking, education level, family history of CAD, hs-CRP, HOMA-IR, diabetes, hypertension, and dyslipidemia (OR: 1.74, 95% CI: 0.79-3.83, P = 0.1721 for DKK3 tertile 2; OR:

The univariable multinomial logistic regression analysis showed that most conventional cardiovascular risk factors, such as age, sex, current smoking, current drinking, physical activity, BMI, HOMA-IR, hypertension, and dyslipidemia, were significantly associated with risks of having < 50% coronary stenosis, whereas age, sex, diabetes, and hypertension were significantly associated with risks of having significant coronary stenosis (Table II). A decreasing DKK3 tertile was associated with significant coronary stenosis with borderline significance (odds ratio, OR: 1.40; 95%, CI: 0.98-1.99, P value = 0.0642).
levels and significant coronary stenosis, we stratified par-

There were seven missing values for current drinking. * Usage of other drugs included statin in one participant and aspirin in two participants. CAD indicates coronary artery disease; METs-h/week, metabolic equivalent hours per week; BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; 2hPG, 2-hour post-load plasma glucose; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment for insulin resistance; and hs-CRP, high-sensitivity C-reactive protein.

Table II. The Risk of Coronary Stenosis in Relation to Cardiovascular Risk Factors in Univariable Multinomial Logistic Regression

| Age (years) | Coronary stenosis < 50% | P value | Coronary stenosis ≥ 50% | P value |
|-------------|-------------------------|---------|-------------------------|---------|
| 1.08 (1.03-1.12) | 0.0011 | 1.14 (1.05-1.23) | 0.0010 |
| 0.60 (0.42-0.86) | 0.0055 | 0.34 (0.19-0.61) | 0.0003 |
| 0.80 (0.55-1.16) | 0.2371 | 0.75 (0.41-1.38) | 0.3518 |
| 1.72 (1.16-2.55) | 0.0073 | 1.55 (0.84-2.86) | 0.1650 |
| 0.65 (0.43-0.99) | 0.0426 | 0.54 (0.29-1.00) | 0.0515 |
| 1.00 (1.00-1.01) | 0.0445 | 1.00 (1.00-1.01) | 0.8689 |
| 1.13 (0.73-1.77) | 0.5838 | 1.11 (0.55-2.25) | 0.7668 |
| 1.12 (1.06-1.18) | <0.0001 | 1.07 (0.99-1.17) | 0.1091 |
| 1.03 (0.94-1.12) | 0.5531 | 1.05 (0.93-1.18) | 0.4539 |
| 1.15 (1.02-1.30) | 0.0221 | 1.19 (1.00-1.41) | 0.0542 |
| 1.10 (0.72-1.68) | 0.6698 | 2.20 (1.20-4.02) | 0.0104 |
| 2.30 (1.60-3.32) | <0.0001 | 2.88 (1.62-5.14) | 0.0003 |
| 1.71 (1.15-2.54) | 0.0086 | 1.88 (0.97-3.66) | 0.0628 |
| 0.97 (0.78-1.21) | 0.7767 | 1.40 (0.98-1.99) | 0.0642 |

2.42, 95% CI: 1.10-5.33, P = 0.0279 for DKK3 tertile 1.

To further explore the relationship between DKK3 levels and significant coronary stenosis, we stratified participants by age, sex, current smoking, current drinking, BMI, diabetes, hypertension, and dyslipidemia, respectively. Associations were consistent among subgroups, and no significant interactions were observed (Figure 2; all P values for interaction > 0.10).

Discussion

In this study, we found that 59 participants had significant coronary stenosis in an asymptomatic cohort of 550 middle-aged Chinese. Those who had lower levels of serum DKK3 had worse profile of metabolic factors such as BMI, HOMA-IR, and hs-CRP and higher proportions of significant coronary stenosis. After full adjustment for potential confounding factors, participants in the lowest
serum DKK3 tertile had a 1.42-fold increased risk of significant coronary stenosis than those in the highest DKK3 tertile. A similar association was also found in all subgroup analysis. Our findings might provide a novel insight into understanding vaso-occlusive cardiovascular diseases in asymptomatic people, with potential targets for disease treatment and prevention.6,19)

Compelling evidence has shown that DKK3 might

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**Table III. The Risk of Coronary Stenosis in Relation to Tertiles of Serum DKK3 Levels in Multivariable Multinomial Logistic Regression**

|                      | Coronary stenosis < 50% |  | Coronary stenosis ≥ 50% |  |
|----------------------|-------------------------|---|-------------------------|---|
|                      | OR (95% CI) | *P* value | OR (95% CI) | *P* value |
| Model 1              |             |           |             |           |
| DKK3 tertile 3       | 1.00        |           | 1.00        |           |
| DKK3 tertile 2       | 0.92 (0.59-1.44) | 0.7233 | 1.81 (0.84-3.90) | 0.1335 |
| DKK3 tertile 1       | 1.08 (0.69-1.68) | 0.7418 | 2.69 (1.26-5.76) | 0.0109 |
| Model 2              |             |           |             |           |
| DKK3 tertile 3       | 1.00        |           | 1.00        |           |
| DKK3 tertile 2       | 0.91 (0.58-1.43) | 0.6949 | 1.68 (0.77-3.66) | 0.1957 |
| DKK3 tertile 1       | 0.94 (0.59-1.48) | 0.7749 | 2.60 (1.20-5.63) | 0.0155 |
| Model 3              |             |           |             |           |
| DKK3 tertile 3       | 1.00        |           | 1.00        |           |
| DKK3 tertile 2       | 0.92 (0.58-1.46) | 0.7303 | 1.74 (0.79-3.83) | 0.1721 |
| DKK3 tertile 1       | 0.88 (0.55-1.41) | 0.5876 | 2.42 (1.10-5.33) | 0.0279 |

Model 1 was adjusted for age and sex. Model 2 was adjusted for BMI, physical activity, current smoking, current drinking, education level, and family history of CAD in addition to model 1. Model 3 was adjusted for hs-CRP, HOMA-IR, diabetes, hypertension, and dyslipidemia in addition to model 2.

Figure 2. Odds ratios (OR) and 95% confidence intervals (CI) of significant coronary stenosis in association with 1-tertile DKK3 decrease in the overall population and in subgroup.
potentially play a protection role against hypertrophic heart disease.\textsuperscript{10,20} In the past two years, Yu and colleagues have demonstrated DKK3 interfering with atherosclerosis through induction in endothelial cell migration\textsuperscript{12} and alteration in atherosclerotic plaque content.\textsuperscript{13} They reported a significant association between serum DKK3 and subclinical carotid atherosclerosis examined by ultrasonography of the carotid arteries, although the predictive value of carotid artery intima-media thickness and carotid plaques used in their study is limited for future CAD.\textsuperscript{21-24}

In this study, contrast-enhanced coronary CT angiography was used to detect significant coronary stenosis. We found a substantially increased risk of significant coronary stenosis in participants with lower levels of serum DKK3 after controlling for CVD risk factors. To the best of our knowledge, this is the first population study to demonstrate associations between serum DKK3 and significant coronary stenosis, adding to the very limited literatures of population studies on DKK3 and atherosclerosis.\textsuperscript{12,13}

In addition, we found that participants with higher DKK3 levels had lower BMI, HOMA-IR, and hs-CRP. These findings are consistent with recent reports showing that DKK3 could inhibit obesity, insulin resistance, and inflammation in mice.\textsuperscript{13,25} Obesity and insulin resistance are conventional cardiovascular risk factors,\textsuperscript{26} and inflammation has been identified to play an important role in atherosclerosis.\textsuperscript{27,28} In addition, participants in tertile 3 of DKK3 levels were older than those in tertile 1 in the present study, which is also consistent with previous reports that blood DKK3 increased in the elderly.\textsuperscript{12,29} Since DKK3 has been reportedly associated with age, BMI, and glucose metabolism,\textsuperscript{28,29} we examined the association between DKK3 and significant coronary stenosis stratified on these variables. No significant interaction was observed (all \(P\) values for interaction > 0.05), indicating a consistent association among subgroups.

Our study adds to the existing evidence by demonstrating that serum DKK3 level was inversely associated with significant coronary stenosis in asymptomatic adults, which might have important clinical and public health implications for developing screening measures and implementing early interventions in high-risk subjects. The National Institute for Health and Care Exellences (NICE) Clinical Guidelines have recommended coronary CT angiography as the first-line test for patients presenting with chest pain due to suspected CAD.\textsuperscript{30} However, CT angiography is not recommended for screening in asymptomatic subjects.\textsuperscript{31} Therefore, the measurement of serum DKK3 levels in these patients could help in the identification of high-risk population who might benefit from coronary CT angiography for early detection of significant coronary stenosis.

This is the first population study demonstrating associations between serum DKK3 and significant coronary stenosis. Comprehensive evaluations of cardiovascular risk factors including lifestyles and inflammatory factors such as hs-CRP were available and controlled as confounding factors. Coronary CT angiography was used to detect coronary stenosis noninvasively in adults without CVD history or clinical manifestations of CVD.\textsuperscript{19,32} Several limitations should be also considered. First, our sample size was relatively small, and insufficient statistical power in subgroups could have existed. Second, due to the cross-sectional design of this study, we are not able to draw conclusions regarding the causal relationship between serum DKK3 and significant coronary stenosis. Third, although many cardiovascular risk factors were adjusted in the multivariable analyses, some residual or undetected confounding could not be ruled out. Fourth, information on the number of plaques in each participant was not recorded and the multiplicity of plaques in relation with the DKK3 cannot be analyzed. Fifth, the selection of participants was based on their status of glucose metabolism, rather than a more general population. Nevertheless, the association between DKK3 and significant coronary stenosis is independent of diabetes status indicated by multivariable analysis and was consistent in participants with different glycemic status indicated by subgroup analysis. Finally, this study only included Chinese adults, which has limited the generalizability of our findings.

**Conclusion**

CAD is a challenging health problem not only because it is one of the major causes of death in adults worldwide but also because it is a silent process till the first clinical manifestation with serious outcomes,\textsuperscript{33} which is even more devastating in patients with diabetes.\textsuperscript{34} Therefore, efforts to identify individuals who are at an elevated risk of developing clinical events such as acute myocardial infarction at a subclinical stage are warranted.\textsuperscript{35} We provide the first evidence that serum DKK3 is inversely associated with the risk of significant coronary stenosis in asymptomatic middle-aged Chinese. Population studies with larger sample sizes and with a longitudinal design recording incident cardiovascular events are also warranted to further examine the relationship between serum DKK3 and development of atherosclerosis.

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**Disclosure**

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**Authors’ contributions:** LW, SL, JN, YB and YuX contributed to study design, data collection, analysis, and interpretation, and drafting of the manuscript. ML, ZZ, TW, JL, YC, MX, WW, SY, MD and GN contributed to data
collection and the editing of the manuscript. YuX and YB critically revised the manuscript for important intellectual content. All authors contributed to the revision of the manuscript and approved the final version of the manuscript.

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