

Original Article

Increased levels of plasma alpha 2-Heremans-Schmid glycoprotein in Yi Chinese patients with lower extremity atherosclerosis diseases due to type 2 diabetes mellitus

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Abstract: The study aimed to investigate the value of plasma alpha 2-Heremans-Schmid glycoprotein (AHSG) levels for predicting the presence and severity of lower extremity atherosclerosis disease (LEASD) in Yi Chinese patients with type 2 diabetes mellitus (T2DM). A total of 128 consecutive patients with T2DM and 120 normal controls were recruited. Plasma AHSG levels were analyzed by enzyme-linked immunosorbent assay (ELISA). LEASD was diagnosed by color Doppler ultrasound examination, and its severity was assessed by the sum of each atherosclerosis (AS) score, including intima-media thickness (IMT), degree of sclerosis, plaques, and luminal stenosis. T2DM subjects had significantly higher AHSG levels than normal controls ($348.88 \pm 48.18 \mu\text{g/ml}$ vs. $283.09 \pm 40.72 \mu\text{g/ml}$, $P < 0.01$). T2DM patients with LEASD showed significantly higher AHSG levels than T2DM patients without LEASD ($363.80 \pm 48.35 \mu\text{g/ml}$ vs. $332.48 \pm 42.66 \mu\text{g/ml}$, $P < 0.01$). The difference remained statistically significant after adjusting for all other potential confounders between groups using covariance analysis. AHSG was positively correlated with homeostasis model assessment-insulin resistance (HOMA-IR) and AS scores by Pearson's correlation analysis ($r = 0.175$, $P < 0.05$; $r = 0.407$, $P < 0.01$, respectively). HOMA-IR was independently associated with AHSG in a multivariate linear regression analysis ($t = 2.150$, $P < 0.05$). Logistic regression analysis revealed that AHSG was an independent risk factor for T2DM and for T2DM complicated by LEASD (OR = 1.070, 95% CI: 1.027-1.116, $P < 0.01$; OR = 1.024, 95% CI: 1.011-1.037, $P < 0.01$, respectively). An increased level of plasma AHSG might serve as a potential predictor of the presence and development of T2DM and LEASD caused by T2DM in Yi Chinese patients.

Keywords: Alpha 2-Heremans Schmid glycoprotein, lower extremity atherosclerosis disease, type 2 diabetes, predictor, Yi Chinese people

Introduction

With the rapid change in lifestyles in China, the number of patients with type 2 diabetes mellitus (T2DM) has increased. In 2010, the prevalence of diabetes in people older than 20 years is 9.7%, and the rate of prediabetes (impaired fasting glucose and impaired glucose tolerance patients) is 15.5% [1]. In addition to impaired insulin secretion from cells, insulin resistance (IR) was found to play a major role in the pathogenesis of T2DM [2]. Macrovascular complications, pathologically based on atherosclerosis (AS), are the leading cause of death in patients with T2DM. In these cases, the arteries of the lower extremities are some of the most commonly affected vessels, and can contribute to arterial occlusion and tissue ischemic necrosis

of the lower extremities and decreased quality of life for patients with T2DM. An early sign of AS is the increased intima-media thickness (IMT) of arterial wall. Early detection in high-risk groups can facilitate effective preventive measures to reduce the incidence of T2DM and its macrovascular complications.

Recent studies have revealed that alpha 2-Heremans-Schmid glycoprotein (AHSG) might affect glucose metabolism and vascular disease. AHSG, also called fetuin-A, is a serum glycoprotein produced primarily in the liver that circulates at high levels [3]. The human AHSG gene resides on chromosome 3q27, which has been mapped as a T2DM locus [4]. Higher serum AHSG concentrations are associated with the manifestation of T2DM in humans [5, 6]

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and in women with gestational diabetes [7]. However, previous studies addressing the function of AHSG in vascular disease have produced conflicting results. Some studies have indicated that AHSG is positively correlated with coronary AS [8] and that increased serum AHSG levels are associated with the degree of arterial stiffness [9]. In addition, patients with high AHSG concentrations have a 4-fold increased risk of acute myocardial infarction (AMI) and ischemic stroke, compared to subjects with low AHSG levels [10]. Conversely, several studies have shown that low levels of AHSG are correlated with the severity of coronary arterial calcification [11, 12], and a negative relationship between AHSG and subclinical AS was found in patients with obstructive sleep apnea syndrome [13]. Another study conducted in patients with chronic lower extremity AS indicated that AHSG is inversely correlated with the severity of arterial calcification [14].

Although AS can present in several arteries simultaneously, the occurrence or development of arterial lesions is not synchronous in all parts of the body [15]. The inconsistency of results might be related to differences in lesion position and underlying diseases in study subjects. Studies of the association of AHSG with lower extremity atherosclerosis disease (LEASD) due to T2DM are limited. Many T2DM patients with vascular lesions have no clinical symptoms, and there is no definitive evidence indicating the need to perform vascular tests. Prediction of LEASD could be essential for improving the survival and clinical outcomes of patients with T2DM. Additionally, the Yi minority is the most populous in Yunnan province, and it has a long history in the southwest border area of China. Therefore, this study aimed to investigate the value of plasma AHSG levels for predicting the presence and severity of LEASD in patients with T2DM among Yi Chinese individuals.

Materials and methods

Subjects

A cohort of 128 T2DM patients (73 males and 55 females, with an average age of 55.48 ± 7.18 years) and 120 normal controls (82 males and 38 females, with an average age of 52.22 ± 5.63 years) (group Con) were recruited from the Departments of Endocrinology and Physical Examinations in the Chuxiong Yi autonomous prefecture hospital of Yunnan province, respec-

tively. The diagnosis of T2DM was based on fasting plasma glucose (FPG) ≥ 7.0 mmol/l and 2 h postprandial plasma glucose (2 h post-75 oral glucose tolerance test) ≥ 11.1 mmol/l. The group Con showed no symptoms of diabetes, hypertension, dyslipidemia, or history of liver or kidney dysfunction. The patients with T2DM and the controls were all unrelated and all belonged to the Yi Chinese ethnic group. This study was conducted in accordance with the declaration of Helsinki. This study was conducted with approval from the Ethics Committee of Kunming Medical University. Written informed consent was obtained from all participants.

Clinical data and blood chemistry measurements

The sex, age, height, body weight, systolic blood pressure (SBP), and diastolic blood pressure (DBP) of the study participants were recorded. Fasting venous blood samples were drawn from participants. After clotting, the samples were immediately centrifuged and stored at -80°C until analysis. Plasma total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), fasting plasma glucose (FPG), blood urea nitrogen (BUN), and creatinine (Cr) levels were measured using standard laboratory techniques with an automatic biochemical analyzer (Olympus AU2700). Fasting insulin (FINS) was measured by radioimmunoassay. The body mass index (BMI) was calculated for all subjects. IR status was evaluated using the homeostasis model assessment-insulin resistance (HOMA-IR) index. The HOMA-IR index was calculated using the following formula: $\text{FINS } (\mu\text{IU/mL}) \times \text{FPG } (\text{mmol/L}) / 22.5$ [16]. Plasma AHSG levels were measured with a commercially available ELISA kit (R&D Systems, Minneapolis, MN, USA).

LEASD assessment

The arteries of the lower extremities in all of the subjects, including the bilateral common femoral arteries, superficial femoral arteries, deep femoral arteries, popliteal arteries, posterior tibial arteries, and dorsalis pedis arteries, were examined using a color Doppler ultrasound instrument (Aloka $\alpha 10$, Japan). The vessel diameter, intraluminal echo, lesion sites, intima-media thickness (IMT), atherosclerotic plaques, and bloodstream characteristics were determined. A diagnosis of LEASD was applied if any

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Table 1. Baseline clinical characteristics and plasma AHSg levels

Variables	Control group (n = 120)	T2DM group (n = 128)	T2DM group	
			Without LEASD (n=61)	With LEASD (n = 67)
Sex (male/female)	82/38	73/55	40/21	33/34
Age (years)	52.22±5.63	55.48±7.18**	53.79±6.59	57.01±7.39 ^Δ
BMI (kg/m ²)	23.48±3.17	24.26±3.04*	24.62±3.30	23.49±2.69
SBP (mmHg)	115.97±8.93	125.87±17.26**	121.51±16.89	129.84±16.75 ^Δ
DBP (mmHg)	75.11±5.19	78.41±10.06**	75.70±9.19	80.88±10.25
TC (mmol/l)	4.50±0.57	5.41±1.42**	4.80±0.92	5.97±1.56 ^{ΔΔ}
TG (mmol/l)	1.44±0.43	2.67±1.39**	2.15±1.17	3.15±1.41 ^{ΔΔ}
LDL-c (mmol/l)	2.51±0.60	3.41±0.96**	2.96±0.68	3.82±1.00 ^{ΔΔ}
HDL-c (mmol/l)	1.31±0.35	1.16±0.65*	1.27±0.46	1.08±0.77 ^{ΔΔ}
BUN (mmol/l)	4.66±1.03	5.31±1.86**	4.60±1.35	5.96±2.02 ^{ΔΔ}
Cr (μmol/l)	72.78±13.11	81.92±23.81**	71.16±14.34	91.72±26.45 ^{ΔΔ}
FPG (mmol/l)	5.02±0.49	9.78±3.20**	8.18±2.80	11.23±2.86 ^{ΔΔ}
HOMA-IR			3.07±2.27	4.30±3.07 ^Δ

All values are presented as the mean value and SD. T2DM = type 2 diabetes, LEASD = lower extremity atherosclerosis disease, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, TC = total cholesterol, TG = triglyceride, LDL-c = low density lipoprotein cholesterol, HDL-c = high density lipoprotein cholesterol, BUN = blood urea nitrogen, Cr = creatinine, FPG = fasting plasma glucose, HOMA-IR = HOMA-insulin resistance index. **P* < 0.05, ***P* < 0.01 compared with the control group; ^Δ*P* < 0.05, ^{ΔΔ}*P* < 0.01 compared with T2DM patients without LEASD.

of the following conditions were met: 1) IMT ≥ 1 mm; 2) IMT < 1 mm but intraluminal echo enhancement; 3) single, multiple, or diffuse plaques detected; and 4) luminal stenosis. The severity of LEASD was assessed by the sum of each of the following lower extremity AS scores [17]: 1) IMT: < 1 mm, 0; 1-1.2 mm, 1; > 1.2 mm, 2; 2) degree of sclerosis: normal, 0; mild sclerosis (IMT < 1 mm but intraluminal echo enhancement; no plaques), 1; moderate or severe sclerosis (mild sclerosis with plaques or luminal stenosis), 2; 3) plaques: undetected, 0; single, 1; multiple, 2; diffuse, 3; 4) luminal stenosis: no significant stenosis of the vessel diameter, 0; 30-50% stenosis, 1; 50-75% stenosis, 2; occlusion (no blood flow), 3.

Statistical analysis

The data are expressed as mean ± SD, and normality tests were performed using the Kolmogorov-Smirnov test. Variables with a non-normal distribution were logarithmically transformed before statistical analysis. Differences between two groups were analyzed using the independent sample t-test. Covariance analysis was employed to adjust all other potential confounders between groups. Pearson's correlation analysis was used to examine the associations between plasma AHSg levels and clinical characteristics. Multivariate linear regression analysis was used to assess independent con-

tributions to plasma AHSg levels. Multivariate logistic regression analysis was performed to evaluate the independent predictors of T2DM and T2DM complicated by LEASD. Similarly, *P* < 0.05 was regarded as statistically significant differences. All statistical analyses were performed using the SPSS11.5 software (Chicago, USA).

Results

Baseline characteristics and measurements

The baseline characteristics and measurements of the study groups are presented in **Table 1**. The control group showed significantly lower age, BMI, SBP, DBP, TC, TG, LDL-c, BUN, Cr, and FPG levels, as well as significantly higher HDL-c levels than the T2DM group (*P* < 0.05 to *P* < 0.01). Then, the T2DM patients were divided into two subgroups: patients without LEASD (n = 61) and patients with LEASD (n = 67). The T2DM controls without LEASD showed significantly lower age, SBP, TC, TG, LDL-c, HOMA-IR, BUN, Cr, and FPG levels, as well as significantly higher HDL-c levels, than those with LEASD (*P* < 0.05 to *P* < 0.01).

Plasma AHSg levels

The T2DM group had significantly higher AHSg levels than the control group (348.88±48.18

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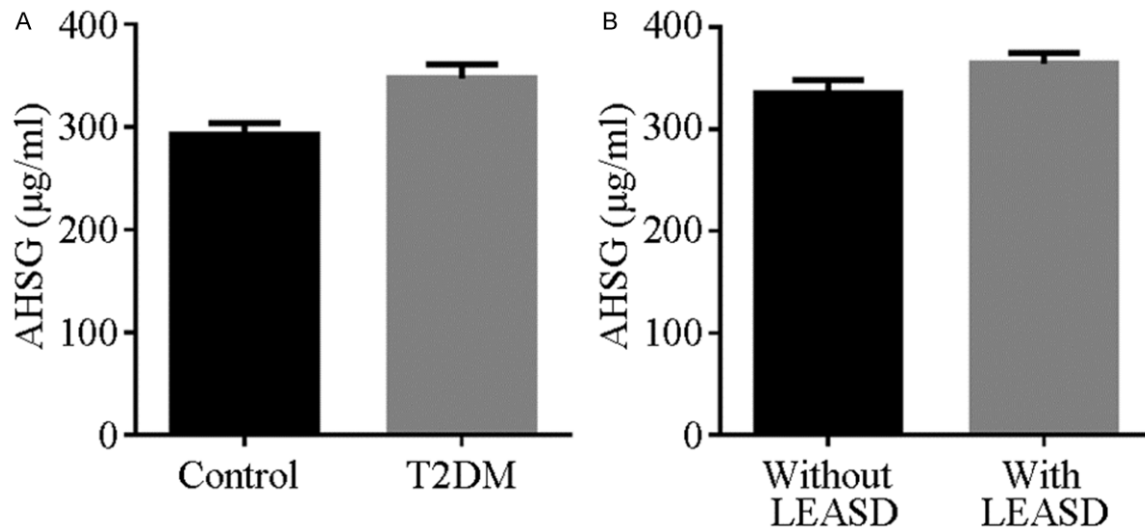


Figure 1. Plasma AHSB levels of the study subjects. Control = normal controls, T2DM = patients with type 2 diabetes, without LEASD = T2DM patients without LEASD, with LEASD = T2DM patients with LEASD.

µg/ml vs. 283.09 ± 40.72 µg/ml, $P < 0.01$) (**Figure 1A**). In addition, T2DM patients with LEASD showed significantly higher AHSB levels than those without LEASD (363.80 ± 48.35 µg/ml vs. 332.48 ± 42.66 µg/ml, $P < 0.01$) (**Figure 1B**). The differences remained significant after adjusting for all other potential confounders using covariance analysis ($P < 0.01$).

Correlation of plasma AHSB levels with clinical parameters

Pearson's correlation analysis revealed that plasma AHSB levels were positively associated with HOMA-IR ($r = 0.175$, $P < 0.05$), AS scores ($r = 0.407$, $P < 0.01$), SBP ($r = 0.196$, $P < 0.01$), DBP ($r = 0.179$, $P < 0.01$), TC ($r = 0.284$, $P < 0.01$), TG ($r = 0.296$, $P < 0.01$), LDL-c ($r = 0.312$, $P < 0.01$), BUN ($r = 0.245$, $P < 0.01$), Cr ($r = 0.273$, $P < 0.01$), and FPG ($r = 0.470$, $P < 0.01$) but negatively with HDL-c ($r = -0.153$, $P < 0.05$) (**Figure 2A-K**). Multivariate linear regression analysis showed that plasma AHSB levels were independently associated with HOMA-IR, BUN, and HDL-c ($t = 2.150$, $P < 0.05$; $t = 3.248$, $P < 0.01$; $t = -1.926$, $P < 0.01$, respectively) (**Table 2**).

Independent factors for T2DM and LEASD-complicated T2DM

Multivariate logistic regression analysis was performed to evaluate the independent factors for T2DM and T2DM complicated by LEASD, respectively. Using backward stepwise analysis, we demonstrated that plasma AHSB levels,

SBP, DBP, TC, TG, LDL-c, and FPG were independently associated with the presence of T2DM (**Table 3**). In addition, AHSB levels, BMI, TG, Cr, and FPG independently contributed to the presence of LEASD-complicated T2DM (**Table 4**).

Discussion

In the present study, we show for the first time that plasma AHSB levels are independently associated with T2DM and LEASD due to T2DM in Yi Chinese patients. This study revealed that AHSB levels were higher in patients with T2DM and patients with T2DM complicated by LEASD than in controls. Moreover, a logistic regression analysis found that AHSB was positively correlated with HOMA-IR and AS scores in correlation analyses and an independent risk factor for T2DM and LEASD due to T2DM. This suggested that increased AHSB might serve as a potential predictor for the presence and development of T2DM and LEASD due to T2DM in Yi Chinese patients. Although the relationship between AHSB and lower extremity peripheral artery disease (PAD) has previously been studied in patients with T2DM, in whom PAD was diagnosed by calculating the ankle brachial index (ABI) and toe brachial index (TBI), these results conflicted with those of other studies [18, 19]. Here, we employed a color Doppler ultrasound instrument for examining both lower extremities of subjects, which provides a more accurate and specific method for detecting early-stage AS, to confirm and extend previous findings. Furthermore, our research subjects

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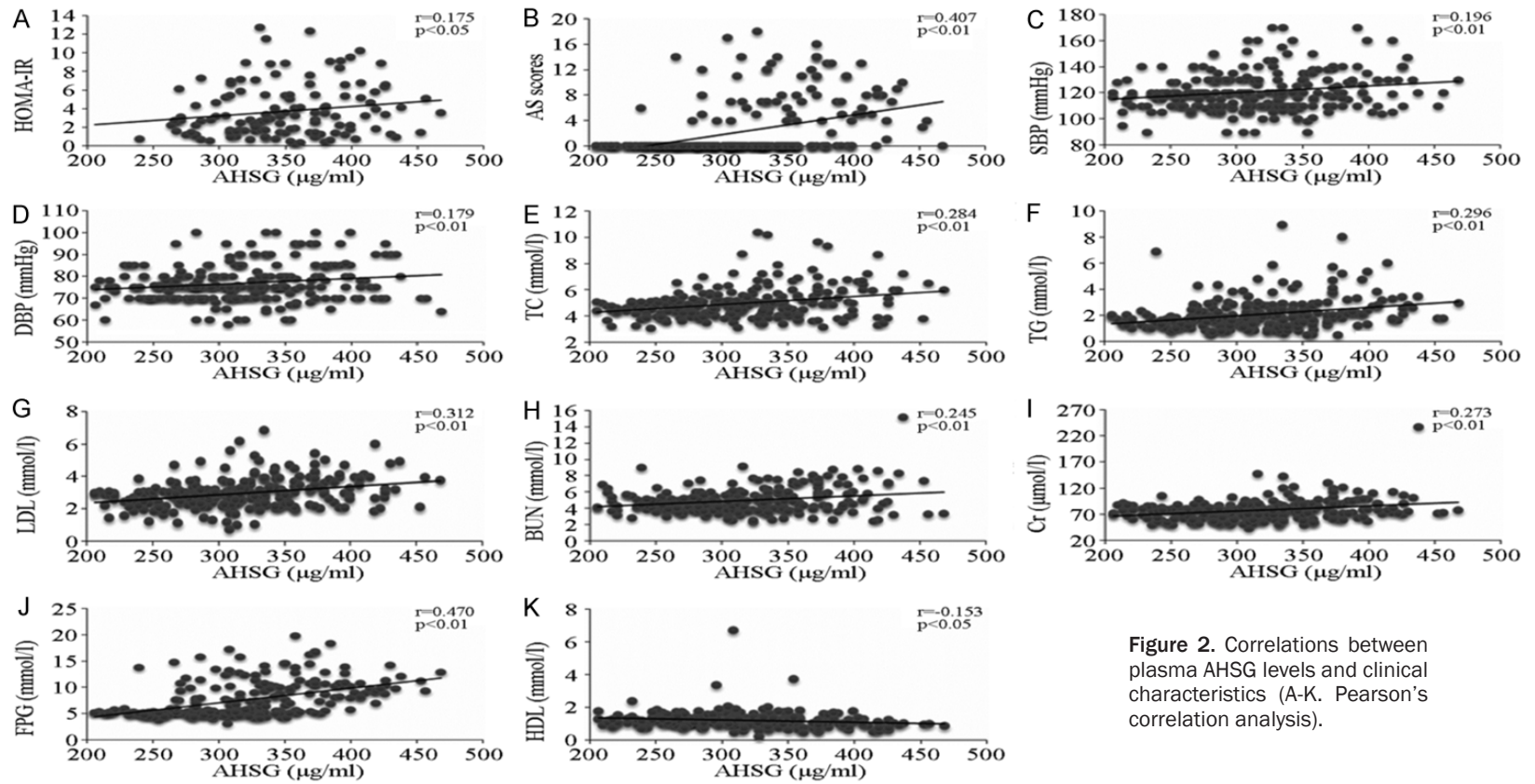


Figure 2. Correlations between plasma AHSG levels and clinical characteristics (A-K. Pearson's correlation analysis).

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Table 2. Multivariate linear regression analysis for the independent factors of AHSG levels

	Unstandardized beta coefficients	Standardized beta coefficients	t	P value
HOMA-IR	3.126	0.181	2.150	0.033
BUN	7.073	0.273	3.248	0.001
HDL-c	-12.092	-0.162	-1.926	0.005

Table 3. Multivariate logistic regression analysis for the independent factors of T2DM

	B	S.E.	P value	OR (95% CI)
AHSG	0.068	0.021	0.001	1.070 (1.027-1.116)
SBP	0.120	0.059	0.041	1.127 (1.005-1.264)
DBP	0.264	0.115	0.021	0.768 (0.613-0.961)
TC	2.682	1.327	0.043	0.068 (0.005-0.921)
TG	2.825	1.151	0.014	16.858 (1.765-161.051)
LDL-c	4.411	1.690	0.009	82.333 (2.997-2261.84)
FPG	4.037	1.050	0.000	56.657 (7.243-443.21)

Table 4. Multivariate logistic regression analysis for the independent factors of T2DM complicated by LEASD

	B	S.E.	P value	OR (95% CI)
AHSG	0.024	0.007	0.000	1.024 (1.011-1.037)
BMI	0.558	0.143	0.000	0.572 (0.432-0.758)
TG	0.734	0.241	0.002	2.084 (1.301-3.341)
Cr	0.068	0.026	0.008	1.070 (1.017-1.125)
FPG	0.552	0.108	0.000	1.736 (1.405-2.147)

showed strong ethnic similarity; all subjects belonged to the Yi minority, and had no migration history or history of marriage with other nationalities within three generations. All resided in the Chuxiong Yi minority autonomous district in Yunnan province.

Currently, T2DM represents a major global public health hazard and, together with obesity, constitutes an important contributor to the expected decline in life expectancy [20]. IR has previously been identified as the major pathophysiologic determinant of T2DM [2]. Our conclusions on the connection between AHSG and T2DM are concordant with previous findings. Brix et al. reported that AHSG levels and HOMA-IR decreased significantly after bariatric surgery-induced weight loss, and that participants with high AHSG levels have an increased risk of incident diabetes [21]. Reinehr et al. demonstrated that adolescents with T2DM showed significantly higher AHSG serum levels than

obese controls without T2DM [22]. Similar results have also been reported in other studies of new-onset T2DM (nT2DM), which showed that nT2DM subjects had significantly higher AHSG levels than normal glucose tolerance (NGT) subjects and that AHSG was positively correlated with HOMA-IR [23]. High AHSG levels were found to be significant-

ly associated with IR in women with T2DM [24]. All of these findings indicate that AHSG is involved in the development of IR and T2DM. This could be explained by the mechanisms of action of insulin and AHSG. Binding of insulin to the insulin receptor leads to glucose uptake through phosphorylation of insulin receptor substrate-1 protein (IRS-1). AHSG inhibits the activity of the insulin receptor tyrosine kinase in muscle and liver tissue by preventing autophosphorylation of tyrosine kinase and IRS-1, inhibits insulin signaling, and induces IR, which results in deterioration of insulin secretion and decompensation of glucose homeostasis [25]. Thus, elevated AHSG levels may be responsible for obesity-induced IR and T2DM.

Nevertheless, the association of AHSG with vascular diseases is controversial. Similar to our findings, AHSG has been found to be positively correlated with carotid artery IMT in patients with non-alcoholic fatty liver disease [26] or in patients with normal renal function [27]. Rittig et al. [28] reported that serum AHSG levels were higher in patients with subclinical AS than in healthy subjects in a middle-aged population. In contrast, low levels of AHSG have been found to be correlated with cardiovascular disease (CVD) in patients undergoing dialysis [29]. Bilgir et al. [30] revealed that decreased serum AHSG is related to coronary artery disease (CAD) including stable angina (SA) and AMI. Lim et al. [31] indicated that a low AHSG concentration is an independent predictor of death after ST elevation AMI. Similarly, conflicting findings have been reported for patients who also suffer from T2DM. Concordant with our data, AHSG was observed to be positively correlated with the risk of CVD among individuals with T2DM [32]. Lorant et al. [18] found that AHSG was higher in T2DM-PAD patients than in T2DM-non-PAD patients. In the Rancho Bernardo study, lower AHSG levels were significantly correlated with a reduced risk of cardiovascular mortality in persons with diabetes [33]. In addition, a subsequent study conducted in pa-

tients with CAD caused by T2DM reported that elevated AHSG could be a predictor for the presence and severity of CAD in patients with T2DM [34]. However, a study by Eraso et al. [19] demonstrated an opposing trend. They found that decreased circulating AHSG was associated with PAD in T2DM, beyond traditional and novel cardiovascular risk factors.

As a calcification inhibitor and a negative acute-phase protein, AHSG has long been considered as a potential protective factor for vascular disease. It maintains solubilization of calcium and phosphorus in the serum and prevents hydroxyapatite deposition in vessel walls [35, 36]. However, AHSG is a physiological inhibitor of insulin receptor tyrosine kinase and is thus associated with IR, metabolic syndrome (MetS), and an increased risk of T2DM. It is well known that T2DM is a risk factor for the development of atherosclerotic lesions and that IR underlies the mechanisms leading to the occurrence of vascular disease in patients with T2DM. Thus, AHSG can also exacerbate vascular disease through metabolic pathways (IR, obesity, and adipocyte dysfunction). The observed discrepancies between research findings might be explained by the dual function of AHSG in vascular disease and its differing roles, depending on the stage of vascular disease. Higher levels of AHSG are associated with vascular risk because its effects to promote IR and dyslipidemia play a primary role in the early stages, whereas the reverse relationship is observed in later-stage patients with established vasculopathy because of its ability to prevent vascular calcium deposition [34, 37]. In the present study, patients with LEASD are mainly comprised of the patients with early-stage vascular disease, who were identified as having LEASD if existing thickening of IMT, sclerosis of vascular wall, and formation of plaques or stenosis were present. In accordance with our results, most studies have tended to demonstrate that patients with vascular lesions due to T2DM show increased AHSG levels. As recently proposed by Vörös et al., AHSG is more likely to be involved in diabetes-related AS via metabolic pathways than via calcification [38].

In summary, our research supports that increased plasma AHSG levels might be used as a novel risk biomarker for the presence and development of T2DM, as well as LEASD due to T2DM. One limitation of this study is that the sample size was relatively small because all

subjects were selected from a minority ethnic group, and larger sample sizes will be necessary for further research to investigate the link between AHSG and macrovascular complication of T2DM in Yi Chinese patients.

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Disclosure of conflict of interest

None.

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