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Gender differences in plasma growth arrest-specific protein 6 levels in adult subjects

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ABSTRACT

Background: Growth-arrest-specific 6 (Gas6) is recognized as a secreted vitamin K-dependent protein, as it interacts with receptor tyrosine kinases of the TAM (Tyro-3, Axl, Mer) family. The plasma Gas6 are important to the inflammatory process, and are involved in diverse human diseases. Few studies have shown plasma Gas6 concentration varies with genders. We determined whether plasma Gas6 concentrations are associated with sex hormones in both genders.
Methods: A total of 589 adult subjects, including 361 male and 228 female were recruited. Plasma Gas6 concentration, biochemical, testosterone, estradiol (E2), and sex hormone-binding globulin were assayed. The indices of free androgen (FAI) and free E2 (FEI) were calculated.
Results: Significantly higher Gas6 concentrations were observed in adult male rather than female ($P < 0.05$). In univariate regression analysis, plasma Gas6 concentrations were positively associated with FAI in male ($\beta = 0.167$, $P = 0.002$) and both E2 and FEI in female ($\beta = 0.384$, $P < 0.001$ and$\beta = 0.292$, $P < 0.001$, respectively). Otherwise, Gas6 concentrations were inversely associated with ages in both genders ($\beta = -0.234$, $P < 0.001$ in male and $\beta = -0.226$, $P = 0.001$ in female, respectively). In multivariate regression analysis, only age in male and E2 in female were independent variables to determine the plasma Gas6 concentrations ($\beta = -0.231$, $P = 0.002$ and $\beta = 0.458$, $P = 0.001$).
Conclusions: Plasma Gas6 is associated with sex hormones in female and ages in male, indicating a potential role of sex hormones and ages involving the Gas6/TAM system.

1. Introduction

Growth-arrest-specific 6 (Gas6) is recognized as a secreted vitamin K-dependent protein, as it interacts with receptor tyrosine kinases of the TAM (Tyro-3, Axl, Mer) family via its C-terminal sex hormone binding globulin (SHBG)-like domain [1]. Gas6 has the highest affinity for Axl among the TAM receptors and their binding can activate the Gas6/Ax1 pathway. Gas6 and soluble form of Axl (sAxl) are present in mice and human circulatory systems and circulate bound to each other in a high affinity complex [2]. Gas6 expression is widespread in many tissues, including immune cells, endothelial cells, vascular smooth muscle cells, and adipocytes [3–5]. The Gas6/TAM system has been implicated in cell survival and proliferation, cell adhesion and migration, homeostasis, and inflammatory cytokine release [1,6]. The Gas6/TAM system has also been implicated in mediating adipocyte survival and proliferation [7,8].

Clinical studies recently indicate that plasma Gas6 concentrations correlate with a number of inflammatory markers among adult patients with systemic inflammatory diseases and cardiovascular disease [9–12]. It is also represented as a surrogate marker of disease activity of autoimmune disorders [13,14]. In our previous studies, we demonstrate that plasma Gas6 concentrations are associated with altered glucose tolerance and inflammation in middle-aged adults [15]. It is also associated with obesity and its related chronic inflammation and metabolic complications in adolescents [16].

However, one study reported the presence of functional androgen-response elements (AREs) in the *GAS6* promoter, and androgen receptor signaling directly regulates Gas6 transcription, which leads to inhibition of vascular calcification in vascular smooth muscle cells [17]. Clauser et al. discovered that plasma Gas6 concentration varies with genders and is decreased with oral contraceptive use [18]. Plasma Gas6 concentrations in premenopausal women without oral contraception are significantly higher than in men, which may be related to up-regulation by estrogens and down-regulation by androgen [18]. Recently, we demonstrated that postmenopausal women have lower plasma Gas6 concentrations than premenopausal women. Endogenous estrogen concentrations

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are directly associated with plasma Gas6 concentrations in both pre- and postmenopausal women [19]. Therefore, these results suggest that plasma Gas6 concentrations might be regulated by sex hormones. Nevertheless, till now, little is known of the clinical significance and association between the Gas6/TAM system, sex hormones and its related binding protein particular in male subjects.

2. Materials and methods

2.1. Subjects and sample collection

A total of 589 apparent healthy adult subjects aged between 18 and 80 y were recruited periodically from the outpatient clinics of Tri-Service General Hospital, Taipei, Taiwan from 2008 to 2011. The participants composed of adult male ($n = 361$) and female ($n = 228$). Other criteria for inclusion into this study were as follows: body mass index (BMI) <35 kg/m^2, absence of infection within the previous weeks, absence of taking contraceptives, hormone replacement therapy, oral anticoagulants and anti-diabetic therapy, including oral hypoglycemic agents, insulin and glucagons-like peptide 1, and absence of malignant tumor history. Exclusion criteria included women who were pregnant or breast feeding; patients with impaired renal function (serum creatinine ≥1.5 mg/dl); patients with abnormal serum aspartate aminotransferase or alanine aminotransferase (2.5 times above the upper normal ranges); patients with acute or chronic pancreatitis; patients with a history of cerebrovascular accident, myocardial infarction or heart failure; patients with autoimmune disorders or psychiatric diseases, including mood disorders and alcoholism; and patients taking concomitant drugs such as beta-blockers, diuretics, cholestyramine or systemic steroids. A 75 g oral glucose tolerance test (OGTT) was performed in all subjects after they had fasted for at least 10 h. According to American Diabetes Association criteria, type 2 diabetes was diagnosed with fasting glucose ≥126 mg/dl or 2 h post-load glucose >200 mg/dl. The institutional review board of the Tri-Service General Hospital approved the protocol and all subjects gave written informed consent.

2.2. Analytic methods

After 10 h of fastening, blood samples were obtained to determine plasma glucose, creatinine, and lipid profiles. Serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C) were measured using the dry, multilayer analytical slide method in the Fuji Dri-Chem 3000 analyzer (Fuji Photo Film Corp.). The intra-assay and inter-assay CVs for LDC-C were 0.8% and 2.5%, respectively. Serum concentrations of high-density lipoprotein cholesterol (HDL-C) were determined by an enzymatic cholesterol assay method after dextran sulfate precipitation. The intra-assay and inter-assay CVs for HDL-C were 1.1% and 1.7%, respectively. The concentrations of HbA_1c were evaluated by the ion-exchange high pressure liquid chromatography (HPLC) method (Bio-Rad Variant II). The intra-assay and inter-assay CVs for HbA_1c were 1.3% and 2.2%, respectively. Plasma glucose concentrations were determined by the glucose oxidase method on a Beckman Glucose Analyzer II (Beckman Instruments). The intra-assay and inter-assay CVs for glucose were 0.6% and 1.5%, respectively.

Plasma testosterone and estradiol (E2) were determined by the radioimmunoassay (BioSource Europe), with detection limit of 0.05 ng/ml and 2 pg/ml, respectively. Intra-assay and inter-assay CVs were <7.0% for both methods. Sex hormone binding globulin (SHBG) was assayed by the immunoradiometric assay (ZenTech s.a.). The intra-assay and inter-assay CVs for SHBG were 2.9 and 4.6% respectively. All concentrations of the above biochemical variables were determined in duplicate, and the values of the two samples were averaged. The free androgen index (FAI) was calculated, the molar ratio of total testosterone/SHBG, which is highly correlated with free testosterone [20]. Similarly, the free E2 index (FEI), the molar ratio of E2/SHBG was calculated, which is also correlated well with free E2 concentrations [20].

Plasma Gas6 protein was measured using DuoSet® ELISA Development kit (R&D Systems) contains the basic components required the development of sandwich ELISA to measure natural and recombinant human Gas6. For each plasma sample, 100 μl was directly transferred to the microtest strip wells of the ELISA plate coated with capture antibody of mouse anti-human Gas6 and incubated for 2 h at room temperature. After three times of washing steps, the detection antibody was added, and the reaction mixture was incubated for 2 h at room temperature. Antibody binding was detected with streptavidin-conjugated horseradish peroxidase and developed with a substrate solution. The reaction was stopped in adding stop solution, and optical density was determined using a microplate reader set at 450 nm. The Gas6 concentration was quantitated by a calibration curve using a human Gas6 standard. Each plasma sample was assayed in duplicate according to the instructions of the manufacturers, and the values were within the linear portion of the standard curve. The intra-assay and inter-assay CVs of Gas6 were 6.5 and 8.5% respectively, mean recovery on 10 patients was 97%, and lower limit of quantification was 0.26 ng/ml).

2.3. Statistical methods

Descriptive results of continuous variables were expressed as means ± SEM. Before statistical analysis, normal distribution and homogeneity of the variables were evaluated using Levene test for quality of variance, and variables were then given a base logarithmic transformation if necessary. The parameters of triglyceride, testosterone, E2, SHBG, FAI, FEI and Gas6 were analyzed and tested for significance on a log scale. An unpaired *t*-test was applied to determine differences in continuous variables between two groups. A univariate and multivariate linear regression analysis was employed with Gas6 as a dependent variable and the other parameters as independent variables. One-way ANOVA was used to determine the trend between Gas6, sex hormones and ages. A two-sided *P*-value <0.05 was considered statistically significant. All statistical analyses were performed using SPSS Statistics 18.0 software.

Table 1
Anthropometric and biochemical data among adult subjects.

	Male ($n = 361$)	Female ($n = 228$)	*P*
Age (y)	46.7 ± 0.8	48.4 ± 1.0	NS
BMI (kg/m^2)	25.5 ± 0.2	25.2 ± 0.2	NS
Waist (cm)	89.2 ± 0.5	85.1 ± 0.6	<0.001
SBP (mm Hg)	131.5 ± 0.9	132.8 ± 1.1	NS
DBP (mm Hg)	83.6 ± 0.6	83.8 ± 0.6	NS
OGTT			NS
FPG (mg/dl)	114.7 ± 1.8	112.7 ± 1.2	NS
2 h BG (mg/dl)	197.1 ± 4.7	182.4 ± 4.2	<0.05
HbA1c (%)	6.1 ± 0.1	6.0 ± 0.1	NS
Diabetes (%)	25.8	23.9	NS
HDL-C (mg/dl)	48.1 ± 1.0	53.1 ± 1.2	<0.001
TC (mg/dl)	189.0 ± 2.2	195.6 ± 2.6	NS
LDL-C (mg/dl)	127.5 ± 2.6	132.5 ± 3.2	NS
Triglyceride (mg/dl)[a]	141.7 ± 4.3	148.3 ± 8.9	NS
Creatinine (mg/dl)	0.9 ± 0.0	0.8 ± 0.0	<0.001
Testosterone (nmol/l)	17.8 ± 0.3	ND	
Estradiol (nmol/l)	ND	56.2 ± 4.0	
FSH (mIU/ml)	ND	39.7 ± 2.1	
SHBG (nmol/l)[a]	20.4 ± 0.6	36.7 ± 1.7	<0.001
FAI	1.1 ± 0.0	ND	
FEI	ND	9.1 ± 0.9	
Gas6 (ng/ml)[a]	22.8 ± 0.7	20.2 ± 0.6	<0.05

Data was showed as mean ± SE
Abbreviations: ND: not done; NS: not significant; BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; OGTT: oral glucose tolerance test; HDL-C: high deNSity lipoprotein-cholesterol; TC: total cholesterol; LDL-C: low density lipoprotein-cholesterol; FSH: follicle-stimulating hormone; SHBG: sex hormone binding globulin; FAI: free androgen index; FEI: free estrogen index; Gas6: growth arrest-specific protein 6.
[a] The logarithms of these variables were used for the analysis.