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The levels of plasma growth arrest-specific protein 6 is associated with insulin sensitivity and inflammation in women

Feng-Chih Kuo[a], *Yi-Jen Hung*[a], *Yi-Shing Shieh*[b,c], *Chang-Hsun Hsieh*[a], *Fone-Ching Hsiao*[a], *Chien-Hsing Lee*[a,*]

[a] *Division of Endocrinology and Metabolism, Department of Internal Medicine, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan*

[b] *School of Dentistry, National Defense Medical Center, Taipei, Taiwan*

[c] *Department of Oral Diagnosis and Pathology, Tri-Service General Hospital, Taipei, Taiwan*

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ABSTRACT

Aims: Vitamin K-dependent growth arrest-specific protein 6 (Gas6) and its receptors of the TAM (TYRO-3/Axl/Mer) family are ubiquitously expressed in immune, cardiovascular, and reproductive systems. They play pivotal roles of regulating tissue homeostasis via anti-inflammatory effects. Recent studies show that the Gas6/TAM system is involved in glucose tolerance-related metabolic disorders. Our aim was to investigate the link between Gas6 protein, insulin sensitivity and inflammatory cytokines in men and women.

Methods: A total of 278 adults (126 men and 152 women) were recruited in this study. Plasma Gas6 concentration and various biochemical, proinflammatory and endothelial markers were measured. Insulin sensitivity was estimated by homeostasis model assessment.

Results: Waist, fasting and 2 h post-load glucose, and glycated hemoglobin (HbA_{1C}) were significantly lower in women than in men. Age, high-density lipoprotein cholesterol, and highly-sensitive C-reactive protein levels were significantly higher in women than in men. Plasma Gas6 levels were negatively correlated with waist ($r = -0.187$, $P = 0.022$), HOMA-IR ($r = -0.171$, $P = 0.035$), interleukin 6 ($r = -0.362$, $P < 0.001$), and E-selectin ($r = -0.216$, $P = 0.008$), while they were positively correlated with insulin sensitivity (QUICKI) ($r = 0.168$, $P = 0.039$) in women, but not in men. Stepwise multiple regression analysis showed that TNF-α was independently correlated with plasma Gas6 levels in both the sexes ($P < 0.001$).

Conclusion: Plasma Gas6 is associated with obesity, insulin sensitivity, inflammation, and endothelial dysfunction in women and may be a general marker of inflammatory conditions in women.

1. Introduction

Metabolic syndrome is a cluster of risk factors including obesity, insulin resistance, dyslipidemia, and hypertension which increases the risk for type 2 diabetes mellitus and cardiovascular disease [1]. Longitudinal data from the Framingham population study have shown that women have a lower risk of coronary artery disease [2] and a recent analysis in Taiwan's population with diabetes from the National Health Insurance (NHI) database indicates that the incidence of

* Corresponding author. Tel.: +886 2 87927182; fax: +886 2 87927183.
E-mail address: doc10383@gmail.com (C.-H. Lee).

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diabetes in men aged 20–59 years was higher than that in age-matched women [3]. Research over the past decade on the role of estrogens indicates that they regulate body fat distribution [4,5], prevent β-cell apoptosis [6], and reduce pro-inflammatory signaling [7,8] with improvement of insulin action [9]. In estrogen receptor α (ERα)-knockout female mice, a critical role of estrogen signaling through ERα on whole-body insulin sensitivity and insulin metabolic signaling in skeletal muscle tissue was shown [10]. Furthermore, the imbalance of ERα/ERβ ratio in the ''metabolic network'' may also lead to the metabolic syndrome [11]. Variations in hormonal status could thus contribute to the sex differences in the risk of developing diabetes and cardiovascular disease, and hence deserve further investigations.

Growth arrest-specific 6 (Gas6) is a new member of the vitamin K-dependent proteins identified from growth-arrested fibroblasts in 1993 [12]. Gas6 and protein S have approximately 42% amino-acid identity and share the same distinctive structural components, including a Gla domain at its amino terminus, followed by four epidermal growth factor (EGF)-like domains, and then by two tandem lamin G domains that are related to those of the sex hormone binding globulin (SHBG) [12]. The Gla domain, a region rich in glutamic acid residues that are γ-carboxylated in a vitamin-K-dependent reaction, binds to phospholipid phosphatidylserine, and stimulates the phagocytosis of apoptotic cells [13]. The C-terminal SHBG-like lamin G domains have been shown to be indispensable for TAM (Tyro-3/Axl/Mer) receptor-binding and for other biological activities [14,15]. Based on sequence similarity, SHBG which binds to both estrogens and androgens with high affinity, may also interact with the TAM receptors [16]. Gas6 was found to interact with all three receptor tyrosine kinases of the TAM family albeit with different affinities. The Axl receptor has the highest affinity for Gas6, followed by Tyro-3 and Mer [17]. The Gas6/TAM system has been recently shown to have a number of diverse functions, including regulation of cell survival, proliferation, migration, adhesion [18], platelet aggregation [19], inflammatory cytokine release, macrophage clearance of apoptotic cells, natural killer cell differentiation [20], and adipocyte development [21], all of which implicate the Gas6/TAM system in the pathogenesis of atherosclerosis, autoimmune disorders, metabolic disorders, and even cancer development [20]. Previous studies from our laboratory have shown that plasma Gas6 levels are associated with adiposity, insulin resistance among overweight and obese adolescents [22] and altered glucose tolerance in middle-aged adults [23]. This may be possibly due to the anti-inflammatory effects of the Gas6/TAM system.

Interestingly, the Gas6/TAM system also modulates gonadotropin-releasing hormone (GnRH) neuron migration [24] and spermatogenesis [25] to improve maturity of reproductive functions. The female sex hormone, estrogen, is particularly known to reciprocally induce Axl expression and provide a survival signal to ER-positive human breast cancer cells [26]. Studies on mouse mammary gland identified that ERα interacted with the estrogen response element (ERE) in the *GAS6* promoter [27]. Additionally, a clinical study showed that women had high baseline Gas6 concentrations and their levels decreased after receiving oral contraceptives [28]. Together, these results suggest a close interaction between the Gas6/TAM system and hormonal status, which could interfere with the pathogenesis of cardiovascular disease and diabetes. However, little is known about the clinical significance of gender-based differences in Gas6 levels. Therefore, we addressed this issue by conducting a cross-sectional study to determine whether plasma Gas6 levels are linked to insulin sensitivity and inflammatory cytokines.

2. Methods

A total of 278 adults (126 men and 152 women) were recruited from the outpatient clinics of Tri-Service General Hospital, Taipei, Taiwan. The sample size was calculated from the formula by Rosner [29]. Since Gas6 values were negatively correlated with endothelial, inflammatory markers and glucose levels in our previous study [23], to detect a correlation between Gas 6 protein and the above variables with a significance level of 0.05 and 90% power, a total of 222 evaluable subjects were needed. Criteria for inclusion into this trial were as follows: 20–75 years of age, body mass index (BMI) $<35\ kg/m^2$, absence of infection within the previous weeks, no consumption of oral anticoagulants and existing anti-diabetic therapy, including oral hypoglycemic agents, insulin and glucagon-like peptide 1, and absence of malignant tumor history. Exclusion criteria included pregnant or breastfeeding women; serum creatinine $\geq$132.6 μmol/L; abnormal serum aspartate aminotransferase or alanine aminotransferase (2.5 times above the upper reference ranges); acute or chronic pancreatitis; a history of cerebrovascular accident, myocardial infarction, or heart failure; autoimmune disorders or psychiatric diseases, including mood disorders and alcoholism; and taking concomitant drugs such as beta-blockers, diuretics, cholestyramine, or systemic steroids. A 75 g oral glucose tolerance test (OGTT) was performed in all patients after they had fasted for at least 10 h.

The institutional review board of the Tri-Service General Hospital approved the protocol and all subjects gave written informed consent.

2.1. *Biomarker assays*

Blood samples were collected following 10 h of fasting. Glucose, insulin, creatinine, inflammatory cytokines and lipid levels were measured. Serum total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C) were measured using the dry, multilayer analytical slide method in the Fuji Dry-Chem 3000 analyzer (Fuji Photo Film Corporation, Tokyo, Japan). Serum levels of high-density lipoprotein cholesterol (HDL-C) were determined by an enzymatic cholesterol assay method after dextran sulfate precipitation. HbA_{1C} was estimated using ion-exchange high-pressure liquid chromatography (HPLC) (BIO-RAD VARIANT II, Los Angeles, CA, USA). Plasma glucose concentrations were determined using the glucose oxidase method on a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA, USA). Plasma insulin was measured using the commercially available immunoradiometric kit (BioSource Europe S.A., Nivelles, Belgium). The intra-assay and inter-assay coefficients of variance (CV) for insulin measurements were 2.2% and 6.5%,