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Preliminary report: Zn-alpha2-glycoprotein genotype and serum levels are associated with serum lipids

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Abstract

Zn-alpha2-glycoprotein (ZAG) is a serum protein implicated in cancer cachexia and lipolysis. Our aim was to investigate serum levels of ZAG and polymorphisms in the ZAG gene in relation to serum lipids in man. Serum levels of ZAG correlated with serum levels of cholesterol ($P = .00088$) in healthy subjects and during weight loss ($P = .059$). The ZAG genotype was associated with total cholesterol ($P = .014$) and low-density lipoprotein cholesterol ($P = .026$) in healthy subjects, and the associations were replicated in an additional cohort ($P = .0017$ and $P = .060$, respectively). Our data indicate that ZAG plays a role in lipid metabolism.

1. Introduction

Zn-alpha2-glycoprotein (ZAG) is a circulating protein implicated in cancer cachexia and lipolysis [1]. Mice that are devoid of ZAG have increased body weight compared with controls [2], and ZAG has been linked to body weight in a mouse model for type 2 diabetes mellitus [3]. A recent study suggests that ZAG is a novel adipokine and that its expression in adipose tissue is down-regulated in obese subjects [1]. Thus, ZAG appears to have diverse metabolic effects. Our objective was to analyze serum levels of ZAG and polymorphisms in the ZAG gene in relation to serum lipids.

2. Methods

In the population-based Swedish Obese Subjects Reference (SOS-Ref) study, 186 healthy subjects were selected for genotyping, and in 228 subjects, serum ZAG levels were analyzed. The ZAG levels were also analyzed in 62 obese subjects before, during, and 2 weeks after a very low calorie diet (VLCD, 450 kcal/d for 16 weeks). Genotyping was also performed in 550 subjects with coronary artery disease and 550 individually age- and sex-matched controls from the Intergene study [4] and 387 patients with myocardial infarction and 387 age- and sex-matched controls from the Stockholm Coronary Atherosclerosis Risk Factor (SCARF) project [5]. The studies were approved by the local ethics committees.

Serum ZAG concentrations were determined by an in-house immunoassay [6] in the VLCD study and by enzyme-linked immunosorbent assay (BioVendor, Modrice, Czech Republic) in the SOS-Ref study. Conserved regions in the ZAG gene were identified by cross-species comparison using PipMaker (http://bio.cse.psu.edu/pipmaker). DNA was

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sequenced with the BigDye Terminator version 3.1 cycle sequencing kit using the ABI Prism 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). The polymorphism rs4215 was genotyped with TaqMan Assays-by-Design, using the ABI Prism 7900HT Sequence Detection System.

In the SOS-Ref study, multivariate linear regression analyses were performed to test for correlation between serum levels of ZAG and lipid levels. Within-person longitudinal dependencies in the VLCD studies were addressed with generalized estimating equations to obtain adjusted standard errors. Tests of parameters using adjusted standard errors were performed using generalized Wald tests. Multivariate linear regression analyses were used to test for association between ZAG genotypes and serum lipids using an additive model where age and sex were covariates.

3. Results and discussion

In healthy subjects, serum levels of ZAG correlated with serum levels of total cholesterol ($\beta = 0.21$, $P = .00088$) and triglycerides (TG) ($\beta = 0.14$, $P = .035$) but not with low-density lipoprotein cholesterol (LDL-C) or high-density lipoprotein cholesterol (HDL-C). During diet-induced weight loss, using a combined analysis of all time points, serum levels of ZAG correlated with LDL-C ($\beta = 2.13$, $P = .033$); and there were borderline correlations to total cholesterol ($\beta = 1.88$, $P = .059$) and HDL-C ($\beta = 1.92$, $P = .055$) but no correlation with serum levels of TG. Furthermore, changes in serum levels of ZAG correlated with changes in serum total cholesterol ($\beta = 0.56$, $P < .0001$) and LDL-C ($\beta = 0.33$, $P < .0001$) in the VLCD study.

Nine polymorphisms were identified in the conserved regions of the ZAG gene, and rs4215, located in an exon, was selected for further analysis. In the SOS-Ref study, rs4215 was associated with total cholesterol and LDL-C but not with HDL-C or TG (Table 1). To replicate these findings, rs4215 was genotyped in subjects from the Intergene and SCARF studies after exclusion of individuals that were on statin treatment or had a coronary event within 90 days before sampling [7]. In the remaining subjects (n = 939), polymorphism rs4215 was once more associated with serum levels of total cholesterol and LDL-C but not with HDL-C or TG (Table 1).

Table 1
Serum lipid levels according to polymorphism rs4215 genotype

SOS-ref	Total cholesterol (mmol/L)	LDL-C (mmol/L)	HDL-C (mmol/L)	TG (mmol/L)
CC n = 76	5.00 ± 0.80*	2.96 ± 0.73†	1.66 ± 0.36	0.84 ± 0.27
CT n = 74	5.29 ± 0.88	3.20 ± 0.82	1.71 ± 0.31	0.85 ± 0.30
TT n = 36	5.51 ± 1.03	3.33 ± 0.99	1.79 ± 0.39	0.87 ± 0.25
Intergene/SCARF				
CC n = 352	5.46 ± 1.05‡	3.50 ± 0.90§	1.40 ± 0.46	1.45 ± 0.80
CT n = 453	5.65 ± 1.06	3.59 ± 0.92	1.40 ± 0.46	1.58 ± 1.00
TT n = 111	5.57 ± 1.02	3.64 ± 0.89	1.35 ± 0.42	1.50 ± 0.76

Analysis of CC vs not-CC using multivariate linear regression where age and sex were covariates.

* $P = .014$.

† $P = .026$.

‡ $P = .0017$.

§ $P = .060$.

There are 4 very recent reports where serum levels of ZAG have been studied [8-11]. Selva et al [8] present a correlation between serum levels of ZAG and adipose tissue ZAG mRNA levels, whereas Ceperuelo-Mallafre et al [9] report the lack of correlation. This makes the relationship between serum levels of ZAG and adipose tissue–derived ZAG unclear. Yeung et al [10] report a correlation between serum levels of ZAG and TG, supporting our data from the healthy individuals. This correlation may arise from the proposed role of ZAG in adipose tissue lipolysis. Stejskal et al [11] investigated serum levels of ZAG in relation to total cholesterol levels but did not find any correlation. In our study, we found stronger relationship between serum levels of ZAG and lipids in the healthy compared with obese subjects. The reason for this is unknown; but we suspect that the difference in number of individuals analyzed may contribute, as we analyzed serum ZAG levels in 228 healthy individuals but only in 62 obese individuals. However, considering the abnormal metabolic profile in obese subjects; it is possible that there are unknown factors that affect lipid levels and disturb the normal relationship between serum ZAG and lipids. Stejskal et al [11] included both healthy subjects and subjects with metabolic syndrome, which may explain the discrepancy between the results of our studies. X-ray crystal structure of ZAG has revealed a binding site for hydrophobic ligands that has been proposed to modify the activity of ZAG [12,13]. It is possible that ligands binding to ZAG alter the properties of the protein and thereby influence ZAG interaction with other proteins or degradation of ZAG, possibly explaining the differences in relationship between serum ZAG levels and lipids in patients with different lipid profiles.

Our findings that serum levels of ZAG and cholesterol correlate in 2 separate studies and that a polymorphism in the ZAG gene is associated with circulating levels of cholesterol in 2 separate cohorts suggest that ZAG is involved in cholesterol metabolism. In obesity, more than half of the cholesterol in the body may be found in adipose tissue [14] and it has been demonstrated that the TG content in adipose tissue is paralleled by cholesterol content [15]. Furthermore, increased lipolysis leads to increased release of cholesterol from adipose tissue [16]. Therefore, it may be speculated that the correlation between serum levels of ZAG and total cholesterol is the result of the increased lipolysis of TG caused by ZAG itself and that the adipocytes adjust their cholesterol content accordingly. On the other hand, we cannot rule out that the observed relationship between ZAG and cholesterol is caused by an unrecognized direct effect of ZAG on cholesterol metabolism.

In conclusion, our data indicate a link between lipids and ZAG that merits further investigation.

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