Int Urol Nephrol
DOI 10.1007/s11255-012-0354-2

NEPHROLOGY - ORIGINAL PAPER

Serum gamma-glutamyltransferase levels are inversely related to endothelial function in chronic kidney disease

Mahmut Ilker Yilmaz · Faruk Turgut · Mehmet Kanbay · Mutlu Saglam · Alper Sonmez · Halil Yaman · Seref Demirbas · Hilmi Umut Unal · Mahmut Gok · Murat Karaman · Seyit Ahmet Ay · Erkan Demirkaya · Adrian Covic · Juan Jesus Carrero

Received: 7 August 2012 / Accepted: 30 November 2012

Abstract
Backgrounds Gamma-glutamyltransferase (GGT) is an enzyme responsible for the extracellular catabolism of the antioxidant glutathione and recently implicated in the pathogenesis of atherosclerosis. Endothelial dysfunction is a prodromal feature of atherogenesis. Since oxidative stress is highly present in uremia and causally linked to endothelial dysfunction, we hypothesized that GGT may be a factor implicated in this process.

Methods Serum GGT and C-reactive protein (CRP) levels, estimated glomerular filtration rate (eGFR), and 24-h proteinuria were measured in 214 nondiabetic stages 3–5 CKD patients. The endothelium-dependent vasodilatation (FMD) of the brachial artery was assessed by using high-resolution ultrasound. We investigated the relationship between FMD and circulating serum GGT.

Results Serum GGT levels were negatively associated with FMD ($r = -0.41$, $p < 0.001$) and eGFR ($r = -0.34$, $p < 0.001$) in univariate analysis.

M. I. Yilmaz · F. Turgut · M. Kanbay · H. U. Unal · M. Gok · E. Demirkaya
Department of Nephrology, Gulhane School of Medicine, Ankara, Turkey

M. Kanbay (✉)
Alparslan Mahallesi, Umit Sokak, No: 25/14, Melikgazi/Kayseri, Turkey
e-mail: drkanbay@yahoo.com

M. Saglam
Department of Radiology, Gulhane School of Medicine, Ankara, Turkey

A. Sonmez
Department of Endocrinology, Gulhane School of Medicine, Ankara, Turkey

H. Yaman
Department of Biochemistry, Gulhane School of Medicine, Ankara, Turkey

S. Demirbas · M. Karaman · S. A. Ay
Department of Internal Medicine, Gulhane School of Medicine, Ankara, Turkey

A. Covic
Nephrology Clinic, Dialysis and Renal Transplant Center, "C.I. PARHON" University Hospital, "Gr. T. Popa" University of Medicine and Pharmacy, Iasi, Romania

J. J. Carrero
Division of Renal Medicine, Department of Clinical Science, Intervention and Technology, Karolinska Institutet, Karolinska University Hospital at Huddinge, 141 86 Stockholm, Sweden

Published online: 15 December 2012

Multivariate regression analysis showed that the association between GGT and FMD persisted after adjustment for age, sex, smoking, renal function (eGFR), inflammation (CRP), proteinuria, and homeostatic model assessment index.
Conclusion Circulating GGT levels significantly associate with endothelial dysfunction, an important early feature of the atherogenic process. GGT might be an early marker of oxidative or other cellular stress that it is possibly directly related to the pathogenesis of endothelial dysfunction.

Keywords Chronic kidney disease · Endothelial dysfunction · Gamma-glutamyltransferase

Introduction

Cardiovascular diseases are a major cause of morbidity and mortality in patients with chronic kidney (CKD) [1]. The mechanisms underlying the increased cardiovascular risk of CKD are not fully explained, but endothelial damage is generally considered a pivotal step in the development of atherosclerosis and subsequent cardiovascular complications [2–4]. Indeed, endothelial dysfunction, as determined by the dilation of the brachial artery following transient occlusion, strongly predicts cardiovascular events independently of arterial pressure levels and other traditional and non-traditional risk factors [5, 6]. Among several relevant contributors to the nearly ubiquitous ED, oxidative stress (also greatly increased in CKD) is an important disruptor of endothelial function in CKD patients [3].

Gamma-glutamyltransferase (GGT) is an enzyme expressed intracellularly in virtually all tissues; the liver being the major source of circulating GGT levels [7]. GGT is responsible for the extracellular catabolism of the antioxidant glutathione (GSH) allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis. Paradoxically, some experimental reports also suggest that cellular GGT may in itself be involved in the generation of reactive oxygen species [8, 9] and altogether a number of epidemiological studies suggest that serum GGT even within its normal range might be an early and sensitive enzyme related to oxidative stress [10].

In clinical practice, an increased serum GGT enzyme activity is conventionally interpreted as a marker of alcohol abuse and liver dysfunction. However, increasing number of prospective studies shows that serum GGT activity directly relates to an increased risk of death, major vascular and non-vascular outcomes, hypertension and diabetes, even after adjustment for alcohol consumption and established risk factors [11–20]. Additionally, well-powered studies have also suggested GGT as a predictor of incident CKD and/or incident microalbuminuria in populations with manifest hypertension or diabetes [21–23]. A study by Postorino et al. [24] demonstrated that GGT levels are important predictors of mortality in dialysis patients. In some of the above-mentioned studies, further adjustments for liver disease and markers of viral hepatitis did not affect the results. Thus, viral hepatitis and alcohol consumption are unlikely to fully explain these associations, supporting the hypothesis that GGT reflects oxidative stress or other pathogenic pathway involved in the process of atherogenesis [25]. Oxidative stress is a prominent feature in uremia and a causal agent in the development of endothelial dysfunction and atherosclerosis [26]. We hypothesized that circulating GGT is related to endothelial dysfunction in CKD 3–4 patients. The present study therefore aims to characterize GGT levels in nondiabetic patients with CKD and investigate plausible associations with flow-mediated dilation (FMD), taken here as a surrogate of endothelial dysfunction.

Materials and methods

Population

Subjects included in this cross-sectional study were prevalent patients with CKD 3–5 referred to the Nephrology outpatient clinics of Gulhane School of Medicine (Ankara, Turkey) between May 2005 and May 2010. All patients received for the first time a diagnosis of CKD, and estimated glomerular filtration rate (eGFR) was calculated by the simplified version of the Modification of Diet, in Renal Disease formula as defined by Levey et al. [27]. Patients taking renin–angiotensin system blockers, oral contraceptives and anticonvulsants, and consuming alcohol were excluded from the study. Patients with established

atherosclerotic complication (coronary artery disease, congestive heart failure, or peripheral vascular disease), nephrotic syndrome, diabetes mellitus, cirrhosis, cholestatic jaundice and on dialysis were neither included in order to reduce confounders that may influence the association between GGT and endothelial function. After exclusion criteria, 214 nondiabetic CKD patients were eventually selected for the study. Body mass index (BMI) (weight in kilograms divided by height in meters squared) was calculated for each subject.

Blood pressure measurements

A physician measured the arterial blood pressure three times after a 15-min resting period in the morning, and mean values were calculated for systolic blood pressure (SBP) and diastolic blood pressure (DBP) for all participants. Hypertension was defined as SBP $>$ 140 mmHg or DBP $>$ 90 mmHg on repeated measurements or the use of antihypertensive drugs.

Laboratory measurements

Blood samples (6–8 ml) from venous blood were collected in the morning between 8:00 a.m. and 9:00 a.m., after an overnight fast from all patients. After clot formation, the samples were centrifuged (4,000 rpm) at room temperature for 10 min. The serum samples were stored at −80 °C until the time of the assay. In all patients, we measured fasting serum GGT, glucose, low-density lipoprotein (LDL) cholesterol, s-albumin, high-sensitivity C-reactive protein (hsCRP), urea, creatinine, and proteinuria by using original kits. The normal reference value of the serum GGT level for a healthy subject was 5–65 U/L in our laboratory. Twenty-four-hour urine collection was performed 3 times, and the average of three 24-h proteinuria measurements was taken as representative of each participant 24-h protein excretion rate. Basal insulin level was measured by the coated tube method (DPC-USA), and an insulin resistance score *homeostasis model assessment-insulin resistance* (HOMA-IR) was calculated [28].

Brachial artery measurements

The endothelium-dependent vasodilatation (FMD) of the brachial artery was assessed by using high-resolution ultrasound according to the method of Celermajer et al. [29]. Measurements of FMD were made by a single observer using an ATL 5000 ultrasound system (Advanced Technology Laboratories, Bothell, WA) with a 12-MHz probe. All vasoactive medications were withheld for 24-h before the procedure. The subjects remained at rest in the supine position for at least 15 min before the examination started. The participant's arm was comfortably immobilized in the extended position to allow consistent recording of the brachial artery 2–4 cm above the antecubital fossa. Three adjacent measurements of end-diastolic brachial artery diameter were made from single two-dimensional frames. All ultrasound images were recorded on S-VHS videotape for subsequent blinded analysis. A pneumatic tourniquet was inflated to 200 mmHg with obliteration of the radial pulse. After 5 min, the cuff was deflated. Flow measurements were made at 60 s after deflation. The maximum FMD dilation diameters were calculated as the average of the three consecutive maximum diameter measurements. The FMD was then calculated as the percentage change in diameter compared with baseline resting diameters. All measurements were performed by a single investigator blinded to clinical and biochemical data of the patients. Intraobserver variability for brachial artery measurements was 3 %.

Statistical analyses

All analyses were conducted using SPSS 15.0 (SPSS Inc., USA). Unless otherwise stated, all data are presented as mean ± standard deviation (SD). The groups were compared using the Student's *t*-test for continuous variables and chi-squared test for categorical variables. Pearson's coefficient of correlation analyses were used to test univariate relations. The nonparametric Spearman's rho coefficient of correlation was used to assess correlations between variables with non-normal distribution. Significant determinants identified from this analysis were studied in a stepwise multiple regression model using the F statistics. All variables associated with these parameters with a level of significance <0.1 were included in the tested model. Variables were forced in the model using a stepwise procedure. A $p < 0.05$ for the final model was considered as statistically significant.

Results

A total of 214 newly diagnosed stages 3–5 CKD patients were included in the study. Seventy-seven (36 %) patients had stage 3 CKD, 63 (29.4 %) stage 4 CKD, and 74 (34.6 %) stage 5 CKD. None of the patients had acute infection at the time of the study. As per protocol, 190 (89 %) patients were not using any antihypertensive drug, 20 (9.3 %) patients were using calcium channel blocker, 2 (0.9 %) patients were using beta-blocker, and 2 (0.9 %) patients were using loop diuretics. None of the patients were on treatment with erythropoietin-stimulating agents. Demographics of the patients were shown in Table 1.

The mean serum GGT level in this population was 39.7 ± 16.4 U/L (range 12.9–92.5 U/L). We divided the patients into two groups according to median serum GGT level (34.1 U/L). Age, gender, BMI, hypertension, and smoking status were similar in both groups (Table 1). Blood pressure, hsCRP, LDL-cholesterol, glucose, and s-albumin levels were also similar (Table 2). Proteinuria and eGFR were significantly higher, and HOMA-IR was significantly lower in the patients with GGT below the median value. Also, FMD levels were significantly higher in patients with low GGT as compared with those with high GGT (6.7 ± 0.8 vs 6.0 ± 0.9, $p < 0.0001$).

Correlation analysis

Serum GGT levels were closely associated with FMD ($r = -0.41, p < 0.001$, Fig. 1) and eGFR ($r = -0.34$, $p < 0.001$), but were not related to age, BMI, blood pressure, HOMA-IR, LDL-cholesterol, hsCRP, or proteinuria.

To determine the independent contribution of serum GGT level to FMD variance, we constructed a series of multiple regression models based on traditional and non-traditional risk factors. All parameters that significantly correlated with the FMD, as well as other risk factors considered physiologically as potentially relevant for FMD, were introduced in a standard multivariate regression analysis with a three step procedure, using the enter method (Table 3). In a first step, we evaluated the independent influence of Framingham risk factors in the association between serum GGT level and FMD: serum GGT level ($\beta = -0.40$; $p < 0.001$) and SBP ($\beta = -0.13$, $p = 0.03$) were the only statistically significant independent predictors of FMD. In a second step, we further adjusted for the presence of proteinuria and eGFR, and this time serum GGT ($\beta = -0.24$, $p < 0.001$) and eGFR ($\beta = 0.49$, $p < 0.001$) remained as statistically significant FMD predictors. In a third step, we further adjusted for the presence of HOMA-IR and hsCRP, and in this final model, serum GGT ($\beta = -0.26$, $p < 0.001$), hsCRP ($\beta = -0.13, p = 0.02$), and eGFR ($\beta = 0.42$, $p < 0.001$) remained as significant and independent predictors of FMD.

Discussion

This cross-sectional study demonstrates, for the first time in CKD and in any other pathology, that serum GGT levels strongly and inversely associate with endothelial function as assessed by FMD. Importantly, this strong inverse association was not confounded by various traditional and non-traditional cardiovascular risk factors. The results are compatible with the hypothesis that the link between GGT and adverse clinical outcomes depends on the fundamental role of this enzyme in oxidative stress mechanism(s).

Several biological mechanisms may explain the association of serum GGT with endothelial function in our study. GGT has been found in atherosclerotic

Table 1 Demographics of the patients according to median GGT values

Parameters	All patients ($n = 214$)	Patients with lower GGT ($n = 107$)	Patients with higher GGT ($n = 107$)
Sex (men)	100	50	50
Age (years)	52 ± 12	52.4 ± 12	52.1 ± 12
BMI (kg/m^2)	25.9 ± 2.7	25.9 ± 2	25.8 ± 2
Hypertension (yes/no)	28/186	15/92	13/92
Smokers (n)	93	45	48

GGT gamma-glutamyltransferase, *BMI* body mass index, no significant differences were found between the groups

Table 2 Laboratory parameters of the patients according to median GGT values

Parameters	All patients ($n = 214$)	Patients with lower GGT ($n = 107$)	Patients with higher GGT ($n = 107$)	p value
SBP (mmHg)	133 ± 10	132 ± 10	135 ± 10	NS
DBP (mmHg)	84 ± 5	84 ± 5	84 ± 4	NS
HOMA-IR	1.6 ± 0.7	1.6 ± 0.7	1.7 ± 0.6	0.04
eGFR (mL/min/ 1.73 m^2)	26 ± 17	33 ± 16	20 ± 15	<0.0001
Proteinuria (mg/24 h)	1949 ± 1137	2068 ± 1153	1830 ± 1114	0.03
hsCRP (mg/L)	20 ± 7	19 ± 6	21 ± 8	NS
LDL-cholesterol (mg/dL)	126 ± 16	125 ± 16	126 ± 17	NS
Glucose (mg/dL)	95 ± 27	94 ± 28	96 ± 26	NS
Albumin (g/dL)	4.0 ± 0.3	4.0 ± 0.3	3.9 ± 0.3	NS
GGT (U/L)	39 ± 16	27 ± 5	52 ± 13	–

GGT gamma-glutamyltransferase, *eGFR* estimated glomerular filtration rate, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *HOMA-IR* homeostasis model assessment-insulin resistance, *hsCRP* high-sensitivity C-reactive protein, *NS* not significant

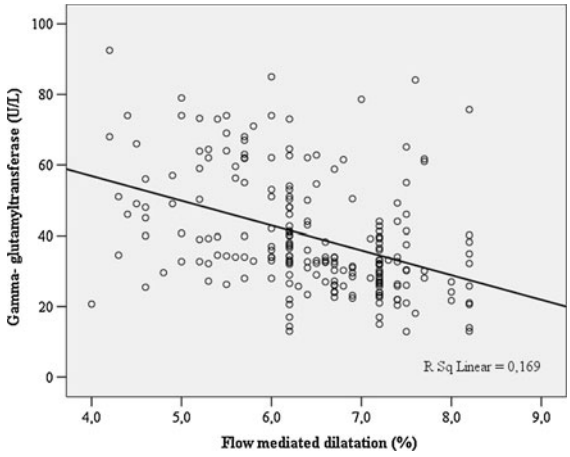

Fig. 1 Linear regression showing the association between serum GGT levels and FMD in nondiabetic nondialyzed CKD patients stages 3–5

plaques and suggested to catalyze oxidation of LDL lipoproteins, contributing to plaque evolution and rupture [30]. GGT is also present on the surface of most cell types and is the enzyme responsible for the extracellular catabolism of the antioxidant glutathione. Circulating GGT has been implicated in the generation of reactive oxygen species, and evidence supports its role as a marker of systemic oxidative stress [10]. In light of this, it is also possible to hypothesize that the strong association of serum GGT with endothelial function in our study might be partially explained by a mechanism related to oxidative stress [30–32]. This conclusion is indirectly supported by prospective studies showing a stronger association of serum GGT with diabetes risk and vascular outcomes than alanine-aminotransferase (ALT), which is instead considered a marker of liver injury but not of systemic oxidative stress [33, 34]. Other recent studies hypothesize that serum GGT activity can mark exposure to various environmental pollutants [35, 36]. Cellular GGT is a pre-requisite for metabolism of GSH conjugates that detoxify xenobiotics to mercapturic acid. According to this concept, serum GGT may increase with increasing exposure to environmental pollutants, which need to be conjugated to GSH. This principle nevertheless may also apply to the detoxification of uremic toxins.

Previous studies reported that GGT associates with age, obesity, serum cholesterol, blood pressure, and microalbuminuria [37–39]. In contrast, our study in a selected population of advanced CKD patients could not observe such associations. When interpreting these results, we should first allude to the design of our study, whose strict exclusion criteria aimed to study putative and unconfounded associations with endothelial dysfunction. Therefore, our selected population may not be representative of the average advanced CKD patients. Additionally, we found an inverse and significant association between serum GGT level and eGFR, being plausible that reduced kidney function per se or retention of other substances alters the overall redox status in general and/or the synthesis and

Table 3 Multiple regression models showing the association between GGT and flow-mediated dilatation in CKD 3–5 patients

	Crude (β, p)	Model 1 (β, p) ($r^2 = 0.22$)	Model 2 (β, p) ($r^2 = 0.43$)	Model 3 (β, p) ($r^2 = 0.45$)
Serum GGT	−0.41 (<0.001)	−0.40 (<0.001)	−0.24 (<0.001)	−0.26 (<0.001)
Age		0.05 (0.36)	0.06 (0.21)	0.04 (0.35)
Sex		0.05 (0.42)	0.04 (0.41)	0.05 (0.27)
Systolic blood pressure		**−0.13 (0.03)**	−0.06 (0.23)	−0.06 (0.23)
LDL-cholesterol		0.10 (0.10)	0.04 (0.40)	0.05 (0.35)
Smoking		0.02 (0.70)	0.03 (0.55)	0.01 (0.82)
Body mass index		0.04 (0.42)	0.04 (0.40)	0.05 (0.28)
eGFR			0.49 (**<0.001**)	0.42 (**<0.001**)
Proteinuria			0.05 (0.29)	0.05 (0.31)
C-reactive protein				**−0.13 (0.02)**
HOMA-IR				−0.02 (0.63)

GGT gamma-glutamyltransferase, *eGFR* estimated glomerular filtration rate, *HOMA-IR* homeostasis model assessment-insulin resistance, *hsCRP* high-sensitivity C-reactive protein

extracellular deposition of GGT in particular. Further studies are needed to clarify this issue. Additional limitations include the lack of screening for hepatitis C antibodies and the cross-sectional nature of our analysis, which cannot preclude causality in these associations. Thus, we cannot rule out the possibility that increased ectoplasmic GGT may be the consequence of intracellular stress in the disturbed endothelium. Nevertheless, the current understanding of the atherogenic process contemplates oxidative stress as an initiating insult in the disturbances that lead to the atheromatous plaque formation [3, 4, 32].

In conclusion, this study in advance CKD patients demonstrates that serum GGT level is strongly associated with endothelial dysfunction, an important early feature of the atherogenic process. Overall, the current evidence suggests that increased serum GGT activity might be a marker rather than a true causal risk factor for vascular outcomes. Thus, we speculate that serum GGT might be an early marker of oxidative or other cellular stress and that it is possibly directly related to the pathogenesis of endothelial dysfunction. Still, serum GGT activity remains a non-specific laboratory test, but our study evidences its potential as an inexpensive marker to monitor endothelial dysfunction and ultimately cardiovascular risk. A recently described methodology based on gel filtration chromatography permitted quantification of four GGT fractions of different molecular weight in the plasma of healthy volunteers [40]. Plasma GGT fraction analysis could improve the specificity of current GGT assays, contributing to a better understanding of the complex links between this enzyme and vascular outcomes.

Acknowledgments We thank the patients and personnel involved in the creation of this patient material. The authors would like to express their sincere appreciation to FAVOR (FMF Arthritis Vasculitis and Orphan Diseases Research/ www.favor.org.tr) web registries at Gulhane Military Medical Academy, Institute of Health Sciences for their supports in epidemiological and statistical advisory and invaluable guidance for the preparation of the manuscript. We acknowledge support from the Gülhane School of Medicine in Turkey, the Swedish Research Council, and the Loo and Hans Osterman Foundation.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Tonelli M, Wiebe N, Culleton B, House A, Rabbat C, Fok M, McAlister F, Garg AX (2006) Chronic kidney disease and mortality risk: a systematic review. J Am Soc Nephrol 17:2034–2047
2. Ross R (1999) Atherosclerosis—an inflammatory disease. N Engl J Med 340:115–126
3. Yilmaz MI, Saglam M, Caglar K, Cakir E, Sonmez A, Ozgurtas T, Aydin A, Eyileten T, Ozcan O, Acikel C, Tasar M, Genctoy G, Erbil K, Vural A, Zoccali C (2006) The determinants of endothelial dysfunction in CKD: oxidative stress and asymmetric dimethylarginine. Am J Kidney Dis 47:42–50
4. Sahin EBF, Karaman M, Ay AS, Saglam M, Cakir E, Yilmaz MI (2010) The relationship between endothelial dysfunction and pentraxin-3 concentration in chronic kidney disease. Turk Nephrol Dial Transpl 19:192–198

5. Perticone F, Ceravolo R, Pujia A, Ventura G, Iacopino S, Scozzafava A, Ferraro A, Chello M, Mastroroberto P, Verdecchia P, Schillaci G (2001) Prognostic significance of endothelial dysfunction in hypertensive patients. Circulation 104:191–196
6. Zoccali C (2006) Endothelial dysfunction and the kidney: emerging risk factors for renal insufficiency and cardiovascular outcomes in essential hypertension. J Am Soc Nephrol 17(Suppl 2):S61–S63
7. Whitfield JB (2001) Gamma glutamyl transferase. Crit Rev Clin Lab Sci 38:263–355
8. Drozdz R, Parmentier C, Hachad H, Leroy P, Siest G, Wellman M (1998) Gamma-glutamyltransferase dependent generation of reactive oxygen species from a glutathione/transferrin system. Free Radic Biol Med 25:786–792
9. Dominici S, Valentini M, Maellaro E, Del Bello B, Paolicchi A, Lorenzini E, Tongiani R, Comporti M, Pompella A (1999) Redox modulation of cell surface protein thiols in U937 lymphoma cells: the role of gamma-glutamyl transpeptidase-dependent H_2O_2 production and S-thiolation. Free Radic Biol Med 27:623–635
10. Lee DH, Blomhoff R, Jacobs DR (2004) Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic Res 38:535–539
11. Caliskan M, Erdogan D, Gullu H, Ciftci O, Yildirim I, Baycan S, Yildirir A, Muderrisoglu H (2007) Association between serum gamma-glutamyltransferase levels and coronary microvascular function in hypertensive patients. J Hypertens 25:2497–2503
12. Caliskan M, Erdogan D, Gullu H, Muderrisoglu H (2008) Higher serum gamma-glutamyltransferase levels are independently associated with impaired coronary microvascular function in patients with dilated cardiomyopathy. Atherosclerosis 201:163–167
13. Emdin M, Pompella A, Paolicchi A (2005) Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: triggering oxidative stress within the plaque. Circulation 112:2078–2080
14. Emdin M, Passino C, Michelassi C, Titta F, L'abbate A, Donato L, Pompella A, Paolicchi A (2001) Prognostic value of serum gamma-glutamyl transferase activity after myocardial infarction. Eur Heart J 22:1802–1807
15. Meisinger C, Döring A, Schneider A, Löwel H, Group KS (2006) Serum gamma-glutamyltransferase is a predictor of incident coronary events in apparently healthy men from the general population. Atherosclerosis 189:297–302
16. Ruttmann E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H (2005) Group VHMaPPS. Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. Circulation 112:2130–2137
17. Lee DH, Silventoinen K, Hu G, Jacobs DR, Jousilahti P, Sundvall J, Tuomilehto J (2006) Serum gamma-glutamyltransferase predicts non-fatal myocardial infarction and fatal coronary heart disease among 28,838 middle-aged men and women. Eur Heart J 27:2170–2176
18. Perry IJ, Wannamethee SG, Shaper AG (1998) Prospective study of serum gamma-glutamyltransferase and risk of NIDDM. Diabetes Care 21:732–737
19. Lee DH, Jacobs DR, Gross M, Kiefe CI, Roseman J, Lewis CE, Steffes M (2003) Gamma-glutamyltransferase is a predictor of incident diabetes and hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Clin Chem 49:1358–1366
20. Wannamethee SG, Shaper AG, Lennon L, Whincup PH (2005) Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. Diabetes Care 28:2913–2918
21. Lee DH, Jacobs DR, Gross M, Steffes M (2005) Serum gamma-glutamyltransferase was differently associated with microalbuminuria by status of hypertension or diabetes: the Coronary Artery Risk Development in Young Adults (CARDIA) Study. Clin Chem 51:1185–1191
22. Ryu S, Chang Y, Kim DI, Kim WS, Suh BS (2007) Gamma-glutamyltransferase as a predictor of chronic kidney disease in nonhypertensive and nondiabetic Korean men. Clin Chem 53:71–77
23. Targher G, Chonchol M, Bertolini L, Rodella S, Zenari L, Lippi G, Franchini M, Zoppini G, Muggeo M (2008) Increased risk of CKD among type 2 diabetics with nonalcoholic fatty liver disease. J Am Soc Nephrol 19:1564–1570
24. Postorino M, Marino C, Tripepi G, Zoccali C (2008) Gammaglutamyltransferase in ESRD as a predictor of all-cause and cardiovascular mortality: another facet of oxidative stress burden. Kidney Int Suppl 111:S64–S66
25. Targher G (2010) Elevated serum gamma-glutamyltransferase activity is associated with increased risk of mortality, incident type 2 diabetes, cardiovascular events, chronic kidney disease and cancer—a narrative review. Clin Chem Lab Med 48:147–157
26. Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM (2002) The elephant in uremia: oxidant stress as a unifying concept of cardiovascular disease in uremia. Kidney Int 62: 1524–1538
27. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 130:461–470
28. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412–419
29. Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, Lloyd JK, Deanfield JE (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 340:1111–1115
30. Franzini M, Corti A, Martinelli B, Del Corso A, Emdin M, Parenti GF, Glauber M, Pompella A, Paolicchi A (2009) Gamma-glutamyltransferase activity in human atherosclerotic plaques–biochemical similarities with the circulating enzyme. Atherosclerosis 202:119–127
31. Paolicchi A, Emdin M, Passino C, Lorenzini E, Titta F, Marchi S, Malvaldi G, Pompella A (2006) Beta-lipoprotein- and LDL-associated serum gamma-glutamyltransferase in patients with coronary atherosclerosis. Atherosclerosis 186:80–85
32. Turgut O, Yilmaz A, Yalta K, Karadas F, Birhan Yilmaz M (2006) Gamma-glutamyltransferase is a promising biomarker for cardiovascular risk. Med Hypotheses 67:1060–1064

33. Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G, Lawlor DA (2009) Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. Diabetes Care 32:741–750
34. Fraser A, Harris R, Sattar N, Ebrahim S, Smith GD, Lawlor DA (2007) Gamma-glutamyltransferase is associated with incident vascular events independently of alcohol intake: analysis of the British Women's Heart and Health Study and Meta-Analysis. Arterioscler Thromb Vasc Biol 27: 2729–2735
35. Lee DH, Steffes MW, Jacobs DR (2008) Can persistent organic pollutants explain the association between serum gamma-glutamyltransferase and type 2 diabetes? Diabetologia 51:402–407
36. Lee DH, Jacobs DR (2009) Is serum gamma-glutamyltransferase a marker of exposure to various environmental pollutants? Free Radic Res 43:533–537
37. Daeppen JB, Smith TL, Schuckit MA (1998) Influence of age and body mass index on gamma-glutamyltransferase activity: a 15-year follow-up evaluation in a community sample. Alcohol Clin Exp Res 22:941–944
38. Nilssen O, Førde OH, Brenn T (1990) The Tromsø Study. Distribution and population determinants of gamma-glutamyltransferase. Am J Epidemiol 132:318–326
39. Diercks GF, van Boven AJ, Hillege JL, de Jong PE, Rouleau JL, van Gilst WH (2002) The importance of microalbuminuria as a cardiovascular risk indicator: a review. Can J Cardiol 18:525–535
40. Franzini M, Ottaviano V, Fierabracci V, Bramanti E, Zyw L, Barsacchi R, Scatena F, Boni C, Mammini C, Passino C, Pompella A, Emdin M, Paolicchi A (2008) Fractions of plasma gamma-glutamyltransferase in healthy individuals: reference values. Clin Chim Acta 395:188–189