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Review

Gamma-glutamyl transferase and the risk of atherosclerosis and coronary heart disease

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ABSTRACT

Gamma-glutamyl transferase (GGT) is a ubiquitous cell surface enzyme that cleaves extracellular glutathione (G-SH) or other gamma-glutamyl compounds. GGT serves to increase the availability of amino acids, primarily cysteine, for intracellular G-SH synthesis and plays a crucial role in maintaining G-SH homeostasis and defense against oxidative stress in organisms. Measurement of circulating GGT activity is widely used for the diagnosis of liver and obstructive biliary diseases and as an indicator of alcohol consumption. Epidemiological studies suggest an association between elevated GGT activity level and a risk of incident coronary heart disease (CHD) or CHD-related mortality. Elevated GGT activity level is associated with a plethora of cardio-metabolic risk factors, including traditional cardiovascular risk factors, metabolic syndrome, systemic inflammation, oxidative stress burden and various comorbidities that incur a negative impact on patient risk profile and prognosis. Experimental studies and studies of human atherosclerotic plaques have revealed not only the presence of catalytically active GGT in atherosclerotic plaques, but also a correlation between GGT activity and indices of plaque instability, suggesting direct involvement in the pathophysiology of atherosclerosis and related clinical events via promotion of pro-oxidant reactions by the enzyme. However, it remains unknown whether GGT plays a direct role in the pathophysiology of atherosclerosis and CHD or is merely a correlate of coexisting cardiovascular risk factors. The exact molecular mechanisms of GGT participation in atherosclerosis or CHD and assessment of GGT-lowering therapies, as well as their impact on clinical outcomes, remain to be investigated in longitudinal studies.

1. Introduction

Gamma-glutamyl transferase (GGT; Enzyme Commission number [EC] 2.3.2.2.) is a ubiquitous enzyme that plays a crucial role in the metabolism of glutathione (G-SH) - the most important cellular antioxidant in humans. While the current nomenclature recommends the use of the name gamma-glutamyl transferase, some authors continue to use the older name gamma-glutamyl transpeptidase. Although cleavage of G-SH by extracts obtained from rat kidney was initially described > 80 years ago [1,2], subsequent studies by Hanes et al. [3] in sheep kidney extracts are credited with the characterization of the transpeptidase reaction and nomenclature of the enzyme. The measurement of circulating GGT activity is widely used for the diagnosis of liver and obstructive biliary diseases and as a marker of alcohol consumption. Aside from its diagnostic uses, GGT has attracted interest mainly for its association with diabetes and metabolic syndrome, cancer, atherosclerosis, and cardiovascular disease. The primary focus of this review is to summarize the current status of knowledge regarding the association of GGT with atherosclerosis and coronary heart disease (CHD) risk. The association between GGT and other cardiovascular diseases such as congestive heart failure, arterial hypertension, embolic disease, stroke, arrhythmias or sudden cardiac death and diabetes has been recently reviewed [4–7] and is therefore not addressed in the current review. After a brief description of the structure and metabolic role of GGT, the review will focus on epidemiological evidence linking GGT with CHD as well as the pathophysiological mechanisms of GGT involvement in atherosclerosis or CHD.

2. GGT structure and function

GGT is a cell surface N-terminal nucleophile hydrolase that cleaves extracellular G-SH and gamma-glutamyl compounds (glutathione-conjugates or other gamma-glutamyl substrates) from various sources. In fact, GGT has wide specificity and cleaves the gamma-glutamyl bond in all substrates in which the glutamate moiety is unfettered. GGT cleaves G-SH by transfer of the gamma-glutamyl moiety from G-SH to various

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acceptors, including amino acids, peptides or water releasing gamma-glutamyl products (or free glutamate) and dipeptide cysteinyl-glycine, the latter of which is further hydrolyzed by dipeptidase into free cysteine and glycine. Apart from reduced G-SH, the other known substrates of GGT are oxidized G-SH (G-S-S-G), glutathione-S-drug compounds, leukotriene C4, glutathione-S-nitric oxide and gamma-glutamyl-taurine [8].

Physiological functions of GGT are only partially known. The most important metabolic action of the enzyme is cleavage of G-SH. The high expression of the enzyme on the apical surface of tissues with transport function – tubular structures such as the proximal tubules of the nephron or the hepatocyte microtubular system – has led to the hypothesis that GGT is involved in the transport of amino acids across cell membranes [9]. However, this hypothesis is weakened by documentation of normal amino acid transport in humans and animals with GGT deficiency. According to current knowledge, GGT is important because it increases the availability of amino acids such as cysteine [10], a rate limiting substrate for intracellular G-SH synthesis (glutamate and glycine are readily supplied in the cell by products of glycolysis). The high activity of GGT on the surface of cells lining the proximal kidney tubules enables cleavage of G-SH present in glomerular filtrate, preventing its elimination from the organism, thereby conserving amino acids - particularly cysteine – for its intracellular synthesis [10]. High G-SH concentrations in the plasma and urine of patients with GGT deficiency [11] – a very rare autosomal recessive disease [12] – and GGT gene knockout mice [13] have been reported. In addition, GGT is involved in the metabolism of leukotrienes (namely conversion of leukotriene C_4 into leukotriene D_4), xenobiotics, neurotransmitters (conversion of gamma-glutamyl-taurine into taurine) and modulation of nitric oxide signaling [14–16]. In various pathological conditions, GGT may be mislocalized and may act on substrates in locations such as serum or interstitial fluids where involvement of the enzyme in pathological processes like ischemia-reperfusion injury, airway hyper-reactivity in asthma, drug nephrotoxicity (through conversion of drug-conjugates to nephrotoxins) or resistance to antitumor drugs has been suggested [10,17]. GGT expression in atherosclerotic plaques is covered in detail later in this review.

Mammalian GGT is a heterodimeric glycoprotein anchored to the outer surface of the plasma membranes of all cells through a small N-terminal transmembrane domain. Human GGT is synthetized as a single 569 amino acid residue polypeptide which is enzymatically inactive. The activation process consists of a post-translational autocleavage reaction catalysed by threonine 381 residue. Mature GGT has a molecular weight of 68 kDa and consists of 2 subunits: a large subunit weighing 46 kDa, responsible for enzyme anchorage on cellular membranes through a hydrophobic transmembrane domain, and a small subunit weighing 22 kDa that carries the catalytic center. The mature enzyme has 7 glucan moieties linked by N-glycosylation bonds localized on the external surface of the protein which are important for proper protein folding and activation proces, in addition to 4 cysteine residues, which stabilize the structure through formation of disulfide bonds [18–20]. The degree of glycosylation differs between molecules, with resulting variations in molecular weight, tissue specificity or disease-related molecular variants of the enzyme. Details of structural organization of GGT are described elsewhere [18,20,21]].

Human GGT is encoded by a multigene family consisting of 7 different genes or pseudogenes located on chromosome 22q11 [22]. The best characterized is the GGT 1 gene, which encodes a single polypeptide that undergoes post-translational changes to form a mature enzyme [12]. GGT 1 gene transcription is controlled by multiple tandemly positioned promoters [23,24]. These promoters and alternative splicing contribute to diversity of molecular forms and tissue specificity of GGT. The GGT 2 gene represents a duplication of GGT 1 gene with 97% nucleotide analogy between both genes and 94% amino acid analogy between their polypeptide products [10]. Some studies have shown that polypeptide products of the GGT 2 gene fail to auto-activate and anchor on plasma membranes, resulting in rapid degradation by cytoplasmic proteases [25]. The only other GGT gene that produces a polypeptide with enzymatic activity is GGT 5 gene (formerly known as γ-glutamyl leukotrienase due to its ability to cleave glutathione-S-conjugate leukotriene C4 to leukotriene D4). The GGT 5 gene polypeptide product has 40% amino acid analogy with the GGT 1 gene product but only 4% enzymatic activity [16,26]. A detailed analysis of genetic variants of the GGT gene family can be found in a review by Heisterkamp et al. [12].

Regulation of GGT expression is complex, poorly understood and outside the scope of this review. However, there is ample evidence that GGT gene expression in animals and humans is controlled by redox mechanisms and signal pathways activated in response to oxidative stress [17,27]. Alcohol is a known inducer of GGT gene expression, possibly through increased oxidative stress caused by its consumption [28]. GGT deficiency is an extremely rare condition, being described in fewer than 10 patients worldwide; however, so far, no mutations in GGT gene 1 have been reported [12]. All described patients with GGT deficiency have had glutathionuria. Study of three patients with GGT deficiency showed complete absence of leukotriene D4 synthesis in monocytes [29]. Experimental studies have shown an up 2500-fold increase in urine G-SH and a 5-fold reduction in plasma cysteine concentration in GGT knockout mice compared with their wild-type counterparts [13]. These data clearly illustrate the central role of GGT in G-SH and cysteine homeostasis.

In healthy humans, measurable GGT activity tends to be low (< 60 U/L). Although the source of circulating GGT is unclear and there is no evidence to support a correlation between circulating and tissue enzyme levels, it is thought to originate predominantly from the liver. The possibility that a portion of GGT may originate from atherosclerotic plaques has also been suggested [30]. Earlier investigations have identified both amphiphilic (either associated with plasma lipoproteins or in the form of multi-enzyme complexes) and hydrophilic forms of the enzyme [31]. It is now known that GGT circulates in multiple forms, differing mainly in the degree of glycosylation. It is estimated that 60–80% of serum GGT from patients with hepatobiliary diseases circulates bound to plasma lipoproteins. GGT activity shows a great degree of variability and is influenced by both genetic and environmental factors [30,32]. Pathological processes, particularly in organs with the highest activity of the enzyme, may lead to elevated GGT levels in the circulation. Circulating GGT levels are markedly increased in patients with liver disease. It has been suggested that the origin of circulating GGT in the presence of cholestatic disorders is of biliary rather than hepatic origin [33]. Franzini et al. [34] and more recently, Fornaciari et al. [35] have identified 4 GGT fractions with different molecular weights: big GGT (with a molecular weight of 2000 kDa), medium GGT (with a molecular weight of 1000 kDa), small GGT (with a molecular weight of 200 kDa) and free GGT fraction (with a molecular weight of 70 kDa). Big GGT consists of membrane microvesicles (microparticles or exosomes) and may be a precursor for smaller fractions (medium and small variants), whereas free GGT represents a free soluble form of the enzyme. It has been shown that at least part of GGT does not need a carrier such as plasma lipoproteins or albumin. It has been proposed that GGT fractions may differ in their diagnostic specificity and may, therefore, help to differentiate between various disease processes despite similar total values [35]. However, this requires further study.

3. Epidemiological evidence

Epidemiological studies suggest an association between elevated GGT activity and almost all aspects of cardiovascular disease. The most extensively investigated aspect is the association of GGT with atherosclerosis and the risk of developing CHD.

3.1. Association of GGT with incident CHD and CHD-related mortality

A number of population-based studies have investigated the association between GGT and stroke, sudden death or liver disease, but in these studies GGT was considered as a surrogate of alcohol consumption [36–38]. The British Regional Heart Study assessed the association between serum GGT activity and cardiovascular risk factors, CHD and all-cause mortality in 7613 middle-aged British men followed for 11.5 years [39]. Of a total of 876 deaths from all causes, 449 (51%) were attributed to cardiovascular disease, with CHD-related deaths accounting for 80% of these. After full adjustment for an array of cardiovascular risk factors and alcohol intake, a significant association remained between GGT and the risk for all-cause mortality (adjusted relative risk [RR] = 1.22, 95% confidence interval [CI] 1.01 to 1.42) and CHD-related mortality (adjusted RR = 1.42 [1.12–1.80]), with both risk estimates calculated for GGT > 24 U/L [the 5th quintile] versus GGT < 24 U/L. The risk of CHD-related mortality was higher (RR = 1.67 [1.03–2.69]) in subjects with pre-existing CHD, particularly in men with definite prior myocardial infarction [39].

More recently, the Framingham Heart Study, investigated the association between circulating GGT levels and the risk of new-onset metabolic syndrome, incident cardiovascular disease –defined as fatal or non-fatal CHD, peripheral or cerebrovascular disease, or heart failure – and death in 3451 participants over a mean follow-up period of 19-years [40]. Incident cardiovascular disease increased from 10.5% in subjects with GGT levels in the 1st quartile, and to 23.8% in subjects with GGT levels in the 4th quartile. The adjusted hazard ratios [HR] for new-onset metabolic syndrome, incident cardiovascular disease or death increased by 26% ($P < 0.05$), 13% ($P \leq 0.01$) and 26% ($P \leq 0.001$), respectively, for each standard deviation increment in the log-GGT [40]. Individuals in the highest GGT quartile showed a 67% increase in the adjusted risk for incident cardiovascular disease. The study emphasized the role of GGT as a marker of metabolic and cardiovascular risk [40].

A third large study, the British Women's Heart and Health Study, investigated the association between GGT level and incident CHD or stroke in 2961 women without CHD or stroke at baseline over 4.6-years of follow-up. Overall, there were 151 cases of incident CHD, translating into a rate of 11.6 incident cases per 1000 women-years. The age-adjusted risk for incident CHD was increased by 28% (HR = 1.28 [1.01–1.62] per unit increment in log-GGT). However, the GGT-incident CHD association was attenuated after full adjustment in a multivariable model (adjusted HR = 1.15 [0.88–1.48] per unit increment in log-GGT) [41]. Finally, a meta-analysis integrated in the same study which included 10 prospective studies – 9 cohort and 1 case-control study – including > 1 million participants confirmed the association between GGT and incident CHD. Pooled analysis of age or age plus sex adjusted studies (6 studies) yielded a HR of 1.53 [1.34–1.76]; $P < 0.001$ per unit increment in natural log-GGT. Pooling fully-adjusted risk estimates (as reported in included publications) yielded a HR of 1.20 [1.02–1.40]; $P < 0.001$, implying a 20% increase in the adjusted risk for an association between GGT and CHD [41]. It must, however, be acknowledged that the meta-analysis by Fraser et al. [41] was limited by high heterogeneity between included studies, marked differences with respect to length of follow-up (ranging from 4.6 to 19.1 years) and the degree of adjustment for cardiovascular risk factors and other confounders. Notably, when two studies of Asian populations were excluded, the degree of heterogeneity was substantially decreased.

A number of subsequent large studies offered further convincing evidence in support of an association between elevated GGT and cardiovascular or CHD-related mortality. A central European cohort of 283,438 individuals investigated the association of GGT with mortality over a median follow-up of 7.6 years. GGT activity was divided into 5 categories as follows: normal low (< 9 U/L for women; < 14 U/L for men); normal high (9–17; 14–27 U/L); moderately elevated (18–26; 28–41 U/L); increased (27–35; 42–55 U/L); and highly elevated (≥ 36; ≥ 56 U/L). The first category served as a reference. Overall, 17,163 (6.1%) deaths were attributed to cardiovascular disease. Of these, 8009 (3295 deaths in women and 4714 deaths in men) were caused by CHD. The adjusted HRs for CHD-related mortality for the 2nd to 5th categories compared with the 1st (normal low) were 1.2 [1.1–1.3], 1.2 [1.1–1.4], 1.4 [1.2–1.6] and 1.4 [1.2–1.5] in women and 1.2 [1.1–1.3], 1.4 [1.3–1.5], 1.6 [1.4–1.7] and 1.7 [1.6–1.9] in men, respectively ($P < 0.001$ for all risk comparisons) [42]. The study showed that GGT was associated with mortality in men and women, with a dose-response relationship between GGT and mortality risk. The association between GGT and mortality was stronger in younger subjects (< 30 years of age) [42].

In another study, longitudinal GGT changes over a period of 6.9 years were assessed in a population-based cohort of 76,113 Austrian men and women with serial GGT measurements and prospective follow-up for a median of 10.2 years. For each unit increment in log scale of baseline GGT, the adjusted risk for CHD-related mortality increased by 80% in men (adjusted HR = 1.80 [1.32–2.46]; $P < 0.001$) and 40% in women (adjusted HR = 1.40 [0.97–2.02]; $P = 0.07$). An increase in GGT of > 9.2 U/L over 7 years was associated with increased CHD mortality in men (adjusted HR = 2.22 [1.68–2.93]; $P < 0.001$) and women (adjusted HR = 1.45 [1.01–2.09]; $P = 0.046$), with both risk estimates calculated per GGT log-unit increase with GGT as a time-varying covariate [43]. In men, the risk of cardiovascular mortality was significantly higher in those with an increase in GGT during follow-up (adjusted HR = 1.40 [1.09–1.81]) compared with these with stable GGT (defined as a change of − 0.7 to 1.3 U/L over 7 years). The study showed that an increase in GGT over time increases the risk of cardiovascular and CHD mortality, independent of the baseline values of the biomarker. The study also showed a stronger association between GGT and mortality in younger compared with older subjects.

Several recent meta-analyses have summarized prospective studies investigating the association between GGT and cardiovascular disease. A 2013 meta-analysis that included 7 studies with 273,141 subjects showed an association between elevated GGT and cardiovascular mortality (RR = 1.52 [1.36–1.70]; $P < 0.001$) for the highest versus lowest GGT quartiles and a RR = 1.76 [1.60–1.94]; $P < 0.001$ per unit increment in log-GGT [44]. The association between GGT and cardiovascular mortality was not significant in the subgroup of subjects of Asian origin. A meta-analysis from 2014 that included 20 studies and 1,054,181 subjects with 15,194 cardiovascular events showed a 23% increase in the risk of cardiovascular disease per each standard deviation increment in log-GGT (adjusted RR = 1.23 [1.16–1.29]) [45]. However, there is a higher degree of overlapping between the studies included in both meta-analyses.

Summarizing the findings of these studies, epidemiological evidence for an association between elevated GGT activity and the risk of incident CHD and CHD-related mortality is strong. The association between GGT and increased risk of CHD or mortality is observed in both sexes and seems to be stronger in younger subjects. Despite the association between GGT and the risk of CHD or mortality, there is no clear evidence that elevated GGT is associated with a risk of acute coronary events such as acute myocardial infarction [46]. Whether there are differences in the strength of the association between GGT and CHD or mortality according to geographic region – specifically, whether the association is weaker in Asian populations – needs further research.

3.2. GGT and cardiovascular and metabolic risk factors

In earlier studies, GGT drew little attention as a marker of cardiovascular risk because it was considered merely a biochemical parameter of excessive alcohol consumption [36–38]. However, two findings led to the hypothesis that elevated GGT per se may signify an increased risk for CHD, regardless of the level of alcohol consumption: first, the finding of considerable variations in circulating GGT activity among subjects consuming similar amounts of alcohol, and second, the

discovery that moderate alcohol consumption reduces the risk of myocardial infarction. Large epidemiological studies have also reported a strong association between elevated GGT and almost all known important cardiovascular risk factors including older age, male gender, body mass index, smoking, physical inactivity, elevated cholesterol, elevated fasting triglycerides, elevated low-density lipoprotein cholesterol, low high-density lipoprotein cholesterol, elevated blood pressure and heart rate, diabetes, metabolic syndrome, elevated fasting blood glucose, hyperuricemia, elevated C-reactive protein and in women, menopause and the use of contraceptive drugs [47–50]. The Framingham Study found a close relationship between elevated baseline GGT and the risk of new onset metabolic syndrome over a mean follow-up of 19 years [40]. A recent meta-analysis of 10 prospective cohort studies with 67,905 participants, including 6595 cases of incident metabolic syndrome, reported a significant association between GGT and metabolic syndrome (RR = 1.88 [1.49–2.38] for individuals in the highest vs. lowest thirds of baseline GGT activity) [51]. The association between GGT and most of the above-cited cardio-metabolic risk factors remains significant after adjustment for alcohol consumption. The underlying mechanisms for this association are poorly understood. However, several putative mechanisms underlying these associations may be offered.

First, several studies have shown a close association between GGT and insulin resistance in the setting of obesity, diabetes, and nonalcoholic fatty liver disease (NAFLD) or in healthy men and women [52–55]. On one hand, insulin resistance is associated with multiple cardio-metabolic factors serving as a risk factor cluster [53]. On the other hand, ample evidence implicates insulin resistance in all stages of atherosclerotic disease, from prerequisite conditions like endothelial dysfunction, to accelerated atherosclerosis progression, plaque vulnerability, and subsequent coronary events [56]. One angiographic study demonstrated an association between insulin resistance and progression of atherosclerosis in non-grafted coronary arteries 5 years after coronary artery bypass surgery [57]. In this regard, insulin resistance may be viewed as a bridging mechanism linking GGT with cardiovascular risk factors and CHD.

Second, NAFLD is the most frequent hepatic disorder and the most common cause of elevated levels of liver enzymes, including GGT [58]. It has been suggested that GGT may represent a link between fatty liver and early atherosclerosis [59].The ongoing epidemic of obesity and its close association with NAFLD is expected to increase the importance of this morbid condition as a contributor to cardiovascular disease and in particular, to CHD. NAFLD is considered the hepatic equivalent of metabolic syndrome because of its association with atherogenic dyslipidemia, obesity, and type 2 diabetes [60]. In fact, NAFLD and metabolic syndrome share common features including arterial hypertension, insulin resistance, elevated triglyceride levels, increased small dense low-density lipoprotein particles, low high-density lipoprotein levels, hyperuricemia, impaired glucose tolerance, obesity, diabetes, chronic inflammation, decreased adiponectin level, increased oxidative stress, hypercoagulability, and impaired fibrinolysis [61]. Patients with NAFLD have elevated levels of angiotensin II - a peptide hormone with pro-oxidant activity that has been implicated in the generation of oxygen-free species and production of pro-inflammatory mediators [62]. Angiotensin II promotes progression of atherosclerosis in a number of ways, including hemodynamic (pressure) effects, endothelial dysfunction, inflammation, blood coagulability and plaque vulnerability [63], all of which are known to predispose to CHD or CHD-related mortality. A large Korean study showed that the association between GGT and cardiovascular mortality was attenuated by adjustment for ultrasound diagnosed fatty liver [64], further supporting the role of NAFLD as a bridging mechanism between elevated GGT and cardiovascular risk. Since cardiovascular disease remains the number one cause of death among subjects with NAFLD, it has been proposed that subjects with NAFLD may benefit from cardiovascular risk evaluation, and potentially, regular cardiovascular disease risk surveillance [65].

Third, there is strong evidence of an association between elevated circulating GGT activity and increased oxidative stress [27]. In addition, oxidative events have been linked to the pathogenesis of atherosclerosis and subsequent cardiovascular events [66,67]. This is intuitive if one considers that increased oxidative stress increases the need for G-SH, the intracellular synthesis of which is dependent on GGT. The CARDIA (Coronary Artery Risk Development in Young Adults) study [68] and the third National Health and Nutritional Examination Survey [69] both showed an inverse association between GGT and circulating antioxidants. Inverse associations between serum GGT concentration and fruit intake or circulating levels of vitamin C or beta-carotene have also been reported [68,70]. Conversely, higher meat consumption is correlated with elevated GGT [68,71] potentially because of ingestion of (heme) iron, which lays a pivotal role in redox reactions, leading to generation of free oxygen species [72]. Positive associations have also been reported between GGT and ferritin – a protein involved in iron-storage [71,73,74]. Evidence from these studies is indirect in the sense that elevated circulating GGT levels may reflect the cellular need for G-SH for protection from heightened oxidative stress and excessive production of oxygen free radical species. Nonetheless, circulating GGT has been reported to correlate with F2-isoprostanes arising from peroxidation of polyunsaturated fatty acids (primarily arachidonic acid), which are widely accepted as in vivo markers of oxidative stress [48], further supporting a link between GGT levels and the degree of oxidative stress in organism. There is also evidence to suggest that redox mechanisms play an important role in induction of GGT and that signaling pathways such as Ras, ERK, p38MAPK and PI3K may also participate in GGT gene expression [17]. Furthermore, the association between alcohol consumption and GGT may be at least partly explained by increased induction of oxygen free radical species by alcohol [28].The possibility that GGT itself may behave as a pro-oxidant is discussed later in this review.

Fourth, elevated GGT has been reported to be closely correlated with markers of systemic inflammation – another important contributor to atherosclerosis and CHD. In the third U.S. National Health and Nutrition Examination Survey, serum GGT activity across all deciles was positively associated with serum concentrations of C-reactive protein after adjustment for race, sex, age, cigarette smoking, alcohol intake, and body mass index [75]. A recent cross-sectional study of 5446 healthy, nondiabetic subjects reported an independent association between GGT and high-sensitivity C-reactive protein, which was present across the entire spectrum of risk and persisted after adjustment for other cardiovascular risk factors [76]. A close correlation has also been reported between GGT, C-reactive protein and metabolic syndrome independent of age, smoking status, alcohol consumption, uric acid, or renal function [77].

Fifth, elevated GGT has been shown to be associated with additional comorbidities and risk factors (or markers) for atherosclerosis and CHD, including incident and chronic kidney disease [78,79], coronary calcification [80] or environmental (air) pollutants [81]. Chronic kidney disease is a well-recognized risk factor for coronary atherosclerosis and increased risk of myocardial infarction [82], and coronary calcification is an equivalent of coronary atherosclerosis that is independently associated with markedly increased risk of cardiovascular disease and myocardial infarction [83]. Air pollutants are increasingly being recognized for their deleterious effect on health, including increased risk of atherosclerosis and CHD [84] or mortality [85].

Emerging evidence suggests that circulating GGT fractions may be selectively associated with specific cardiovascular risk factors. Using the Offspring Cohort of the Framingham Heart Study, Franzini et al. [86] showed that circulating GGT fractions differ in the strength of their correlation with specific cardiovascular risk factors. Thus, big-GGT correlated with plasma triglycerides, whereas medium, small, and free fractions correlated with alcohol consumption; medium and small GGT correlated with C-reactive protein and big and free fractions also correlated with plasminogen activator inhibitor-1. Furthermore, body mass

index, blood pressure, glucose and triglycerides correlated with big and free GGT fractions. Overall, big-GGT was the fraction to correlate most frequently with cardiovascular risk factors [86].

Although the relationship between GGT and cardiovascular risk factors seems to be genetically determined, evidence for this is limited. Whitfield et al. [30]. suggested that significant correlations between GGT and risk factors such as body mass index, serum lipids, plasma lipoproteins, glucose, insulin or blood pressure were more attributable to genetic factors that affect both these risk factors and GGT than to environmental factors. Moreover, a study in baboons showed a significant impact of genetic factors on the relationship between circulating albumin – another marker of liver function - and cardiovascular risk factors, but not on the relationship of GGT with these factors [32]. This issue, however, remains largely under-investigated.

In conclusion, evidence from epidemiological, clinical and experimental studies shows that GGT is associated with an array of cardiometabolic risk factors and comorbidities, with an established role in the development of atherosclerosis, CHD or CHD-related clinical events. It has been demonstrated that factors, conditions or comorbidities associated with cardio-metabolic risk tend to cluster in subjects with elevated GGT activity. In this regard, GGT may be seen as a marker of increased cardiovascular risk in general. GGT activity is controlled by both genetic and environmental factors. In the absence of specific diseases affecting tissues or organs, particularly those with the highest GGT activity, elevated GGT activity may reflect increased stress on the cells from various sources. The intricate association between GGT and cardio-metabolic risk factors raises questions as to whether GGT is directly involved in the pathophysiology of atherosclerosis and CHD (a risk factor) or is simply an epiphenomenon of co-existing cardio-metabolic risk factors (a risk marker).

3.3. GGT, alcohol consumption and atherosclerotic risk

GGT is considered a sensitive marker of alcohol use. Studies in large populations have shown that even moderate drinking is associated with higher levels of GGT activity than abstainers [87]. Alcohol consumption and obesity – another frequent factor associated with alcohol use and elevated GGT activity – seem to be additive in elevating GGT activity because they may synergistically increase metabolic burden and liver injury [88]. Thus exclusion of alcohol consumers and overweight individuals from the reference population would possibly yield upper normal limits of GGT activity < 60 U/L [88].

Alcohol consumption was considered to potentially modify the association between GGT and the risk of CHD. For this reason, epidemiological studies that have assessed the association between GGT and the risk of CHD have adjusted for alcohol use. The current view is that moderate drinking has protective effects with respect to atherosclerosis and CHD risk. Studies that have investigated the association between alcohol use and atherosclerosis did not show a negative impact of alcohol on initiation or progression of atherosclerosis [89–92]. A recent systematic review of 13 cardiac biomarkers showed that moderate alcohol consumption was associated with favorable changes in several of these biomarkers, including higher levels of high density lipoprotein cholesterol and adiponectin and lower levels of fibrinogen, providing indirect evidence for a protective effect of moderate alcohol use on CHD risk [93]. A recent study of 1,937,360 patients from the CALIBER (Cardiovascular research using Linked Bespoke studies and Electronic health Records) programme showed that heavy drinking conferred an increased risk of unheralded coronary death, heart failure, cardiac arrest, intracerebral hemorrhage and peripheral arterial disease but a lower risk of CHD entities, such as myocardial infarction and stable angina [94]. Thus, although the relationship between GGT, alcohol and the risk for atherosclerosis and CHD is complex, the atherosclerotic risk clustered in subjects with elevated GGT levels seems to be independent and unrelated to alcohol per se.

3.4. GGT and outcome in patients with CHD

Population-based studies have signalled a higher risk for CHD-related mortality in individuals with pre-existing CHD, particularly in those with documented prior myocardial infarction [39]. Coronary angiographic studies have supported, to a degree, the hypothesis that pre-existing CHD may strengthen the association of GGT with mortality. A study of 469 patients with angiographic CHD [95] showed a significantly higher risk of cardiac mortality (25.2% vs. 13.9%) at 6 years in subjects with GGT > 40 U/L versus those with GGT < 40 U/L in the subset of patients with previous myocardial infarction (n = 262), but not in those without. The highest risk for cardiac events was observed over the first 2 years of follow-up. A combination of higher GGT (> 40 U/L), previous myocardial infarction and multivessel disease identified a subgroup of patients (n = 168) with the highest risk for cardiac events at 6 years [95]. This association remained significant after adjustment for potential confounders, including alcohol consumption. The Ludwigshafen Risk and Cardiovascular Health Study was another study to investigate the association between GGT and mortality in 2556 subjects with angiographic CHD and 699 subjects without, over a mean follow-up of 7.75 years [96]. In the total cohort and in subjects with angiographic CHD, all-cause and cardiovascular mortality increased progressively with increasing GGT activity. Analysis of subjects with angiographic CHD showed that in subjects with GGT activity in the 2nd to 4th quartiles versus those with activity in the 1st quartile the adjusted risk for all-cause mortality increased by 10% (P = 0.486), 25% (P = 0.082) and 59% (P < 0.001) and the risk for cardiovascular mortality increased by 29% (P = 0.174), 55% (P = 0.009) and 77% (P = 0.001), respectively. However, the risk increase for all-cause or cardiovascular mortality in subjects with angiographic CHD did not differ to the risk in the entire cohort [96]. In another study of 1152 participants undergoing rehabilitation after an acute coronary syndrome, the risk of fatal or non-fatal coronary events was 21%, 32% and 75% higher for patients in the 2nd to 4th GGT quartiles compared with the 1st quartile (P for trend = 0.024) at 8-year follow-up [97]. Notably, the risk of all-cause mortality was slightly higher than the risk of cardiovascular mortality. A recent study from our group investigated the association between GGT and mortality or cardiovascular events in 5501 patients with angiographic CHD. In patients with GGT activity in the 1st, 2nd and 3rd tertiles, respective 3-year all-cause mortality was 7.1%, 7.2% and 13.9% and cardiac mortality was 4.1%, 3.6% and 7.9%. After adjustment, GGT was independently associated with a 30% (P < 0.001) and 21% (P = 0.005) increase in the adjusted risk of all-cause and cardiac mortality, respectively, for each standard deviation increment in log-GGT [98]. In another recent study of 2534 patients with acute coronary syndromes treated with percutaneous coronary intervention, we found a 24% increase (P = 0.002) in 3-year mortality for each standard deviation increment in log-GGT [99]. However, when cause-specific mortality was analysed, GGT was only associated with the risk of non-cardiac mortality (adjusted HR = 1.35 [1.09–1.66]; P = 0.005) and not with the risk of cardiac mortality (adjusted HR = 1.16 [0.97–1.38]; P = 0.098) [99].

Studies investigating the association between GGT and extent of coronary atherosclerosis have shown mixed results. A recent study of 442 patients with stable CHD showed a weak but significant correlation between GGT activity and extent of coronary atherosclerosis as assessed by the SYNTAX score [100]. Another study of 259 subjects undergoing computed tomography angiography showed that GGT correlated with atherosclerotic plaque burden and noncalcified plaques [101], which are known to be prone to rupture and subsequent coronary events. Conversely, a study by Saely et al. [102] which included 1000 patients who underwent coronary angiography, showed that GGT was associated with metabolic syndrome but not with the extent of coronary atherosclerosis, irrespective of the association between GGT and mortality [102].

Elevated GGT has been implicated in several other conditions in

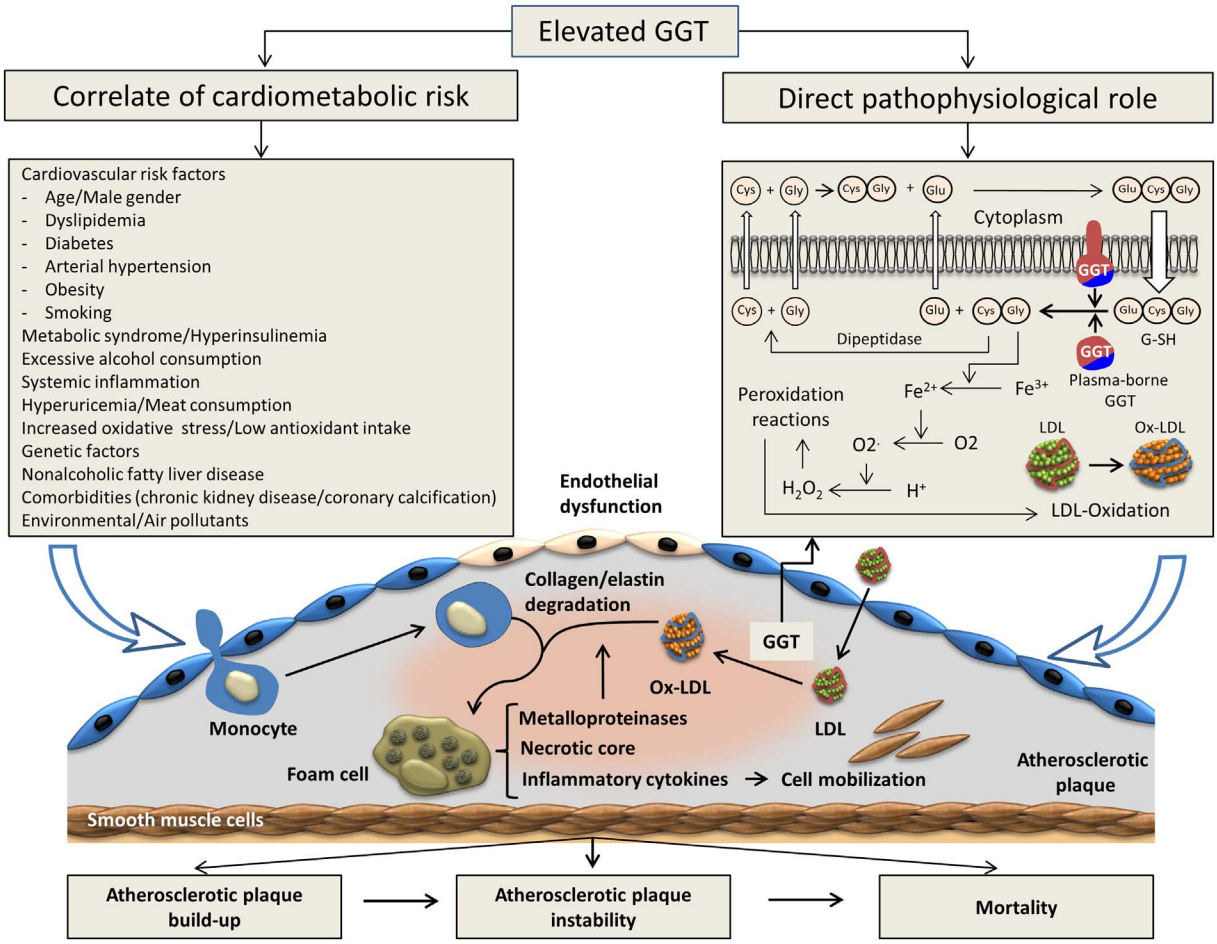

Fig. 1. Proposed mechanisms of participation of gamma-glutamyl transferase (GGT) in the pathophysiology of coronary heart disease. Cys = cysteine; Glu = glutamic acid; Gly = glycine; GSH = glutathione; LDL = low-density lipoprotein; oxLDL = oxidized LDL.

CHD. For example, studies have shown an association between elevated GGT activity and the risk of major adverse cardiac events [103], no-reflow [104] or contrast-induced acute kidney injury [105] after primary percutaneous coronary intervention in patients with acute myocardial infarction. Other studies have reported higher GGT levels in patients with syndrome X [106] microvascular dysfunction [107], endothelial dysfunction [108], carotid artery plaques [109], carotid intima media thickness [110], arterial stiffness [111] coronary slow flow [112] or higher risk of in-stent restenosis after coronary stent implantation [113]. Although such associated conditions impact unfavorably on prognosis in patients with CHD, causality has not been proven.

In conclusion, available evidence suggests that elevated GGT levels are associated with increased risk of mortality in patients with CHD. However, many aspects of the association between elevated GGT and outcomes of such patients remain unclear. In the absence of comparative studies in subjects with and without CHD, the hypothesis that pre-existing CHD may strengthen the association between GGT and mortality remains untested. One possibility is that subjects with CHD are at a generally increased risk of cardiovascular events and CHD-related mortality. Cardiovascular risk factors – known to be more frequent in subjects with CHD – are closely correlated with GGT. Thus, dissecting the intricate relationship and interdependence between cardiovascular risk factors, GGT, and CHD remains difficult. Thus, at present it remains unknown whether GGT per se contributes to poorer outcome in subjects with CHD, or is simply an epiphenomenon of cardiovascular risk factors clustered in subjects with elevated levels of the enzyme. Some studies have suggested a stronger association of GGT with all-cause than with cardiac mortality [97], or even loss of the association between GGT and cardiac mortality after adjustment for cardio-metabolic risk factors [99]. Furthermore, angiographic studies may offer limited adjustment for factors and comorbidities other than cardiovascular risk factors. These factors, however, may contribute to increased overall mortality by increasing the risk of non-cardiac mortality. Although some studies have shown an association between GGT and progression of coronary calcium [80] no studies have assessed whether subjects with higher GGT level show accelerated progression of coronary atherosclerosis compared to those with lower levels of the enzyme. Finally, it has been suggested that percutaneous coronary intervention may abolish or at least attenuate the association of GGT with coronary events [95]. Moreover, contemporary patients with CHD are treated with statins or other secondary prevention measures which may further attenuate the association between GGT and outcome. Experimental data suggests that statins may supress GGT expression in aortic atherosclerotic plaques in mice [114]. These drugs also stabilize atherosclerotic plaques and reduce coronary events, and in high doses, promote atherosclerosis regression. These factors may lead to attenuation of the association between GGT with coronary events or cardiac mortality in contemporary patients with CHD.

4. Mechanisms of association between GGT and the risk of CHD

Knowledge of the underlying mechanisms of the association between GGT and increased risk of CHD and CHD-related outcomes is incomplete and remains hypothetical. In principle, however, these mechanisms may belong to 2 categories. First, GGT activity is known to

be closely correlated with cardio-metabolic risk. As discussed earlier in this review, an elevated circulating level of GGT was closely correlated with traditional cardiovascular risk factors, systemic inflammation, metabolic syndrome, increased oxidative stress and several co-morbidities, all of which increase the risk of CHD or CHD-related adverse events. Thus, a worse cardiovascular risk profile may explain the increased risk of CHD in subjects with elevated GGT activity levels. Some studies suggest that GGT does not offer incremental risk information beyond that provided by concomitant cardiovascular risk factors [99,115,116]. Second, there is evidence, mostly obtained in the experimental setting, that GGT may be involved directly in the pathophysiology and promotion of atherosclerosis through generation of reactive oxygen species, and in turn, by oxidative reactions initiated by these species in atherosclerotic plaques (Fig. 1). Stark et al. [117] were the first investigators to propose that G-SH cleavage catalysed by GGT leads to production of cysteinyl-glycine dipeptide, which retains the thiol group and is a more reactive and stronger reducing agent than G-SH (Fig. 1). Subsequent studies have confirmed that cysteinyl-glycine, but not G-SH, is responsible for generation of reactive oxygen species [118]. The cysteinyl-glycine moiety acts as a strong reducing agent of iron from ferric (Fe^{3+}) to ferrous (Fe^{2+}) form, which participates in the production of reactive oxygen species including super-oxide and hydrogen peroxide [119,120].These species have multiple cellular targets and promote (per)oxidation of several cellular components, including low-density lipoproteins [121].

Studies suggest that these reactions occur within atherosclerotic plaques and they represent the most widely accepted mechanism for direct participation of GGT in the pathophysiology of atherosclerosis. Research from Pisa University in Italy has contributed greatly to our understanding of the role of GGT in the development of atherosclerosis, demonstrating the presence of catalytically active GGT within cerebral, carotid and coronary artery atherosclerotic plaques, co-localized with oxidized lipids and CD68 + foam cells [121–123]. Franzini et al. [124] demonstrated significantly higher GGT activity in atheroma from the carotid artery compared with normal arterial tissue and demonstrated a serum-like GGT protein, indicating a potential serum origin of GGT found in atherosclerotic plaques. Moreover, this study showed the presence of GGT mRNA transcribed from the GGT-1 gene within the carotid plaques. In addition, analysis of plaque extracts revealed the presence of free and protein-bound cysteinyl-glycine dipeptide, offering further evidence for the occurrence of pro-oxidant reactions in atherosclerotic plaques catalysed by GGT reaction products [124]. More recently, Pucci et al. [125] showed a link between GGT activity in atherosclerotic plaques and indices of plaque instability in a study of 65 patients undergoing carotid plaque endarterectomy. Big-GGT fraction was the only fraction found in plaques and plaque big-GGT correlated with plaque cholesterol content and plasma big- and free- GGT fractions. Importantly, higher big-GGT activity was found in thin-cap fibroatheromas and this correlated with histologic indices of plaque instability such as larger necrotic zone, higher cholesterol content and greater macrophage infiltration [125].These studies strongly suggest a role of GGT in the pathophysiology of atherosclerosis, including plaque progression, instability and rupture [124,125].

5. Concluding remarks and perspective

Large epidemiological studies suggest an association between elevated circulating GGT levels and the risk of incident CHD or CHD-related mortality. However, the evidence linking GGT activity with acute coronary events is weaker and inconsistent. Ample evidence suggests an inherent association between GGT and a plethora of cardio-metabolic risk factors, including traditional cardiovascular risk factors, metabolic syndrome, systemic inflammation, oxidative stress burden and various comorbidities that incur a negative impact on prognosis. In this regard, GGT seems to fulfil Vasan's criteria as a biomarker of cardiovascular risk [126]. Experimental studies and studies involving human atherosclerotic plaques have demonstrated the presence of catalytically active GGT in human atherosclerotic plaques and a correlation between GGT activity and indices of plaque instability. These studies support the possibility of direct involvement of GGT in the pathophysiology of atherosclerosis and related clinical events. Conversely, a number of factors speak against such a role. Such factors include the intricate relationship between GGT and cardio-metabolic risk factors, the lack of consistent evidence of an association between GGT and acute coronary events or extent of coronary atherosclerosis, and the failure to improve risk prediction for CHD or CHD-related outcomes by adding GGT to conventional cardiovascular risk factors. Thus, the crucial question of whether GGT plays a direct role in the pathophysiology of CHD or is simply an epiphenomenon of coexisting cardiovascular risk has not been definitively answered and Hill's criteria [127] for causality between GGT and CHD are not fulfilled. It also may be hypothesized that GGT serves as a bridging mechanism linking cardiovascular risk factors with atherosclerosis or CHD. Exploration of molecular mechanisms of GGT involvement in the pathophysiology of CHD and eventual use of interventions to reduce circulating GGT activity and longitudinally assess clinical outcomes need to be addressed in future studies. Although strong GGT inhibitors are available, most of them are highly toxic for human use. Clarification of the role of GGT in human health, and particularly in cardiovascular disease, will remain a fascinating research field in the foreseeable future.

Conflict of interest

None.

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None.

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