Long-Term Cumulative Exposure to High γ-Glutamyl Transferase Levels and the Risk of Cardiovascular Disease: A Nationwide Population-Based Cohort Study

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Background: Elevated γ-glutamyl transferase (γ-GTP) levels are associated with metabolic syndrome. We investigated the association of cumulative exposure to high γ-GTP with the risk of cardiovascular disease (CVD) in a large-scale population.
Methods: Using nationally representative data from the Korean National Health Insurance system, 1,640,127 people with 4 years of consecutive γ-GTP measurements from 2009 to 2012 were included and followed up until the end of 2019. For each year of the study period, participants were grouped by the number of exposures to the highest γ-GTP quartile (0–4), and the sum of quartiles (0–12) was defined as cumulative γ-GTP exposure. The hazard ratio for CVD was evaluated using the Cox proportional hazards model.
Results: During the 6.4 years of follow-up, there were 15,980 cases (0.97%) of myocardial infarction (MI), 14,563 (0.89%) of stroke, 29,717 (1.81%) of CVD, and 25,916 (1.58%) of death. Persistent exposure to high γ-GTP levels was associated with higher risks of MI, stroke, CVD, and death than those without such exposure. The risks of MI, stroke, CVD, and mortality increased in a dose-dependent manner according to total cumulative γ-GTP (all P for trend <0.0001). Subjects younger than 65 years, with a body mass index <25 kg/m², and without hypertension or fatty liver showed a stronger relationship between cumulative γ-GTP and the incidence of MI, CVD, and death.
Conclusion: Cumulative γ-GTP elevation is associated with CVD. γ-GTP could be more widely used as an early marker of CVD risk, especially in individuals without traditional CVD risk factors.

Keywords: Gamma-glutamyltransferase; Cardiovascular diseases; Metabolic syndrome; Nation-wide study

INTRODUCTION

Globally, cardiovascular disease (CVD) is the most important cause of death [1]. According to the World Health Organization, approximately 17.9 million people died from CVD in 2019, amounting to 32% of all deaths worldwide [2]. According to an epidemiological study based on nationwide data in Korea, CVD-related deaths and hospitalizations have steadily risen over the past decade, reaching 123 per 100,000 individuals in 2018 [3, 4]. Accordingly, early screening for CVD risk and targeting of high-risk groups is crucial, and identifying new risk markers could be an important healthcare strategy for preventing CVD [5,6].

Gamma glutamyl transferase (γ-GTP) has been considered as a surrogate marker for hepatobiliary disease due to its significant increase in cholestatic liver disease or drug-induced liver damage [7,8]. However, γ-GTP is also related to several other disease states as a marker for cellular antioxidant deficiency [7,9]; hence, its elevation is not solely linked to liver disease [7,8]. High γ-GTP levels are associated with aspects of metabolic syndrome, such as fatty liver, obesity, and insulin resistance [8-10]. Studies in populations undergoing angiography and diagnosed with coronary artery disease have likewise shown that γ-GTP is associated with CVD [11-13]. Moreover, meta-analyses have reported that elevated γ-GTP is a predictor marker for cardiovascular mortality [14,15]. However, previous individual studies were based on small patient populations and did not reflect the longitudinal cumulative levels of γ-GTP. In addition, there is a lack of studies investigating the relationship between γ-GTP and CVD or mortality, and the interaction between various factors such as sex, age, diabetes mellitus (DM), hypertension (HTN), dyslipidemia, or obesity and γ-GTP on the risk of incident myocardial infarction (MI), stroke, and mortality.

This study aimed to address these gaps in the literature by analyzing the long-term association between cumulative exposure to high γ-GTP levels and incident CVD and mortality using data from a large-scale, nationwide, population-based database with 10 years of consecutive health examinations. We also performed subgroup analyses to evaluate the impact of various factors such as age, sex, smoking, drinking, underlying HTN, dyslipidemia, obesity, and fatty liver.

METHODS

Data source and subjects

As a non-profit institution, the National Health Insurance Ser-

vice (NHIS) of the Republic of Korea is a single insurer and mandatory national health insurance system that includes approximately 97% of Korean residents. The remaining 3% of low-income earners are supported by the Medical Aid Program, which is integrated into the NHIS database. Therefore, the Korean NHIS database is representative of the entire population of Korea [16,17].

The NHIS database includes an eligibility database, medical treatment database, health checkup information, and medical care institution database. The eligibility database houses all demographic data (e.g., age, sex, socioeconomic variables, type of eligibility). The medical treatment database includes treatment data, with diagnosis information coded according to the International Classification of Disease, 10th edition (ICD-10). It also includes information about any intervention, prescription, or hospitalization by medical service providers. The health checkup information is composed of general health examinations, questionnaires on lifestyle and behavior, and measured data such as height, weight, waist circumference (WC), blood pressure, and the results of various laboratory tests such as the blood cell count, renal function tests, fasting blood glucose, lipid profile, and liver function markers including γ-GTP.

Our study initially included 1,760,062 people more than 40 years old who underwent health checkups for 4 consecutive years from 2009 to 2012. Patients diagnosed with MI or stroke from January 1, 2002 to the date of the last health checkup in 2012 were excluded. Ultimately, 1,640,127 participants were included in this study and were followed up with until December 31, 2019.

Measurements and definitions

Height, weight, and WC were measured in the health examination. Body mass index (BMI) was calculated as the subject's weight in kilograms divided by the square of their height in meters. Obesity was defined as BMI ≥ 25 kg/m^2 [18]. Information about smoking and alcohol consumption was obtained by questionnaire, with heavy alcohol consumption defined as ≥ 30 g/day. Physical activity was defined as performing more than 20 minutes of strenuous activity ≥ 3 times/week or more than 30 minutes of moderate physical activity ≥ 5 times/week. The hospitals that performed the health checkups were certified by the NHIS and regularly monitored. Participants in the lowest income quartile and those receiving Medical Aid were grouped together as the low-income group.

Blood pressure was measured after participants had rested in a seated position for at least 5 minutes. Blood sampling for

measurement of serum fasting glucose, total cholesterol, triglyceride, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol levels was conducted after an overnight fast. Estimated glomerular filtration rate (eGFR) was calculated using the equation from the Modification of Diet in Renal Disease study: eGFR$=175\times$serum creatinine$^{-1.154}\times$age$^{-0.0203}$, and was further multiplied by 0.742 for women [19]. The fatty liver index (FLI) was used to access the degree of fatty liver and was calculated as reported in a previous study [20]. Participants were defined as having fatty liver if their FLI was $\geq$60. The γ-GTP level was measured in international units per liter (IU/L). Participants were defined as having high γ-GTP for a given year if they were in the highest quartile (Q4) that year, dependent on sex [21]. The cut-off values of the quartile of γ-GTP were set differently for each year of the health checkup period and sex. The number of times a participant ranked in Q4 over 4 years of annual health examinations was counted (range of 0 to 4). In the next step, to evaluate the cumulative effect of γ-GTP elevation, CVD risk was investigated according to the cumulative sum of γ-GTP quartiles over the 4-year health checkup period. Each quartile was scored from 0 to 3, and the cumulative sum over the 4-year health checkup period was obtained (range, 0 to 12) (Supplemental Fig. S1).

The presence of diabetes was determined based on at least one claim with the ICD-10 codes E11–E14 and a prescription of anti-diabetic medication or a fasting glucose level $\geq$126 mg/dL. The presence of HTN was defined as at least one claim per year with the ICD-10 code I10–I13, or I15 and a prescription of anti-hypertensive agents or systolic/diastolic blood pressure $\geq$ 140/90 mm Hg. The presence of dyslipidemia was determined based on at least one claim per year with the ICD-10 code E78 and a prescription of a lipid-lowering agent or total cholesterol level $\geq$240 mg/dL. The presence of chronic kidney disease was defined as at least one claim per year. MI was defined as codes I21–I22 and hospitalization. Stroke was defined as a code of I63–I64 and brain computed tomography or magnetic resonance imaging at the same time as hospitalization. CVD included MI and stroke. The mortality database used was provided by Statistics Korea, a government agency.

Statistical analysis

Baseline characteristics are presented as the mean±standard deviation, median (interquartile range [IQR]) or number (%). Continuous variables were compared using analysis of variance and categorical variables were compared using the chi-square test. The incidence rates of primary outcomes were calculated

by dividing the number of incident cases by the total follow-up duration (person-years). Hazard ratios (HRs) and 95% confidence intervals (CIs) were estimated using Cox proportional hazards analysis for MI, stroke, CVD, and overall mortality based on cumulative exposure to high γ-GTP levels. The proportional hazards assumption was evaluated by the Schoenfeld residuals test with the logarithm of the cumulative hazards function based on Kaplan-Meier estimates for cumulative development of diseases. Kaplan-Meier survival analysis was used to calculate the incidence probability of MI, CVD, stroke, and death according to the number of exposures to high γ-GTP levels, and the log-rank test was used for between-group comparisons. A multivariable-adjusted proportional hazards model was used to account for confounding factors. Model 1 was non-adjusted; Model 2 was adjusted for age and sex; Model 3 was additionally adjusted for smoking, drinking, and physical activity status; Model 4 was additionally adjusted for HTN, dyslipidemia, diabetes, and BMI; and Model 5 was additionally adjusted for alanine transaminase (ALT). Subgroup analyses were conducted according to age, sex, smoking, amount of drinking, exercise, HTN, dyslipidemia, BMI, and FLI. We conducted clinically relevant subgroup analyses and calculated *P* values for interactions between γ-GTP and subgroups in the development of MI, stroke, CVD, and death using Cox regression analysis. Statistical analyses were performed using SAS version 9.4 (SAS institute, Cary, NC, USA), and a *P* value <0.05 was considered to indicate significance.

Patients or public involvement

Patients or the public were not involved in the design, conduct, reporting, or dissemination plans of our research.

Ethics approval and consent to participate

The study adhered to the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of Soongsil University (SSU-202007-HR-236-01). Due to the retrospective nature of the study, the requirement to obtain informed consent was waived by the Institutional Review Board of St. Mary's Hospital.

RESULTS

Baseline characteristics of the study population

The baseline characteristics of the study population are described in Table 1. The study population was divided according to exposure to high γ-GTP levels over 4 years of consecutive

Table 1. Baseline Characteristics of the Study Population

Characteristic	No. of exposures to high γ-GTP					P value
	0 (*n*=1,012,181)	1 (*n*=178,269)	2 (*n*=114,454)	3 (*n*=112,388)	4 (*n*=222,835)	
Age, yr	50.0±7.5	50.4±7.3	50.2±7.2	50.2±7.2	50.1±7.0	<0.0001
Male sex	747,583 (73.9)	116,915 (65.6)	79,656 (70.0)	82,252 (73.2)	168,824 (75.8)	
WC, cm	80.2±7.9	81.8±8.4	82.9±8.3	83.9±8.2	84.8±8.2	<0.0001
BMI, kg/m^2	23.5±2.7	24.3±2.9	24.6±3.0	24.9±3.0	25.2±3.1	<0.0001
Smoking						<0.0001
Non-smoker	494,013 (48.8)	88,861 (49.9)	51,886 (45.3)	46,141 (41.1)	83,157 (37.3)	
Ex-smoker	255,956 (25.3)	40,668 (22.8)	27,287 (23.8)	27,295 (24.3)	50,715 (22.8)	
Current smoker	262,212 (25.9)	48,740 (27.3)	35,281 (30.8)	38,952 (34.7)	88,963 (39.9)	
Drinking						<0.0001
None	470,468 (46.5)	74,544 (41.8)	41,425 (36.2)	35,014 (31.2)	56,365 (25.3)	
Mild	492,330 (48.6)	88,934 (49.9)	60,254 (52.6)	61,788 (55.0)	125,421 (56.3)	
Heavy	49,383 (4.9)	14,791 (8.3)	12,775 (11.2)	15,586 (13.9)	41,049 (18.4)	
Regular exercise	257,743 (25.5)	43,908 (24.6)	28,244 (24.7)	27,615 (24.6)	50,829 (22.8)	<0.0001
Income						<0.0001
Medical aid, Q1	209,201 (20.7)	44,333 (24.9)	27,179 (23.8)	25,753 (22.9)	48,682 (21.9)	
Q2	176,984 (17.5)	34,177 (19.2)	20,603 (18.0)	18,778 (16.7)	35,696 (16.0)	
Q3	230,174 (22.7)	38,701 (21.7)	25,697 (22.5)	25,691 (22.9)	52,291 (23.5)	
Q4	395,822 (39.1)	61,058 (34.3)	40,975 (35.8)	42,166 (37.5)	86,166 (38.7)	
SBP, mm Hg	121.5±13.4	123.5±13.7	124.9±13.8	125.9±14.0	127.2±14.2	<0.0001
DBP, mm Hg	76.5±9.4	77.8±9.6	78.8±9.6	79.5±9.7	80.4±9.9	<0.0001
DM	58,944 (5.8)	16,575 (9.3)	12,841 (11.2)	14,480 (12.9)	34,611 (15.5)	<0.0001
HTN	193,902 (19.2)	47,031 (26.4)	34,122 (29.8)	36,638 (32.6)	79,985 (35.9)	<0.0001
Dyslipidemia	135,524 (13.4)	35,285 (19.8)	25,653 (22.4)	26,754 (23.8)	58,442 (26.2)	<0.0001
eGFR, mL/min/1.73 m^2	89.6±35.7	89.7±34.3	89.9±35.3	90.1±34.6	90.6±33.0	<0.0001
Glucose, mg/dL	96.4±19.5	99.8±23.7	102.0±26.0	103.8±27.5	107.0±30.6	<0.0001
Total cholesterol, mg/dL	195.2±33.4	200.1±35.6	201.8±36.8	202.8±37.1	205.8±38.4	<0.0001
HDL-C, mg/dL	54.6±18.3	54.5±17.3	54.1±17.9	53.9±17.3	54.5±17.7	<0.0001
LDL-C, mg/dL	116.9±31.7	117.8±34.3	117.5±36.2	116.6±37.0	115.4±39.5	<0.0001
TG, mg/dL	105.8 (105.7–105.9)	123.5 (123.1–123.8)	135.1 (134.6–135.5)	145.24 (144.8–145.7)	163.4 (163.0–163.8)	<0.0001
ALT, IU/L	19.5 (19.48–19.51)	23.7 (23.69–23.8)	26.0 (25.94–26.1)	28.1 (28.02–28.19)	32.8 (32.76–32.91)	<0.0001
AST, IU/L	22.4 (22.37–22.39)	24.8 (24.76–24.84)	26.1 (26.05–26.15)	27.4 (27.29–27.41)	30.7 (30.7–30.81)	<0.0001
FLI	22.1±18.3	33.2±22.8	39.7±23.7	45.8±24.3	56.5±24.5	<0.0001
<30	734,880 (72.6)	90,600 (50.8)	45,278 (39.6)	34,229 (30.5)	39,875 (17.9)	
<60	226,554 (22.4)	60,616 (34.0)	43,076 (37.6)	42,685 (38.0)	72,746 (32.7)	
≥60	50,747 (5.0)	27,053 (15.2)	26,100 (22.8)	35,474 (31.6)	110,214 (49.5)	

Values are expressed as mean±standard deviation, number (%), or median (interquartile range).

γ-GTP, γ-glutamyl transferase; WC, waist circumference; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; HTN, hypertension; eGFR, estimated glomerular filtration rate; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; ALT, alanine aminotransferase; AST, aspartate transaminase; FLI, fatty liver index.

Table 2. The Risk of Incident MI, Stroke, CVD, and Death according to the Cumulative Number of Exposures to High γ-GTP

Events	Cumulative no. of exposures to high γ-GTP	No. of events/ total no.	IR[a]	Model 1	Model 2	Model 3	Model 4	Model 5
MI	0	9,026/1,012,181	1.40	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
	1	1,849/178,269	1.64	1.17 (1.1–1.23)	1.22 (1.16–1.29)	1.23 (1.17–1.29)	1.13 (1.07–1.19)	1.12 (1.07–1.18)
	2	1,255/114,454	1.74	1.24 (1.17–1.32)	1.29 (1.21–1.37)	1.30 (1.22–1.38)	1.15 (1.08–1.22)	1.14 (1.08–1.21)
	3	1,253/112,388	1.77	1.27 (1.19–1.34)	1.30 (1.23–1.38)	1.31 (1.23–1.39)	1.14 (1.07–1.21)	1.12 (1.06–1.20)
	4	2,597/222,835	1.86	1.33 (1.27–1.39)	1.37 (1.31–1.43)	1.37 (1.31–1.43)	1.15 (1.10–1.21)	1.13 (1.08–1.19)
Stroke	0	7,875/1,012,181	1.22	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
	1	1,751/178,269	1.55	1.27 (1.21–1.34)	1.33 (1.26–1.40)	1.31 (1.24–1.38)	1.22 (1.16–1.28)	1.23 (1.16–1.29)
	2	1,110/114,454	1.54	1.25 (1.18–1.34)	1.32 (1.24–1.41)	1.29 (1.21–1.38)	1.17 (1.10–1.25)	1.18 (1.11–1.26)
	3	1,240/112,388	1.75	1.43 (1.35–1.52)	1.51 (1.42–1.60)	1.46 (1.37–1.55)	1.29 (1.22–1.38)	1.31 (1.23–1.39)
	4	2,587/222,835	1.85	1.51 (1.45–1.58)	1.63 (1.56–1.70)	1.55 (1.48–1.62)	1.33 (1.27–1.39)	1.35 (1.29–1.42)
CVD	0	16,455/1,012,181	2.57	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
	1	3,506/178,269	3.12	1.22 (1.17–1.26)	1.27 (1.23,1.32)	1.27 (1.22–1.32)	1.17 (1.13–1.22)	1.17 (1.13–1.22)
	2	2,295/114,454	3.19	1.24 (1.19–1.30)	1.30 (1.24,1.36)	1.29 (1.23–1.35)	1.16 (1.11–1.21)	1.16 (1.11–1.21)
	3	2,418/112,388	3.43	1.34 (1.28–1.40)	1.39 (1.33,1.45)	1.37 (1.32–1.43)	1.21 (1.15–1.26)	1.21 (1.15–1.26)
	4	5,043/222,835	3.62	1.42 (1.37–1.46)	1.49 (1.44,1.53)	1.45 (1.40–1.50)	1.23 (1.19–1.27)	1.23 (1.19–1.28)
Death	0	13,209/1,012,181	2.05	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
	1	2,775/178,269	2.45	1.20 (1.15–1.25)	1.32 (1.26–1.37)	1.32 (1.26–1.37)	1.34 (1.28–1.39)	1.31 (1.26–1.37)
	2	2,018/114,454	2.78	1.36 (1.30–1.43)	1.50 (1.43–1.57)	1.50 (1.43–1.57)	1.53 (1.46–1.60)	1.49 (1.42–1.57)
	3	2,305/112,388	3.24	1.59 (1.52–1.66)	1.74 (1.66–1.82)	1.73 (1.66–1.81)	1.76 (1.69–1.85)	1.72 (1.64–1.80)
	4	5,609/222,835	3.99	1.95 (1.89–2.02)	2.20 (2.13–2.27)	2.17 (2.10–2.24)	2.20 (2.13–2.27)	2.11 (2.03–2.19)

Values are expressed as hazard ratio (95% confidence interval). Model 1: non-adjusted; Model 2: adjusted for age, sex; Model 3: adjusted for model 2+status of smoking, drinking, physical activity; Model 4: adjusted for model 3+hypertension, dyslipidemia, and body mass index; Model 5: adjusted for model 4+alanine transaminase.

MI, myocardial infarction; CVD, cardiovascular disease; γ-GTP, γ-glutamyl transferase; IR, incidence rate.

[a]Per 1,000 person-years.

health checkups. Both the overall γ-GTP level and the range of each quartile were significantly higher in men compared to women. For males, the cut-off value of γ-GTP for the highest quartile was 55 to 56 IU/L and for females, it was 23 IU/L. The cut-offs of γ-GTP are described in detail in Supplemental Table S1. Of the 1,640,127 participants, 1,012,181 (61.7%) were never exposed to high γ-GTP levels, while 222,835 (13.6%) persistently had exposure to high γ-GTP over 4 years. Although there was little age difference, subjects with a higher number of exposures to high γ-GTP were more likely to be obese, current smokers, or current alcohol drinkers, and to have a lower level of physical activity. Those with a higher number of exposures to high γ-GTP also included a higher proportion of low-income participants, had a higher FLI, and had a higher proportion of those with traditional cardiovascular risk factors such as HTN,

DM, or dyslipidemia.

Cumulative γ-GTP and the incidence of MI, stroke, CVD, and death

During a follow-up period of 6.4 years (IQR, 6.2 to 6.6), there were 15,980 cases (0.97%) of MI, 14,563 cases (0.89%) of stroke, 29,717 cases (1.81%) of CVD, and 25,916 cases (1.58%) of death. Persistent exposure to high levels of γ-GTP was associated with elevated risks of MI (HR, 1.33; 95% CI, 1.27 to 1.39), stroke (HR, 1.51; 95% CI, 1.45 to 1.58), CVD (HR, 1.42; 95% CI, 1.37 to 1.46), and all-cause death (HR, 1.95; 95% CI, 1.89 to 2.01) compared to those not exposed to high γ-GTP. The results remained similar after adjusting for factors such as age, sex, drinking, diabetes, HTN, dyslipidemia, BMI, and ALT (Table 2). In survival curve analysis, persistent exposure to high levels of
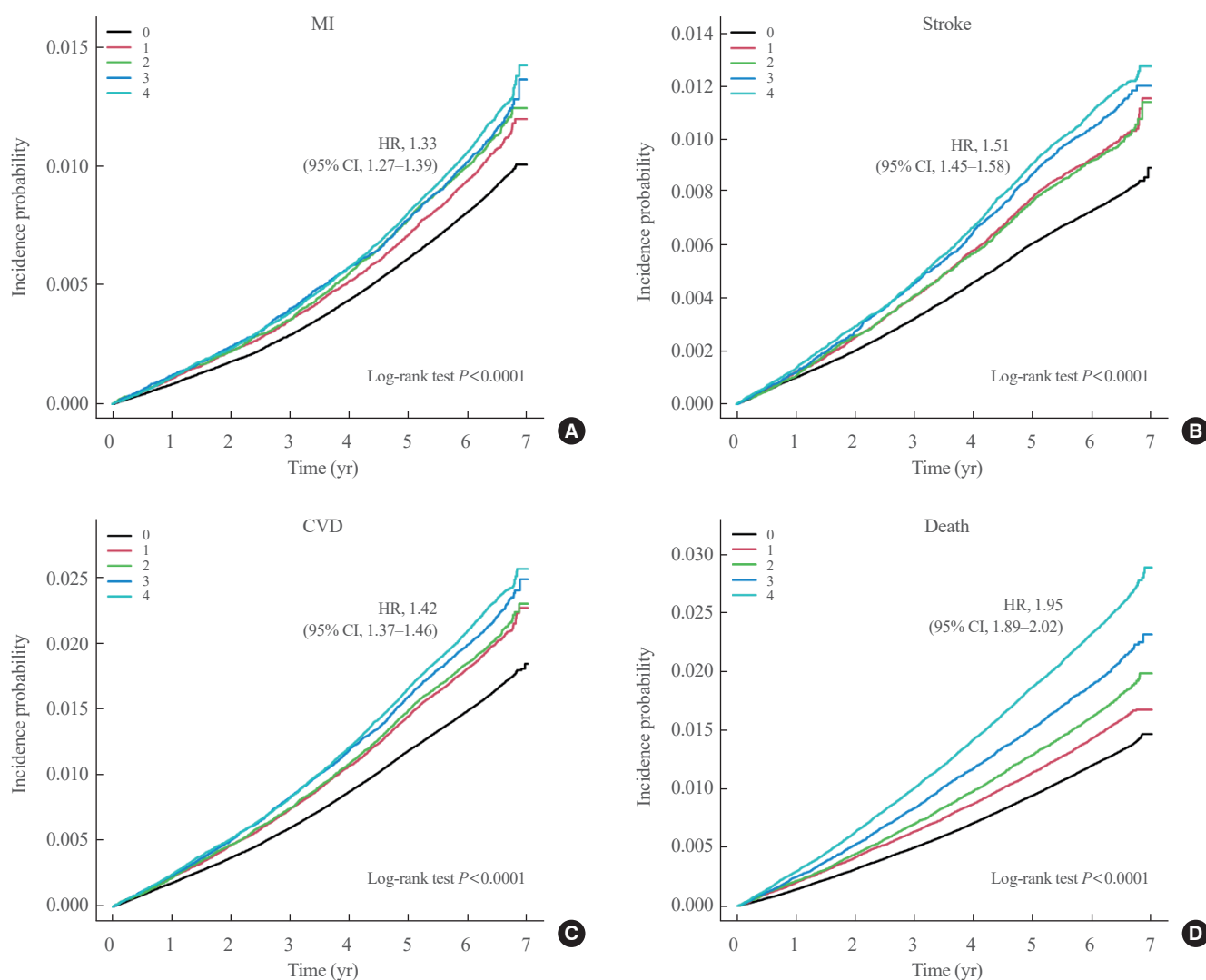
Fig. 1. Survival curve analysis for (A) myocardial infarction (MI), (B) stroke, (C) cardiovascular disease (CVD), and (D) death according to number of exposures to high γ-glutamyl transferase (γ-GTP). HR, hazard ratio; CI, confidence interval.

γ-GTP was associated with an increased probability of incident MI, stroke, CVD and death compared to no exposure to high γ-GTP (all log-rank P values <0.0001) (Fig. 1). Additionally, the risks of MI, stroke, CVD, and all-cause mortality increased in a dose-dependent manner with an increasing cumulative sum of γ-GTP quartiles over the follow-up period. Specifically, the HR increased from 1.10 to 1.34 for MI, from 1.15 to 1.73 for stroke, from 1.13 to 1.51 for CVD, and from 1.12 to 2.48 for mortality with an increasing cumulative sum of γ-GTP quartiles (Fig. 2).

Cumulative γ-GTP and development of MI, stroke, CVD, and death among participant subgroups

Subgroup analysis demonstrated stronger relationships to exist

between cumulative γ-GTP level and the development of MI, stroke, CVD, and death in subjects under 65 years of age (P for interaction=0.0001 for MI and stroke; and P for interaction <0.0001 for CVD and death). Similarly, a stronger relationship was found in female subjects (P for interaction <0.0001 for MI, stroke, and CVD; and P for interaction=0.0171 for death). Non-smokers also showed a stronger relationship between the cumulative γ-GTP exposure and the development of MI, CVD, and death, but not stroke (P for interaction=0.0212 for MI; P for interaction=0.0049 for CVD; and P for interaction=0.0019 for death, vs. P for interaction=0.0978 for stroke). Alcohol drinkers likewise showed a strong relationship between cumulative γ-GTP exposure and development of MI, stroke, CVD, and death (P for interaction=0.0003 for MI; P for interaction=0.0006 for
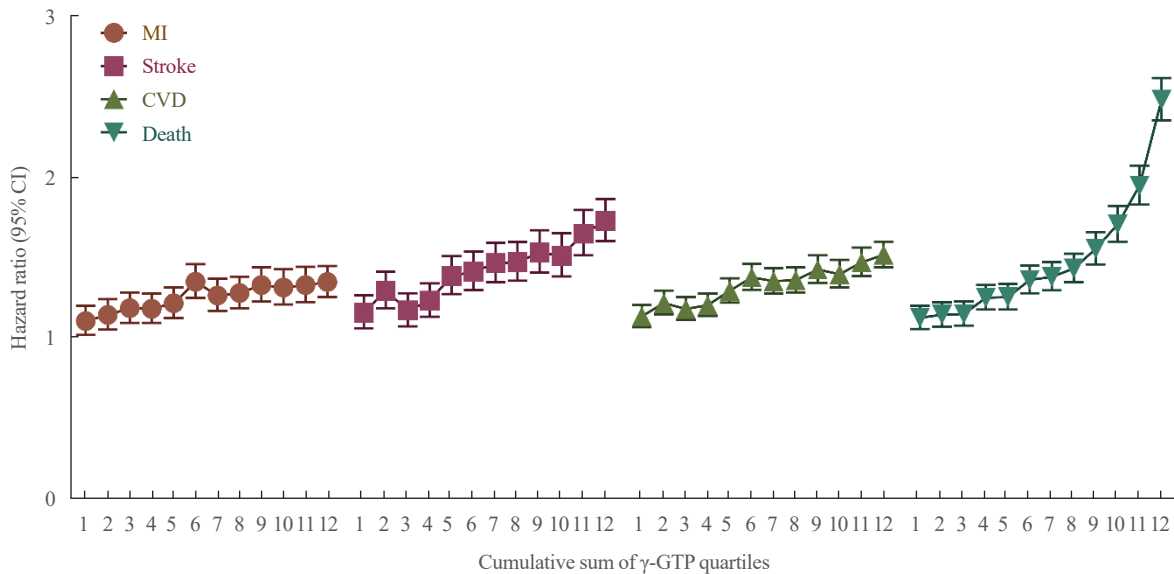
Fig. 2. Association between cumulative sum of γ-glutamyl transferase (γ-GTP) quartiles and the development of myocardial infarction (MI), stroke, cardiovascular disease (CVD) and death. The hazard ratio was adjusted for age, sex, status of smoking, drinking, physical activity, and alanine transaminase. CI, confidence interval.

stroke; and *P* for interaction <0.0001 for CVD and death) (Fig. 3). Participants with an absence of HTN showed a strong relationship between γ-GTP and development of MI, stroke, CVD, and death (all *P* for interaction <0.0001), and those with an absence of dyslipidemia showed a similar pattern (all *P* for interaction <0.0001). Participants who had a BMI of less than 25 kg/m^2 and FLI of less than 30 also showed an augmented effect modification in the relationship between cumulative γ-GTP exposure and CVD or mortality (Figs. 3, 4, Supplemental Table S2).

DISCUSSION

We found that persistent exposure to elevated γ-GTP levels was linked to CVD and mortality. Specifically, those exposed to high γ-GTP levels had 1.55 times higher risk of MI, 1.67 times higher risk of stroke, 1.61 times higher risk of CVD, and 2.16 times higher risk of all-cause mortality compared to those without high γ-GTP exposure. This pattern remained consistent after adjusting for multiple confounding factors, including traditional cardiovascular risk factors. In subgroup analyses, γ-GTP levels were associated with the development of CVD or mortality, especially in young, female, non-smoking, and non-obese participants and those without HTN, dyslipidemia, or fatty liver.

Previous studies have shown that γ-GTP elevation is associated with not only liver disease, but also metabolic syndromes, obesity, and insulin resistance [7-9]. Several small studies have

linked γ-GTP to CVD, but these studies did not account for the cumulative exposure to γ-GTP over time [11-15]. In a study conducted in China with a large study population (*n*=12,504), elevated γ-GTP levels were positively associated with an increased risk of recurrence for stroke, ischemic stroke, and combined vascular events [22]. The study mainly focused on stroke events, and the follow-up duration was quite short (within a year). Our study, in contrast, showed that cumulative exposure to γ-GTP was associated with CVD and overall mortality in a large population with a long-term observation period. To our knowledge, this is the first study to demonstrate an association between cumulative exposure to high γ-GTP and CVD outcomes, rather than research analyzing γ-GTP levels measured once at a specific time point.

In particular, we found that higher levels of γ-GTP were associated with higher risks of MI, stroke, CVD, and death, with the risk rising gradually in a dose-dependent manner. This dose-response relationship could support a causal relationship between γ-GTP and CVD [23]. Although the mechanism of association between γ-GTP and CVD risk is not fully understood, the usual explanation has two parts [7,24]. First, γ-GTP activity is also associated with systemic inflammation or oxidative stress [25] and the γ-GTP level is closely correlated with cardiometabolic risk factors such as diabetes, obesity, non-alcoholic fatty liver disease, and metabolic syndrome. Second, there is evidence that γ-GTP might be directly involved in the pathophysiology and
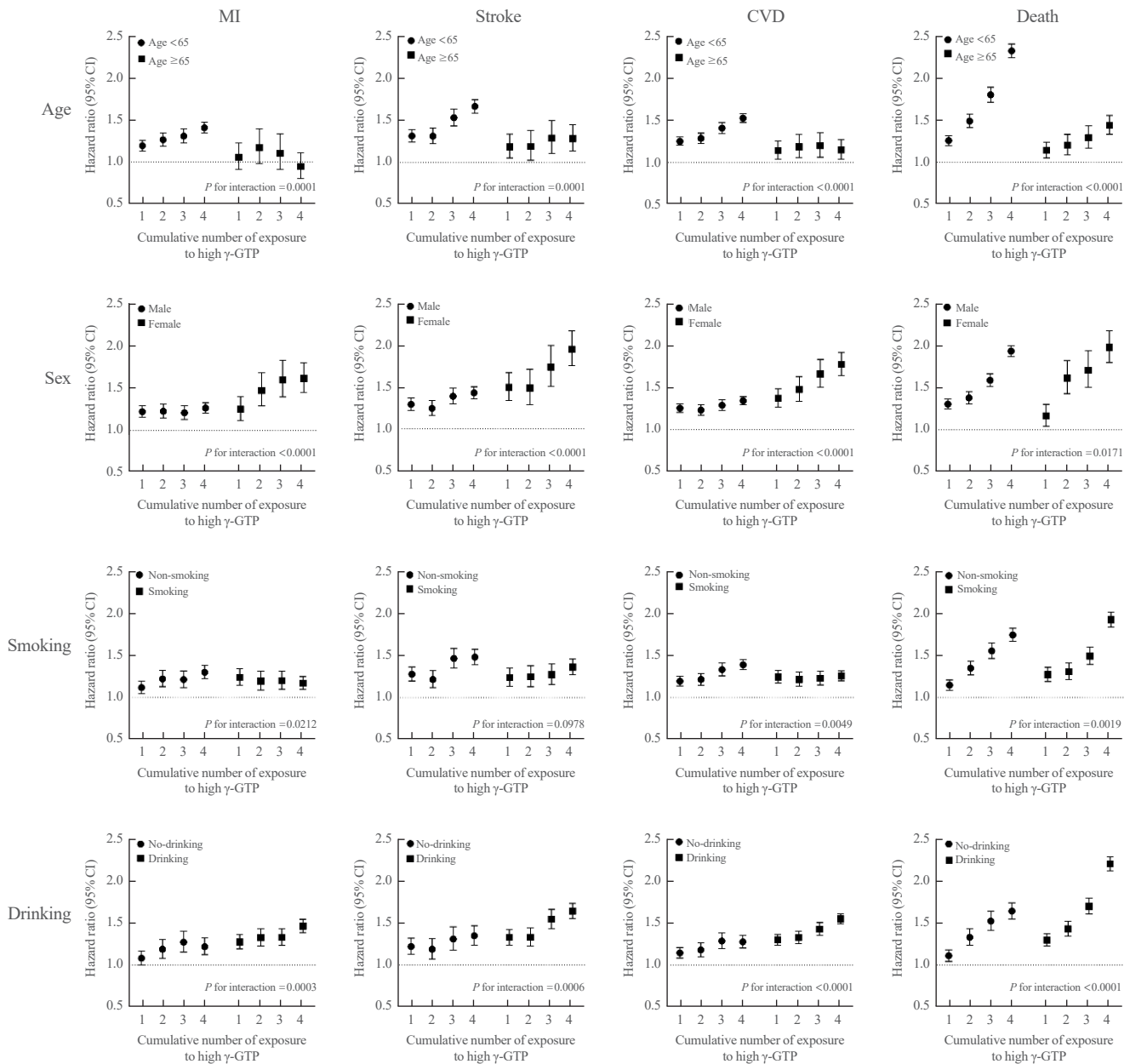
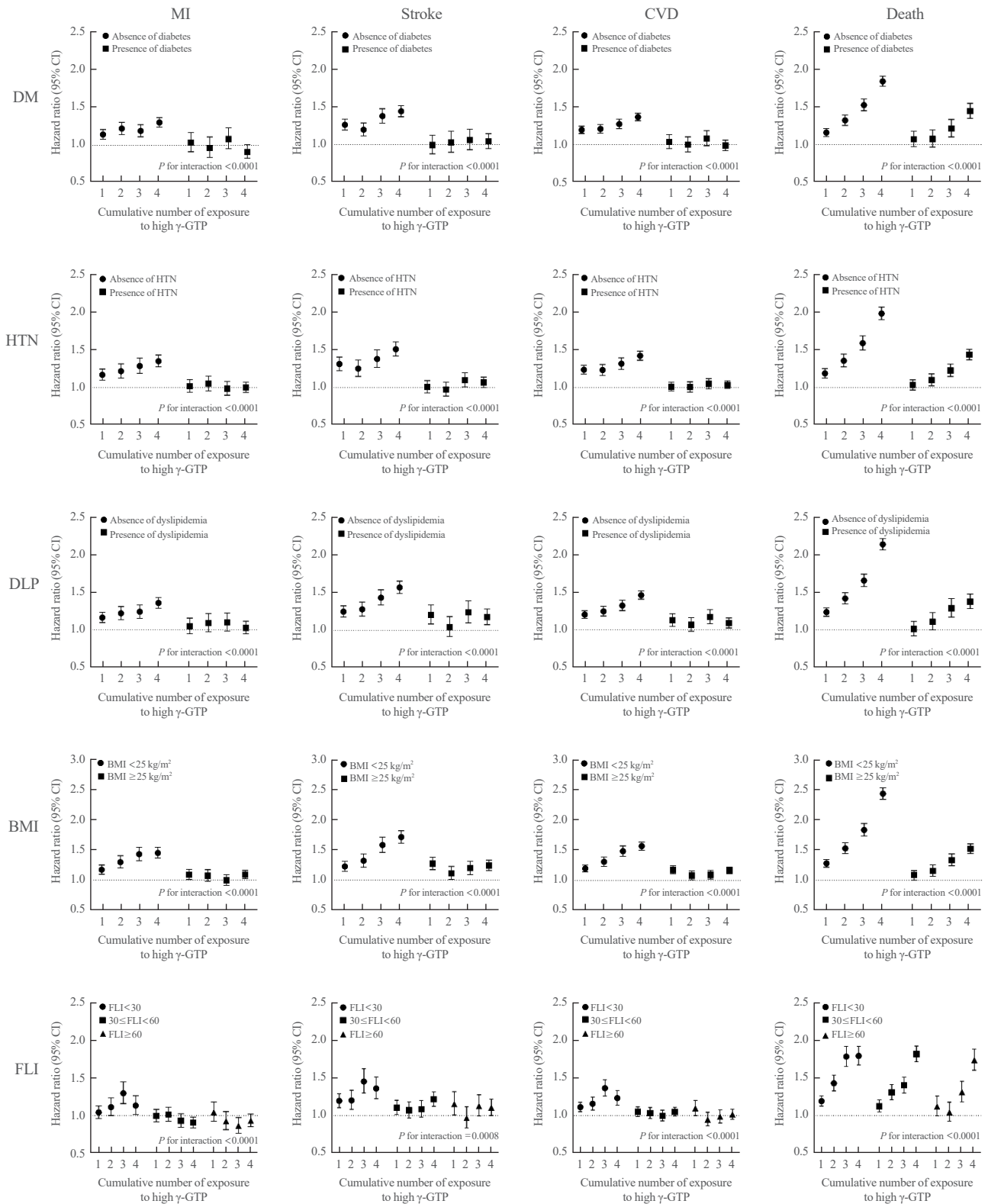**Fig. 3.** Association between cumulative number of exposures to high γ-glutamyl transferase (γ-GTP) and the development of myocardial infarction (MI), stroke, cardiovascular disease (CVD), and death according to age, sex, smoking and drinking. CI, confidence interval.

promotion of atherosclerosis. Evidence for this has mostly been obtained in experimental settings, and indicates that atherosclerosis progresses through the generation of reactive oxygen species that is initiated by the action of γ-GTP within atherosclerotic plaques [24,26].

In our study, we found that the population without traditional CVD risk factors, with the exception of alcohol consumption [27], exhibited a stronger relationship between γ-GTP and CVD. A nationwide study of the Korean population aged 20 to

84 years similarly found that younger patients showed a stronger association between γ-GTP levels and total stroke, ischemic stroke, and intracerebral hemorrhage [28]. Notably, some research has suggested that telomere length may play a role in the pathogenic pathway of elevated γ-GTP levels, especially in young patients. Bijnens et al. [29] suggested that a doubling in serum γ-GTP was associated with 7.8% shorter buccal telomeres and increased cardiometabolic risk in 18- to 30-year-old adults. In addition to age, a stronger correlation between γ-GTP

Fig. 4. Association between cumulative number of exposures to high γ-glutamyl transferase (γ-GTP) and the development of myocardial infarction (MI), stroke, cardiovascular disease (CVD), and death according to diabetes mellitus (DM), hypertension (HTN), dyslipidemia (DLP), body mass index (BMI), and fatty liver index (FLI). CI, confidence interval.

and CVD was observed in subjects without other cardiometabolic risks. This might be because γ-GTP has a weaker effect than other potent traditional risk factors for CVD. For people who already have traditional risk factors, those traditional risk factors are more important than γ-GTP for developing CVD, but for those who do not have traditional risk factors, γ-GTP is relatively important as a risk factor. A cross-sectional study from Korea suggested a role for γ-GTP in CVD risk [30]. The authors observed a strong relationship between impaired glucose tolerance (IGT) and obesity only among the population with high γ-GTP, and consequently hypothesized that γ-GTP has a critical role in the progression of IGT. Applying this hypothesis to our findings, γ-GTP might also play an important role in the development of CVD, especially the early stage of the disease before traditional risk factors develop. This potential causal effect implies that γ-GTP could be used as an early marker to predict CVD.

The observation of different patterns of associations between γ-GTP and MI, stroke, CVD, and death according to the alcohol consumption could be due to strong association between alcohol consumption and γ-GTP [31]. In another national cohort study from Korea, γ-GTP was associated with higher CVD mortality in rare drinkers [32]. In another study, which conducted a meta-analysis, γ-GTP was associated with the risk of CVD mortality regardless of alcohol consumption. The authors of these two papers suggested that γ-GTP is associated with CVD independently of alcohol intake [33]. However, our study showed conflicting results. In another Korean national cohort study, the association between higher γ-GTP and mortality was more pronounced in alcohol drinkers. The association between γ-GTP and CVD mortality according to alcohol consumption remains unclear and requires future study.

There are some limitations of our study. First, despite our attempts to adjust for various confounding factors, the influence of factors such as medication and lifestyle (including diet) could not be fully controlled for. In particular, this could explain the distinct difference between the study groups in mortality compared to other outcomes and the inconsistent results before and after adjusting for confounders, particularly for mortality. Even though we adjusted for liver disease with ALT, this may not have been sufficient for assuming that we controlled for all cases of liver disease in the study population. Secondly, the study population was limited to Koreans, so it would be difficult to generalize our results to populations with diverse ethnicities. Furthermore, 4 years might have been a short period for assessing γ-GTP levels. To overcome this limitation, the follow-up

period was set at 6 years. As the follow-up period from the index date was 6 years, the total observation period was 10 years. In a meta-analysis on γ-GTP and stroke, the observation period of most studies was about 10 years [34]. Therefore, the cumulative γ-GTP information of our study for 4 years with an observation period of 6 years can be considered sufficient to analyze CVD outcomes. Third, there is a limitation inherent in the operational definition: namely, we defined diseases only based on disease codes or medications [35,36], which may lead to bias. Lastly, although some literature supports the existence of a dose-response relationship and causality [23], it would be inappropriate to interpret our findings as demonstrating a causal relationship between γ-GTP and CVD. Hence, it is necessary to conduct a well-designed prospective study with a large population in the near future. Despite these limitations, our study has the distinctive strength of having been conducted on a well-defined large-scale, nationwide, population-based database, with adjustment for many confounding factors and a long-term cohort follow-up period.

In conclusion, our findings suggest that elevated γ-GTP levels are associated with CVD, and furthermore that γ-GTP could be used as an early marker to predict CVD, especially in populations without traditional cardiometabolic risk factors such as aging, smoking, HTN, dyslipidemia, fatty liver, and obesity. This early prediction marker could be helpful in reducing the occurrence of CVD through timely interventions. To fully realize its potential, a well-designed large-scale prospective study is necessary in the future.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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AUTHOR CONTRIBUTIONS

Conception or design: K.H., J.S.Y. Acquisition, analysis, or interpretation of data: H.S.B., B.K., S.H.L., D.J.L., H.S.K., S.A.C., K.H., J.S.Y. Drafting the work or revising: H.S.B., B.K., S.H.L.,

D.J.L., H.S.K., S.A.C., K.H., J.S.Y. Final approval of the manuscript: H.S.B., B.K., S.H.L., D.J.L., H.S.K., S.A.C., K.H., J.S.Y.

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