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Association of gamma-glutamyl transferase and alanine aminotransferase with type 2 diabetes mellitus incidence in middle-aged Japanese men: 12-year follow up

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ABSTRACT

Aims/Introduction: To prospectively investigate whether simultaneous elevation of gamma-glutamyl transferase (GGT) and alanine aminotransferase (ALT) is associated with the increase of type 2 diabetes mellitus incidence independent of alcohol drinking, body mass index and triglycerides.

Methods: A total of 2,775 Japanese male workers who had no history of type 2 diabetes mellitus were followed. High GGT and ALT were defined as the top tertiles (GGT cutpoint: 49 IU/L, ALT cutpoint: 28 IU/L). Three groups were created using these dichotomized GGT and ALT cutpoints: both low, either high or both high. Multivariable Cox proportional hazards models were carried out adjusted for potential confounding factors.

Results: A total of 276 type 2 diabetes mellitus cases were identified during 12 years (27,040 person-years) of follow up. Participants with simultaneously elevated GGT and ALT had a significantly higher incidence of type 2 diabetes mellitus, even after adjustment for fasting insulin and fasting blood glucose compared with the group without GGT or ALT elevation. Similar associations were observed in non- or light-to-moderate alcohol drinkers, as well as in participants with normal weight. However, the association was weaker in participants with triglycerides <150 mg/dL. We then evaluated whether the addition of GGT and ALT would improve the prediction of type 2 diabetes mellitus incidence, and found that their inclusion significantly increased the C-statistic, net reclassification improvement and integrated discrimination improvement.

Conclusions: Simultaneous elevation of GGT and ALT was significantly associated with type 2 diabetes mellitus incidence, independent of potential confounding factors, including alcohol drinking and obesity, although the association might require concomitant elevation of triglycerides. Inclusion of GGT and ALT improved type 2 diabetes mellitus risk prediction.

INTRODUCTION

The epidemic of diabetes mellitus is a serious global public health issue[1]. In Japan, the prevalence of diabetes mellitus is expected to increase from 7.9 to 9.8% during the next two decades[2]. Understanding the pathophysiological pathways leading to type 2 diabetes mellitus would be important for planning effective and efficient prevention programs.

The liver plays an important role in the regulation of blood glucose levels, especially in the fasting state[3], and liver injury indicated by the elevation of blood alanine aminotransferase (ALT) or gamma-glutamyl transferase (GGT) levels has been

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reported to increase the incidence of type 2 diabetes mellitus[4–7]. These enzymes would be markers of underlying liver pathology, such as liver fat accumulation or excessive alcohol intake[8,9], although a recent Mendelian randomization study suggested that ALT might be a causal factor[10]. Thus, we decided to explore the associations of ALT and GGT with the development of type 2 diabetes mellitus in a cohort of middle-aged Japanese men, not only by statistically controlling for potential confounding factors, but also by carrying out several stratified analyses by important confounding or mediating variables, including alcohol intake, obesity and hypertriglyceridemia[11–14].

Furthermore, it would be clinically relevant to examine the simultaneous elevation of ALT and GGT levels, and its relationship with the development of type 2 diabetes mellitus incidence. Although previous cross-sectional studies have shown that the combination of higher GGT and ALT had a positive association with type 2 diabetes mellitus[15,16], prospective studies on that issue are limited. As a statistically significant association does not necessarily indicate usefulness for prediction, we also evaluated the incremental predictive value of the liver enzymes.

In the present study, the association of elevated GGT and/or ALT with the development of type 2 diabetes mellitus was examined prospectively with stratification by alcohol drinking, obesity or elevated triglyceride (TG) levels. We evaluated the predictive value of adding GGT and ALT for type 2 diabetes mellitus incidence at 10 years, which has not been previously done.

METHODS

Study population

The Aichi Workers' Cohort Study established in 1997 is an ongoing prospective study of diabetes mellitus and cardiovascular disease in Aichi Prefecture, located in central Japan.

In 2002, 6648 Japanese civil servants aged 35–66 years participated in the wave 2 baseline survey by responding to self-administered questionnaires and providing their mandatory annual health checkup data[17]. The present study was first restricted to 5,177 male participants because of the small number of female type 2 diabetes mellitus cases observed during the follow-up period. The following participants were then excluded sequentially: (i) 842 who did not give consent to our use of medical history or health checkup data; (ii) 448 prevalent type 2 diabetes mellitus cases at baseline, defined as self-reported medication use or baseline fasting blood glucose (FBG) level ≥126 mg/dL or glycated hemoglobin ≥6.5% (with the USA National Glycohemoglobin Standardization Program method); (iii) 1,108 with missing values for baseline fasting insulin concentration, FBG or smoking status; and (iv) four individuals aged ≥65 years at baseline. Thus, 2,775 participants were left for the analysis.

The study protocol was approved by the Bioethics Review Committee of Nagoya University School of Medicine, Nagoya, Japan (approval number: 504–4).

Measurement of exposures, confounders and outcomes

GGT, ALT and other laboratory measures

Blood was drawn after at least 8 h of fasting. Serum samples were collected and stored at −80°C until biochemical assay. All assays were carried out at a commercial laboratory using the standard procedures. ALT and GGT were measured by the consensus method of the Japan Society of Clinical Chemistry. TG and total cholesterol (TC) were enzymatically determined. High-density lipoprotein cholesterol (HDL-c) was measured by the phosphotungstate method. FBG was enzymatically determined by the hexokinase method. Insulin concentration was measured by a solid-phase radioimmunoassay (RIABEAD II; Abbott Japan Co. Ltd., Tokyo, Japan).

Covariates

Baseline weight and height were obtained at the annual health checkup. Height was measured in the standing position to the nearest 0.1 cm, and bodyweight was measured to the nearest 0.1 kg. The body mass index (BMI) was computed as bodyweight (kg) divided by the square of height (m) and used as a continuous variable in the analyses. Information on alcohol consumption was self-reported. Participants responded to items regarding the frequency of drinking, type of alcohol and the amount of each type of alcohol per occasion. Alcohol intake (g/day) was estimated based on the responses to these items. Non- or light-to-moderate drinking was defined as those who responded that alcohol intake was <20 g/day[18]. Current smoking status was dichotomized as non-current smoker or current smoker. Physically active individuals were defined as those who engaged in a moderate or vigorous leisure-time exercise for a total of ≥60 min for ≥4 days per month. A family history of diabetes was defined as one or more first-degree relatives reported to have the disease.

Ascertainment of incident type 2 diabetes mellitus

The participants were followed through 31 December 2014. The person-years were calculated from baseline to the date of censoring, ascertainment of incident type 2 diabetes mellitus or the end of the follow up, whichever came first. Participants were censored when they died or retired from the workplace, except for those who agreed to provide their health history information to the researchers after their retirement (46.7% of retirees). Incident type 2 diabetes mellitus cases were ascertained through annual mandatory health checkups at workplaces until retirement and questionnaire surveys during employment, as well as after retirement. For the former, the health checkup data were annually reviewed, and type 2 diabetes mellitus incidence was defined to occur when the FBG level first became ≥126 mg/dL or glycated hemoglobin became ≥6.5%. The glycated hemoglobin test was provided only to employees aged 40, 45, 50 and 55 years until 2007, and to those with positive urinary glucose after 2008. A self-administered questionnaire survey was carried out approximately biennially between 2004 and 2014. Participants were asked to

report their medical histories of selected conditions including type 2 diabetes mellitus. The participants who reported a type 2 diabetes mellitus history were requested to provide the detailed contact information of the physicians who took charge of their disease management. The participants' medical records were confirmed with their physicians when participants gave their written consent.

Statistical analysis

First, participants were divided into two groups by the upper cut-off values of ALT and GGT tertiles: low ALT 5–27 and high ALT 28–578 IU/L; low GGT 8–48 and high GGT 49–1,121 IU/L. Subsequently, participants were grouped into three groups by the combination of the above two liver markers: "both low," "either high" and "both high." Those with only high ALT and those with only high GGT were collapsed, as type 2 diabetes mellitus incidence in these categories was low (<10). Cox proportional hazards regression models according to the combination of ALT and GGT were carried out to estimate hazard ratios (HRs) and respective 95% confidence intervals (95% CIs) for the risk of type 2 diabetes mellitus. The first model was adjusted for potential baseline confounders, including continuous variables of age, alcohol intake (g/day), logarithmically transformed (Ln-) BMI, Ln-TG, Ln-HDL-c and Ln-TC, as well as categorical variables of current smoking status (yes/no), regular physical activity (yes/no) and family history of diabetes mellitus (yes/no). The second model was further adjusted for Ln fasting insulin, Ln-GGT or Ln-ALT and a categorical variable for FBG levels (<100, 100–109, ≥110 mg/dL). Stratified analyses were carried out by the presence/absence of excessive drinking (alcohol intakes ≥20 g/day)[18], overweight (BMI ≥25 kg/m^2)[19] and hypertriglyceridemia (TG ≥150 mg/dL), as well as by low HDL-c (HDL-c < 40 mg/dL), high TC (TC ≥ 240 mg/dL)[20], high FBG (FBG ≥ 100 mg/dL)[21] and high fasting insulin levels (fasting insulin ≥ 10 μU/mL)[22].

We also evaluated the incremental predictive value of adding Ln-GGT or/and Ln-ALT for the 10-year risk of type 2 diabetes mellitus incidence by the C-statistic, continuous integrated discrimination improvement (IDI) and net reclassification improvement (NRI) with 95% CI. The C-statistic is the area under the receiver operating curve among individuals with different event times, and the values 0.70 to <0.80, and >0.80 are judged to have acceptable and excellent discrimination, respectively[23,24]. NRI and IDI are alternatives to the increase in the C-statistic for evaluating improvement in the performance of different two models by the addition of new markers[24,25]. The base model included potential confounding variables listed in Table 2. Calculation of these indicators was based on 300 iterations of perturbation resampling. To evaluate the predictive value for the combination of GGT and ALT, their interaction term was also included.

Statistical analyses were carried out using IBM SPSS Statistics for Windows software, Version 24.0 (Armonk, NY, USA) and R version 3.4.3 (Vienna, Austria) for Windows (http://cran.r-project.org/). A *P*-value <0.05 was considered to be significant.

RESULTS

Baseline characteristics

The mean age of the participants in the present study was 48.1 years. Participants in the highest GGT category were older and more likely to be current smokers (Table 1). They also had significantly higher alcohol intake, BMI, and blood levels of triglycerides, fasting insulin levels and FBG. Participants in the highest ALT category were also significantly older, and had higher BMI and blood levels of triglycerides, fasting insulin levels and FBG. Alcohol intake and smoking status did not differ among categories of ALT tertile.

Association between GGT or ALT and type 2 diabetes mellitus incidence

During the 12-year follow-up period of 2,775 Japanese male workers (27,040 person-years), 276 developed type 2 diabetes mellitus. The overall crude incidence rate was 10.2 per 1,000 person-years. It was confirmed that most of the potential confounding variables were associated with type 2 diabetes mellitus incidence, except for regular physical activity and total cholesterol (Table 2).

The incidence rates became higher according to the increase in the GGT or ALT categories (Table 3). Multivariable-adjusted HRs of type 2 diabetes mellitus for men in the highest GGT or ALT categories compared with the lowest tertile were significantly higher than unity. Further adjustment for fasting insulin, FBG and each liver enzyme attenuated the significant association of GGT with type 2 diabetes mellitus, but not for the association of ALT.

Stratified analyses by drinking status, BMI and triglycerides

The incidence rates of type 2 diabetes mellitus in categories created by the combination of GGT and ALT were 6.1, 11.6 and 17.1 per 1,000 person-years in "both low", "either high" and "both high" categories, respectively (Table 4). The positive association remained significant in the fully-adjusted model including fasting insulin and FBG. The significant associations were also observed in non- or light-to-moderate drinkers and in individuals with normal weight, suggesting that there are direct associations independent of these conditions associated with elevated GGT and ALT (*P* for difference in the associations according to drinking status and BMI >0.05). In addition, we observed significant associations of the combination of GGT and ALT with type 2 diabetes mellitus incidence in participants with a normal TC range, FBG <100 mg/dL and fasting insulin <10 μU (Table S1). However, the association might have been confounded by the presence of hypertriglyceridemia or low HDL-c. Namely, the association of the combination of GGT and ALT appeared to be significant only in participants with hypertriglyceridemia or low HDL-c.

Table 1 | Baseline characteristics of the participants according to gamma-glutamyl transferase and alanine aminotransferase levels, Aichi cohort study 2002, Japan

	Gamma-glutamyl transferase (IU/L)			*P*-value	Alanine aminotransferase (IU/L)			*P*-value
	Tl (8–27) (n = 950)	T2 (28–48) (n = 862)	T3 (49–1,121) (n = 963)		T1 (5–18) (n = 983)	T2 (19–27) (n = 842)	T3 (28–294) (n = 950)	
Age (years)	47.0 ± 7.3	48.5 ± 7.2	49.0 ± 6.5	<0.01	48.5 ± 7.2	48.5 ± 7.0	47.5 ± 6.9	<0.01
Family history of diabetes (yes)	124 (13.1)	128 (14.8)	120 (12.5)	0.30	129 (13.1)	99 (11.8)	144 (15.2)	0.10
Regular physical activity (yes)	207 (21.8)	174 (20.2)	200 (20.8)	0.70	223 (22.7)	174 (20.7)	184 (19.4)	0.20
Current smoker (yes)	285 (30.0)	296 (34.3)	403 (41.8)	<0.01	347 (35.3)	285 (33.8)	352 (37.1)	0.36
Alcohol intake (g/day)	9.1 ± 16.2	14.9 ± 18.9	26.8 ± 26.0	<0.01	16.4 ± 21.6	17.1 ± 22.0	17.7 ± 22.9	0.48
<20 g/day	817 (86.0)	627 (72.7)	474 (49.2)	<0.01	695 (70.7)	589 (70.0)	634 (66.7)	0.14
≥20 g/day	133 (14.0)	235 (27.3)	489 (50.8)		288 (29.3)	253 (30.0)	316 (33.3)	
Fasting blood glucose (mg/dL)	91.6 ± 9.2	93.3 ± 8.8	95.1 ± 9.9	<0.01	92.4 ± 9.4	93.6 ± 9.5	94.2 ± 9.4	<0.01
<100 mg/dL	797 (83.9)	681 (79.0)	689 (71.5)	<0.01	810 (82.4)	643 (76.4)	714 (75.2)	<0.05
100–109 mg/dL	112 (11.8)	139 (16.1)	189 (19.6)		123 (12.5)	150 (17.8)	167 (17.6)	
≥110 mg/dL	41 (4.3)	42 (4.9)	85 (8.8)		50 (5.1)	49 (5.8)	69 (7.3)	
Body mass index (kg/m^2)†	22.2 (22.1–22.4)	23.3 (23.1–23.4)	23.7 (23.5–23.9)	<0.01	22.1 (22.0–22.3)	23.0 (22.9–23.2)	24.1 (23.9–24.2)	<0.01
Triglycerides (mg/dL)†	94.9 (92.0–97.9)	114.4 (110.5–118.5)	145.9 (140.9–151.2)	<0.01	95.5 (92.5–98.6)	116.1 (112.2–120.3)	144.5 (139.4–149.7)	<0.01
HDL-c (mg/dL)†	55.1 (54.2–56.0)	53.8 (52.9–54.8)	54.9 (54.0–55.8)	0.12	57.6 (56.7–58.6)	54.2 (53.2–55.1)	52.0 (51.2–52.8)	<0.01
Total cholesterol (mg/dL)†	199.1 (197.2–201.1)	207.8 (205.6–209.9)	214.7 (212.5–216.9)	<0.01	199.8 (197.8–201.8)	207.6 (205.5–209.7)	214.5 (212.3–216.8)	<0.01
Fasting insulin (μU/mL)†	5.6 (5.4–5.9)	6.4 (6.1–6.6)	7.1 (6.8–7.4)	<0.01	5.1 (4.9–5.3)	6.2 (6.0–6.5)	8.1 (7.7–8.5)	<0.01
GGT (IU/L)†	19.7 (19.4–20.0)	36.1 (35.7–36.5)	86.4 (83.7–89.2)	<0.01	27.0 (26.1–27.9)	38.1 (36.6–39.6)	61.4 (58.7–64.3)	<0.01
ALT (IU/L)†	17.3 (16.9–17.7)	23.3 (22.7–24.0)	31.8 (30.7–32.8)	<0.01	14.0 (13.8–14.2)	22.4 (22.2–22.6)	41.6 (40.7–42.6)	<0.01

Data are reported in mean ± standard deviation or number (%), unless otherwise specified. *P*-values are from analysis of variance for continuous variables, and the χ^2-test for categorical variables. †Geometric mean and the 95% confidence interval. ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; HDL-c, high-density lipoprotein cholesterol; T, tertile.

Table 2 | Crude hazard ratios and the 95% confidence intervals for the incidence of type 2 diabetes mellitus according to potential confounding variables, Aichi cohort study 2002–2015, Japan

		Crude HR (95% CI)
Age (years)	Per 1 year	1.0 (1.0–1.1)
Family history of diabetes (yes)		1.9 (1.4–2.5)
Regular physical activity (yes)		0.8 (0.6–1.1)
Current smoking status (yes)		1.5 (1.2–2.0)
Alcohol intake (g/day)	Per 1 g/day	1.0 (1.0–1.0)
Ln-body mass index (kg/m^2)	Per 1 SD	1.4 (1.3–1.6)
Ln-triglycerides (mg/dL)	Per 1 SD	1.4 (1.3–1.6)
Ln-HDL-c (mg/dL)	Per 1 SD	0.7 (0.7–0.8)
Ln-total cholesterol (mg/dL)	Per 1 SD	1.1 (0.9–1.2)
Fasting blood glucose		
<100 mg/dL		REF
100–109 mg/dL		3.2 (2.4–4.2)
≥110 mg/dL		6.8 (5.0–9.3)
Ln-fasting insulin (μU/mL)	Per 1 SD	1.4 (1.3–1.6)

Total sample n = 2,775. Standard deviation (SD) for logarithmically transformed (Ln)(body mass index) = 0.1; SD for Ln(triglycerides) = 0.6; SD for Ln(high-density lipoprotein cholesterol) = 0.3; SD for Ln(total cholesterol) = 0.2; SD for Ln(fasting insulin) = 0.7. HDL-c, high-density lipoprotein cholesterol; REF indicates reference.

Incremental predictive value of adding GGT or/and ALT

The base model that included traditional risk factors for type 2 diabetes mellitus showed a C-statistic of 0.747 (95% CI 0.717–0.778; Table 5). The addition of Ln-GGT or/and Ln-ALT to the base model increased the C-statistic, and significantly improved IDI and NRI ($P < 0.05$). However, further adding GGT to the model (base + ALT) did not significantly improve IDI ($P = 0.159$) nor NRI ($P = 0.106$) anymore.

DISCUSSION

The present study showed that simultaneous elevation of GGT and ALT was significantly associated with the development of type 2 diabetes mellitus, even after adjustment for fasting insulin and FBG levels. Furthermore, the association was observed in non- or light-to-moderate drinkers or in participants with normal weight, totally excluding the possibility of confounding by these variables. Interestingly, the significant association was observed in participants with elevated triglyceride levels, but not in participants with normal triglyceride levels.

The present prospective study is consistent with and extends the findings of previous cross-sectional studies[15,16]. Adjustment for additional variables, such as fasting insulin and fasting blood glucose, was also carried out. Furthermore, stratified analyses were carried out by alcohol drinking status, overweight and hypertriglyceridemia in the present study. Significant associations observed in non- or light-to-moderate drinkers, as well as in individuals with normal weight, indicated that elevation of ALT and GGT would increase type 2 diabetes mellitus risk independent of alcohol intake and obesity. However, the

Table 3 | Crude incidence rates, multivariable-adjusted hazard ratios and the 95% confidence intervals for the incidence of type 2 diabetes mellitus according to tertiles of gamma-glutamyl transferase or alanine aminotransferase, Aichi cohort study 2002–2015, Japan

	Gamma-glutamyl transferase (IU/L)			Continuous HR per 1 SD	Alanine aminotransferase (IU/L)			Continuous HR per 1 SD
	T1 (8–27)	T2 (28–48)	T3 (49–1,121)		T1 (5–18)	T2 (19–27)	T3 (28–294)	
	(n = 950)	(n = 862)	(n = 963)		(n = 983)	(n = 842)	(n = 950)	
No. incidents	59	71	146		67	71	138	
Person-years	9,691	8,325	9,025		9,764	8,234	9,043	
Crude incidence rate	6.1	8.5	16.2		6.9	8.6	15.3	
HR 1	REF	1.1 (0.8–1.5)	2.0 (1.4–2.8)	1.4 (1.2–1.6)	REF	1.1 (0.8–1.6)	1.7 (1.2–2.3)	1.4 (1.2–1.5)
HR 2		1.0 (0.7–1.4)	1.4 (1.0–2.1)	1.1 (0.9–1.3)		1.0 (0.7–1.4)	1.3 (0.9–1.8)	1.2 (1.1–1.4)

Hazard ratio (HR) 1: adjusted for age, family history of diabetes (yes/no), regular physical activity (yes/no), current, smoking status (yes/no), alcohol consumption (g/day), logarithmically transformed (Ln) body mass index (kg/m2), Ln triglycerides, Ln high-density lipoprotein cholesterol and Ln total cholesterol. HR 2: adjusted for same covariates as HR 1 and Ln fasting insulin, fasting blood glucose as categories (<100, 100–109 and ≥110) and Ln gamma-glutamyl transferase or Ln alanine aminotransferase (mutually adjusted). Standard deviation for Ln (gamma-glutamyl transferase) = 0.7; standard deviation for Ln(alanine aminotransferase) = 0.5. REF, reference; T, tertile.

Table 4 | Association of categories of gamma-glutamyl transferase, alanine aminotransferase and their combination with type 2 diabetes mellitus incidence stratified by drinking status, obesity and triglycerides, Aichi cohort study 2002–2015, Japan

	Gamma-glutamyl transferase (IU/L)		Alanine aminotransferase (IU/L)		Combination of gamma-glutamyl transferase and alanine aminotransferase		
	Low (T1 + T2) (8–48)	High (T3) (49–1,121)	Low (T1 + T2) (5–27)	High (T3) (28–294)	Both low	Either high	Both high
All participants							
	(*n* = 1,812)	(*n* = 963)	(*n* = 1,825)	(*n* = 950)	(*n* = 1,417)	(*n* = 803)	(*n* = 555)
No. incidents	130	146	138	138	89	90	97
Person-years	18,016	9,025	17,998	9,043	1,4174	7,665	5,201
Crude incidence rate	7.2	16.2	7.7	15.3	6.3	11.7	18.7
HR 1	REF	1.9 (1.4–2.4)	REF	1.6 (1.2–2.1)	REF	1.6 (1.2–2.1)	2.3 (1.7–3.2)
HR 2		1.4 (1.1–1.0)		1.3 (1.0–1.7)		1.4 (1.1–1.9)	2.0 (1.5–2.8)
Drinking status							
<20 g/day	(*n* = 1,444)	(*n* = 474)	(*n* = 1,284)	(*n* = 634)	(*n* = 1,107)	(*n* = 514)	(*n* = 297)
No. incidents	108	75	85	98	69	55	59
Person-years	14,475	4,432	12,890	6,018	11,218	4,928	2,761
Crude incidence rate	7.5	16.9	6.6	16.3	6.2	11.2	21.4
HR 1	REF	1.9 (1.4 –2.5)	REF	2.1 (1.5–2.9)	REF	1.6 (1.1–2.3)	2.8 (1.9–4.1)
HR 2		1.3 (0.9–1.8)		1.7 (1.2–2.3)		1.4 (0.9–2.1)	2.4 (1.7–3.6)
≥20 g/day	(*n* = 368)	(*n* = 489)	(*n* = 541)	(*n* = 316)	(*n* = 310)	(*n* = 289)	(*n* = 258)
No. incidents	22	71	53	40	20	35	38
Person-years	3,540	4,593	5,108	3,025	2,956	2,737	2,440
Crude incidence rate	6.2	15.5	10.4	13.2	6.8	12.8	15.6
HR 1	REF	2.1 (1.2–3.4)	REF	1.0 (0.6–1.5)	REF	1.6 (0.9–2.8)	1.7 (0.9–3.0)
HR 2		1.9 (1.1–3.3)		0.7 (0.5–1.3)		1.4 (0.8–2.4)	1.6 (0.9–2.9)
Body mass index							
<25 kg/m^2	(*n* = 1,485)	(*n* = 662)	(*n* = 1,539)	(*n* = 608)	(*n* = 1,216)	(*n* = 592)	(*n* = 339)
No. incidents	93	80	104	69	69	59	45
Person-years	14,886	6,375	15,332	5,929	12,257	5703	3,300
Crude incidence rate	6.2	12.5	6.8	11.6	5.63	10.3	13.6
HR 1	REF	1.9 (1.3–2.6)	REF	1.6 (1.2–2.2)	REF	1.6 (1.2–2.3)	2.2 (1.5–3.4)
HR 2		1.5 (1.1–2.2)		1.3 (0.9–1.8)		1.5 (1.0–2.2)	2.0 (1.3–3.0)
≥25 kg/m^2	(*n* = 327)	(*n* = 301)	(*n* = 286)	(*n* = 342)	(*n* = 201)	(*n* = 211)	(*n* = 216)
No. incidents	37	66	34	69	20	31	52
Person-years	3,130	2,650	2,666	3113.7	1,917	1,966	1,901
Crude incidence rate	11.8	24.9	12.8	22.2	10.4	15.8	27.4
HR 1	REF	1.9 (1.2–2.9)	REF	1.4 (1.0–2.0)	REF	1.4 (0.8–2.5)	2.3 (1.3–3.9)
HR 2		1.3 (0.8–2.1)		1.2 (0.8–1.8)		1.3 (0.7–2.3)	2.0 (1.1–3.4)
Triglyceride							
<150 mg/dL	(*n* = 1,398)	(*n* = 513)	(*n* = 1,397)	(*n* = 514)	(*n* = 1,134)	(*n* = 527)	(*n* = 250)
No. incidents	90	51	91	50	64	53	24
Person-years	13,894	4,941	13,876	4,959	11,374	5,022	2439
Crude incidence rate	,6.5	10.3	6.6	10.1	5.6	10.6	9.8
HR 1	REF	1.4 (1.0–2.0)	REF	1.4 (1.0–2.0)	REF	1.7 (1.2–2.5)	1.5 (0.9–2.5)
HR 2		1.2 (0.8–1.7)		1.2 (0.8–1.8)		1.5 (1.0–2.1)	1.5 (0.9–2.4)
≥150 mg/dL	(*n* = 414)	(*n* = 450)	(*n* = 428)	(*n* = 436)	(*n* = 283)	(*n* = 276)	(*n* = 305)
No. incidents	40	95	47	88	25	37	73
Person-years	4,122	4,084	4,122	4,084	2,800	2,643	2,763
Crude incidence rate	9.7	23.3	11.4	21.6	8.9	14.0	26.4
HR 1	REF	2.4 (1.6–3.6)	REF	1.8 (1.3–2.6)	REF	1.5 (0.9–2.5)	2.9 (1.8–4.6)
HR 2		1.7 (1.1–2.6)		1.4 (0.9–2.1)		1.4 (0.8–2.3)	2.5 (1.5–4.0)

HR 1: adjusted for age, family history of diabetes (yes/no), regular physical activity (yes/no), current, smoking status (yes/no), alcohol consumption (g/day), logarithmically transformed (Ln) body mass index (kg/m2), Ln triglycerides, Ln high-density lipoprotein cholesterol and Ln total cholesterol. HR 2: adjusted for the same covariates as HR 1 and Ln fasting insulin, fasting blood glucose as categories (<100, 100–109 and ≥110), and Ln gamma-glutamyl transferase or alanine aminotransferase (mutually adjusted), except relevant strata variables. REF, reference; T, tertile.

Table 5 | Incremental predictive value of gamma-glutamyl transferase or/and alanine aminotransferase for type 2 diabetes mellitus incidence at 10 years, Aichi cohort study 2002–2015, Japan

Prediction model	C-statistic (95% CI)	IDI (95% CI)	*P*-value	NRI (95% CI)	*P*-value
Base model	0.747 (0.717–0.778)	REF	<0.05	REF	<0.05
Base model + Ln-GGT	0.754 (0.724–0.784)	0.007 (0.001–0.018)		0.130 (0.055–0.198)	
Base model	0.747 (0.715–0.779)	REF	<0.05	REF	<0.01
Base model + Ln-ALT	0.754 (0.723–0.785)	0.010 (0.002–0.022)		0.130 (0.051–0.212)	
Base model	0.747 (0.714–0.780)	REF	<0.01	REF	<0.01
Base model + Ln-GGT +Ln- ALT + Int	0.755 (0.723–0.788)	0.012 (0.006–0.026)		0.134 (0.056–0.207)	
Base model + Ln-GGT	0.754 (0.723–0.785)	REF	<0.05	REF	0.060
Base model + Ln-GGT + Ln- ALT + Int	0.755 (0.725–0.786)	0.004 (0.000–0.015)		0.135 (–0.004–0.186)	
Base model + Ln-ALT	0.754 (0.724–0.785)	REF	0.159	REF	0.106
Base model + Ln-ALT + Ln- GGT + Int	0.755 (0.725–0.785)	0.002 (0.000–0.010)		0.096 (–0.039–0.156)	

Total sample n = 2,775. Base model included age, family history of diabetes (yes/no), regular physical activity (yes/no), current, smoking status (yes/no), alcohol consumption (g/day), body mass index (kg/m2), triglycerides, high-density lipoprotein cholesterol, total cholesterol, fasting insulin, fasting blood glucose as categories (<100, 100–109 and ≥110). ALT, alanine aminotransferase, CI, confidence interval; GGT, gamma-glutamyl transferase; Int, interaction for logarithmically transformed gamma-glutamyl transferase × logarithmically transformed alanine aminotransferase; IDI, integrated discrimination improvement; Ln, logarithmically transformed; NRI, net reclassification improvement; REF, reference; T, tertile.

association was observed only in participants with high triglyceride levels. This finding might indicate that the simultaneous presence of hepatic fat accumulation (high TG) and liver injury (high ALT), as well as oxidative stress (high GGT), is etiologically involved in the development of type 2 diabetes mellitus[13,14,26,27].

The implications of the present findings are threefold. First, participants with elevated ALT and GGT had approximately double the risk of type 2 diabetes mellitus incidence, independent of confounding variables, including alcohol intake, obesity, fasting insulin and FBG. Liver injury expressed by elevation of both GGT and ALT should be avoided to prevent type 2 diabetes mellitus. Second, investigations are warranted in those with elevated GGT and ALT for the cause of their elevation, so as to plan type 2 diabetes mellitus preventive measures. Finally, the molecular pathway leading from the concomitant elevation of triglycerides, ALT and GGT to type 2 diabetes mellitus development should be elucidated. We previously reported that the degree of intrahepatic fat accumulation was positively associated with blood levels of ALT independent of homeostasis model assessment for insulin resistance, which indicated that liver fat per se leads to liver damage[28] and a state of increased oxidative stress[3,29–31].

The association between non-alcoholic fatty liver disease and type 2 diabetes mellitus incidence could be explained by intrahepatic fat-led systemic inflammation and insulin resistance[14,32–34]. However, it remains to be elucidated whether early signs of liver damage by intrahepatic fat or non-alcoholic fatty liver disease itself would lead to type 2 diabetes mellitus, or if insulin resistance would be responsible for intrahepatic fat accumulation[35].

Regarding the incremental predictive value, a cross-sectional study in Singapore and a prospective study in the Netherlands implied that the addition of liver enzymes improved the predictive value for type 2 diabetes mellitus[15,36]. The present study extended the evidence to a long-term prospective cohort study.

The strengths of the present study include the prospective cohort design with a 12-year follow-up period. Fasting insulin and FBG were included as a covariate, and stratified analyses were carried out by important variables. Furthermore, the predictive value of GGT and ALT were evaluated by reliable indicators, such as the C-statistic, IDI and NRI. However, there were several limitations. First, it was not possible to differentiate non-drinkers who might have quit drinking due to health problems. We included non-drinking to be collapsed with light-to-moderate drinking in the present study. This might be a reason for the slightly higher incidence rate of type 2 diabetes mellitus in that group than in excessive drinking when GGT and/or ALT was high. Second, while creating categories for the combination of GGT and ALT, the category with only high ALT or GGT was collapsed, as type 2 diabetes mellitus incidence was not considered enough. Future studies are required to investigate the main effect of each liver enzyme and their joint effect on type 2 diabetes mellitus incidence. Third, women could not be evaluated because of the small sample size in the present study. Much larger studies are required to investigate the associations in women.

In conclusion, a concomitant increase of ALT and GGT was associated with a higher incidence of type 2 diabetes mellitus during a 12-year follow-up period in middle-aged Japanese men, independent of alcohol drinking and weight. The positive association was not observed in men with normal triglyceride levels.

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DISCLOSURE

The authors declare no conflict of interest.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1 | Association of categories of gamma-glutamyl transferase, alanine aminotransferase and their combination with type 2 diabetes mellitus incidence stratified by high-density lipoprotein cholesterol, total cholesterol, fasting blood glucose and fasting insulin, Aichi cohort study 2002–2015, Japan.