Prediction of new onset of diabetes mellitus during a 10-year period by using a combination of levels of alanine aminotransferase and γ-glutamyl transferase

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Abstract. Levels of alanine aminotransferase (ALT) and γ-glutamyl transferase (GGT) have been reported to be associated with increased risk of diabetes mellitus (DM). However, whether a combination of levels of ALT and GGT predicts new onset of DM better than does ALT or GGT alone in both males and females has not fully been addressed. We investigated the relationship between the combination of ALT and GGT and DM development during a 10-year follow-up period in 13,919 subjects (male/female: 8,983/4,936; age 48 ± 10 years) who received health examinations. During the 10-year period, 617 males (6.9%) and 153 females (3.1%) had new onset of DM. Multivariable Cox proportional hazard models with a restricted cubic spline showed that hazard ratios (HRs) of DM development increased with higher levels of ALT and GGT at baseline in both sexes after adjustment of confounding factors. When divided into 4 subgroups of high (H-) and low (L-) levels of ALT (male/female: 27/21 U/L) and GGT (male/female: 43/23 U/L) using cutoff values shown by receiver operating characteristic curve analyses, the adjusted HR in the H-ALT/H-GGT group was significantly higher than HR in the L-ALT/L-GGT group as the reference in males (HR [95% confidence interval]: 1.73[1.36–2.20], p < 0.001) but was not significantly higher in females (1.50 [0.97–2.33], p = 0.065). The addition of the combination of H-ALT/H-GGT to traditional risk factors with and without H-ALT or H-GGT alone significantly improved the discriminatory capability for predicting development of DM. In conclusion, the combination of H-ALT/H-GGT efficiently predicts development of DM in male individuals but not significantly in female individuals.

Key words: Diabetes mellitus, Alanine aminotransferase, γ-glutamyl transferase, Liver enzyme

THE PREVALENCE RATE of diabetes mellitus (DM) is 9.3% in adults, and approximately 463 million people suffer from DM worldwide [1]. The number of people with DM is expected to increase further [1]. Since DM causes various complications including nephropathy, neuropathy and cardiovascular disease, identification of individuals who have a high risk of DM is very important. On the other hand, approximately 1.5 billion people worldwide have chronic liver disease such as nonalcoholic fatty liver disease (NAFLD) [2]. NAFLD is diagnosed in approximately 10% to 30% of adults by health examinations [3], and the number of patients with NAFLD has recently been increasing [4]. Since the global burden of chronic liver disease and DM is substantial, it is important to investigate the relationship between DM and chronic liver disease.

Chronic liver disease has recently been reported to be a risk factor of DM [5-8]. It has been reported that several biomarkers for liver function, including alanine aminotransferase (ALT), γ-glutamyl transferase (GGT) and aspartate aminotransferase (AST), are associated with a 1.3~1.5-fold increased risk of DM [9]. Recent meta-analyses showed associations of the risk for development of DM with levels of ALT and GGT but not level
of AST [10, 11]. An elevated level of ALT has been reported to be associated with lipid accumulation in the liver [12], which may cause insulin resistance [13] and secondary pancreatic β-cell failure [14]. On the other hand, it has been reported that GGT is involved in the generation of reactive oxygen species [15] and is associated with several inflammatory factors [16]. Therefore, a combination of high levels of ALT and GGT might be a strong predictor of the risk for DM. It has recently been reported that a simultaneous elevation of the level of ALT and GGT was significantly associated with the development of DM in middle-aged Japanese subjects (only males, \( n = 2,775 \)) [17]. However, since the distribution of concentrations of ALT and GGT differs in males and females, the effect of liver enzymes on the risk for development of DM may differ by sex [18]. Therefore, whether the combination of levels of ALT and GGT predicts new onset of DM in both males and females has not been fully addressed. In the present study, we investigated the association of new onset of DM during a 10-year period with the combination of levels of ALT and GGT as well as each single level at baseline in a general population including both male and female subjects.

Methods

This study was a project of the Broad-range Organization for REnal, Arterial and cardiac studies by Sapporo Medical University Affiliates (BOREAS) investigators and was designed as the BOREAS-DM3 study. The study was performed with the approval of the institutional ethical committee of Sapporo Medical University (Number: 30-2-32) and conformed to the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all of the subjects.

Study subjects and clinical outcome

All of the subjects (\( n = 28,990 \)) who received annual medical checkups at Keijinkai Maruyama Clinic, Sapporo, Japan in 2006 were enrolled [19, 20]. A flow chart of the study subjects is shown in Fig. 1. Exclusion criteria were the diagnosis of DM at baseline and the absence of laboratory data including hemoglobin A1c (HbA1c), fasting plasma glucose (FPG), ALT and GGT at baseline. After exclusion, a total of 13,919 subjects (male/female: 8,983/4,936) who received health examinations at least once during the period from 2007 to 2016 contributed the analysis. The average number of annual examinations during the period from 2007 to 2016 was 6.0, and the number of subjects who continuously received health examinations every year was 5,696 (40.6%, male/female: 3,689/1,967).

The development of DM during a 10-year follow-up period was diagnosed in accordance with the guidelines of the American Diabetes Association [21]: HbA1c ≥6.5%, FPG ≥126 mg/dL or self-reported use of anti-diabetic drugs.

Measurements

Medical examinations were performed after an overnight fast. Body mass index (BMI) was calculated as body weight (kg) divided by height (meter) squared. HbA1c level was presented as the National Glycohemoglobin Standardization Program (NGSP) equivalent value. Estimated glomerular filtration rate (eGFR) was calculated by an equation for Japanese: eGFR (mL/min/1.73 m²) = 194 × serum creatinine \((-1.094) \times \text{age} \times 0.739 \; (\text{if female}) \) [22]. Information on regular alcohol drinking habit (≥3 times/week), current smoking habit, family history of DM, and use of drugs for DM, dyslipidemia and hypertension was obtained by a self-administered questionnaire survey.

Hypertension was diagnosed as systolic blood pressure ≥140 mmHg, diastolic blood pressure ≥90 mmHg or self-reported use of anti-hypertensive drugs. Dyslipidemia was diagnosed as triglycerides ≥150 mg/dL, high-density lipoprotein cholesterol <40 mg/dL, low-density lipoprotein cholesterol ≥140 mg/dL or self-reported use of anti-dyslipidemic drugs.

Statistical analysis

Numeric variables are expressed as means ± SD for normal distribution parameters or medians (interquartile ranges) for skewed distribution parameters. Normality of distribution in each parameter was examined using the Shapiro-Wilk W test. Intergroup differences of demographic parameters were tested by the chi-square test. Differences between data in multiple groups were examined by one-way analysis of variance. The ability of ALT, GGT, FPG or HbA1c at baseline to predict new onset of DM was investigated by using receiver operating characteristic (ROC) curve analysis. The area under the curve (AUC) was calculated, and the cutoff values of ALT and GGT were obtained by the Youden index [23]. The relationship between hazard ratio (HR) for the development of DM and level of ALT or GGT at baseline was analyzed by a multivariable Cox proportional hazard model with a restricted cubic spline after adjustment of confounders including age, BMI, FPG, habits of alcohol drinking and smoking, family history of DM and diagnosis of dyslipidemia and hypertension at baseline. In addition, subjects were divided into subgroups of high (H-) and low (L-) levels of ALT and GGT using cutoff values determined by ROC curve analyses in both male and female subjects. Furthermore, both male and female
subjects were divided into four subgroups: L-ALT/L-GGT, L-ALT/H-GGT, H-ALT/L-GGT and H-ALT/H-GGT groups. The rate of cumulative incidence of new onset of DM was analyzed by the log-rank test of Kaplan-Meier curves. HRs, 95% confidence intervals (CIs) and Akaike’s Information Criterion (AIC) after adjustment of the confounders were also calculated in the subgroups. To compare the discrimination for development of DM between the models adjusted for confounders as traditional risk factors for DM with and without H-ALT, H-GGT or H-ALT/H-GGT, C-statistics analogous to the AUC were estimated using the method of DeLong et al. [24]. Moreover, the increased discriminatory value was examined by net reclassification improvement (NRI) and integrated discrimination improvement (IDI) [25]. A p value <0.05 was considered statistically significant. All data were analyzed by using EZR [26] and R3.6.2.

Results

Characteristics of the study subjects

Characteristics of the recruited subjects are shown in Table 1. Male subjects had larger BMI, higher blood pressures, higher levels of albumin, uric acid, FPG, HbA1c and triglycerides and lower levels of eGFR than did female subjects. Levels of ALT, AST and GGT were significantly higher in males than in females. The frequencies of habits of current smoking and regular alcohol drinking were significantly lower in females than in males.

Incidence of new onset of DM during the follow-up period

Among the 13,919 subjects (male/female: 8,983/4,936), 617 male subjects (6.9%) and 153 female subjects (3.1%) developed new onset of DM during a 10-year period. The mean follow-up period was 9.5 years, and follow-up summation was 93,556 (male/female: 60,427/33,129) person-years. The cumulative incidence rate of new onset of DM was 80 (95% CI: 75–86) (male/female: 99 [92–107]/46 [39–54]) per 1,000 person-years.

Prediction of new onset of DM by levels of ALT and GGT

ROC curve analyses for predicting new onset of DM showed that cutoff points of ALT level at baseline in males and females were 27 U/L (AUC: 0.66) (Fig. 2A) and 21 U/L (AUC: 0.64) (Fig. 2B), respectively. Cutoff
points of GGT level at baseline in males and females were 43 U/L (AUC: 0.66) (Fig. 2C) and 23 U/L (AUC: 0.66) (Fig. 2D), respectively. Sensitivities, specificities, positive predictive values (PPVs) and negative predictive values (NPVs) of the cutoff levels of ALT and GGT for prediction of new onset of DM are shown in Supplementary Table S1.

On the other hand, cutoff points of FPG level at baseline in males and females were 100 mg/dL (AUC: 0.82) (Supplementary Fig. S1A) and 93 mg/dL (AUC: 0.80) (Supplementary Fig. S1B), respectively. Cutoff points of HbA1c level at baseline in males and females were 5.6% (AUC: 0.84) (Supplementary Fig. S1C) and 5.6% (AUC: 0.84) (Supplementary Fig. S1D), respectively.

Levels of ALT and GGT at baseline and new onset of DM

Box plots and histograms of levels of ALT and GGT in both sexes are shown in Supplementary Fig. S2 and Fig. 3, respectively. Multivariable Cox proportional hazard models with a restricted cubic spline showed that HRs of DM development increased with a higher ALT level at baseline in both males (Fig. 3A) and females (Fig. 3B) and a higher GGT level at baseline in both males (Fig. 3C) and females (Fig. 3D) after adjustment for age, BMI, FPG, habits of alcohol drinking and smoking, family history of DM and diagnosis of dyslipidemia and hypertension at baseline.

Level of ALT was a significant risk for new onset of DM in both males and females in unadjusted Cox proportional hazard analyses (Supplementary Table S2). After adjustment of age, BMI, FPG, habits of alcohol drinking and smoking, family history of DM and diagnosis of dyslipidemia and hypertension, the risk of ALT level (per 1 U/L) was significant in males (HR: 1.008, 95% CI: 1.005–1.011, p < 0.001) but not in females (HR: 1.003, 95% CI: 0.993–1.014, p = 0.530).

Level of GGT was a significant risk for new onset of

| Table 1 Characteristics of the subjects at baseline |
|---------------------------------|--------|---------|--------|
| | All   | Male   | Female |
| n                              | 13,919 | 8,983   | 4,936  |
| Age, years                     | 48 ± 10| 48 ± 10 | 48 ± 10| <0.001 |
| Body mass index                | 23.1 ± 3.3 | 24.0 ± 3.1 | 21.7 ± 3.3 | <0.001 |
| Systolic blood pressure, mmHg  | 117 ± 16 | 120 ± 16 | 111 ± 16 | <0.001 |
| Diastolic blood pressure, mmHg | 75 ± 11 | 77 ± 11 | 70 ± 10 | <0.001 |
| Alcohol drinking habit         | 6,451 (46) | 5,152 (57) | 1,299 (26) | <0.001 |
| Current smoking habit          | 4,798 (34) | 3,893 (45) | 905 (19) | <0.001 |
| Family history of DM           | 2,406 (17) | 1,408 (16) | 998 (20) | <0.001 |
| Comorbidity                    |        |        |        |
| Hypertension                   | 1,320 (9) | 971 (11) | 349 (7) | <0.001 |
| Dyslipidemia                   | 572 (4) | 373 (4) | 199 (4) | 0.005 |
| Biochemical data               |        |        |        |
| ALT, U/L                       | 21 (15–31) | 25 (18–36) | 15 (12–20) | <0.001 |
| AST, U/L                       | 21 (18–26) | 22 (19–28) | 19 (16–22) | <0.001 |
| GGT, U/L                       | 31 (19–56) | 42 (27–72) | 18 (14–26) | <0.001 |
| Albumin, g/dL                  | 4.3 ± 0.2 | 4.4 ± 0.2 | 4.3 ± 0.2 | <0.001 |
| eGFR, mL/min/1.73 m²           | 84 ± 16 | 83 ± 14 | 87 ± 15 | <0.001 |
| FPG, mg/dL                     | 90 ± 9 | 92 ± 9 | 86 ± 8 | <0.001 |
| Hemoglobin A1c, %              | 5.2 ± 0.4 | 5.2 ± 0.4 | 5.2 ± 0.4 | <0.001 |
| Total cholesterol, mg/dL       | 203 (182–227) | 203 (183–227) | 202 (180–227) | 0.018 |
| Triglycerides, mg/dL           | 92 (63–136) | 109 (99–109) | 67 (50–94) | <0.001 |
| HDL cholesterol, mg/dL         | 59 (49–71) | 54 (46–64) | 68 (59–79) | <0.001 |
| LDL cholesterol, mg/dL         | 120 (101–141) | 122 (102–143) | 117 (97–139) | <0.001 |

Variables are expressed as number (%), means ± SD or medians (interquartile ranges).

AST, aspartate aminotransferase; ALT, alanine aminotransferase; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; FPG, fasting plasma glucose; GGT, γ-glutamyl transpeptidase; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
DM in both males and females in unadjusted Cox proportional hazard analyses (Supplementary Table S3). After adjustment of age, BMI, FPG, habits of alcohol drinking and smoking, family history of DM and diagnosis of dyslipidemia and hypertension, the risk of GGT level (per 1 U/L) was significant in males (HR: 1.001, 95% CI: 1.000–1.002, \( p = 0.007 \)) but not in females (HR: 1.000, 95% CI: 0.996–1.005, \( p = 0.860 \)).

Combination of levels of ALT and GGT at baseline and hazard risk of new onset of DM

Basal characteristics and cumulative incidence of DM in the 4 subgroups divided by cutoff values of ALT and GGT at baseline determined by ROC curve analyses in all of the subjects as well as in both male and female subjects are shown in Table 2. There were significant differences in parameters, including age, FPG and HbA1c, among the 4 groups in all of the subjects as well as in both male and female subjects.

Kaplan-Meier survival curves showed that there were significant differences in rates of cumulative incidence of new onset of DM among the L-ALT/L-GGT, L-ALT/H-GGT, H-ALT/L-GGT and H-ALT/H-GGT groups in all of the subjects (log-rank test: \( p < 0.001 \)) (Fig. 4A) as well as in male subjects (Fig. 4B) and female subjects (Fig. 4C) when the subjects were divided by sex.

In all of the subjects, after adjustment of age, BMI, FPG, habits of alcohol drinking and smoking, family history of DM and diagnosis of dyslipidemia and hypertension, HR in the H-ALT/H-GGT group (HR: 1.71, 95% CI: 1.39–2.10, \( p < 0.001 \)) was significantly higher than HR in the L-ALT/L-GGT group as the reference
Fig. 3  Hazard ratios of the development of DM by ALT and GGT at baseline.

A–D. Hazard ratios (HRs) for the development of diabetes mellitus (DM) by alanine aminotransferase (ALT) in males (A) and females (B) and by γ-glutamyl transferase (GGT) in males (C) and females (D) at baseline using multivariable Cox proportional hazard models with a restricted cubic spline after adjustment of age, body mass index, fasting plasma glucose, habits of alcohol drinking and smoking, family history of DM and diagnosis of dyslipidemia and hypertension at baseline during a 10-year follow-up period. The reference values of ALT (male/female: 7/4 U/L) and GGT (male/female: 12/9 U/L) were used as minimum values. Solid line: HR, dashed line: 95% confidence interval (CI). Histograms of levels of ALT and GGT are also shown. In the histograms, outlier levels of ALT (male/female: >70/>35 U/L) (Supplementary Figs. S2A, B) and GGT (male/female: >140/>50 U/L) (Supplementary Figs. S2C, D) were excluded in images since the number of subjects was very small in each column.
(Supplementary Table S4). There was no significant interaction between sex and ALT/GGT category \( (p = 0.760) \). When the subjects were divided by sex, the adjusted HR in the H-ALT/H-GGT group was significantly higher than HR in the L-ALT/L-GGT group as the reference in males \( \text{HR}: 1.73, 95\% \text{ CI}: 1.36–2.20, p < 0.001 \) but was not significantly higher in females \( \text{HR}: 1.50, 95\% \text{ CI}: 0.97–2.33, p = 0.065 \) (Table 3). Sensitivities, specificities, PPVs, NPVs and adjusted HRs of the combination of H-ALT/H-GGT for prediction of new onset of DM are shown in Supplementary Table S1.

**Discriminatory capacity of the addition of H-ALT, H-GGT or H-ALT/H-GGT for predicting development of diabetes mellitus**

The addition of H-GGT or H-ALT/H-GGT, but not H-ALT, to traditional risk factors for development of DM, including age, sex, BMI, habits of smoking and alcohol drinking, family history of DM, and diagnosis of hypertension and dyslipidemia, modestly but significantly increased the AUC (Table 4). The incorporation of H-ALT, H-GGT or H-ALT/H-GGT led to significant improvements of the discriminatory capacity for predicting development of DM in the NRI and IDI (Table 4). The addition of H-GGT to traditional risk factors with H-ALT alone significantly improved the discriminatory capacity in the NRI, and the incorporation of H-ALT into traditional risk factors with H-GGT alone significantly improved the discriminatory capacity in the NRI and IDI (Table 4).

Summary of reclassification for the addition of H-ALT/H-GGT to traditional risk factors is shown in Table 5. When the traditional model with H-ALT + H-GGT was used, 21 subjects were correctly reclassified into a higher risk category, whereas 21 subjects were inappropriately reclassified into a lower risk category among subjects who had developed DM. On the other hand, 852 subjects were correctly reclassified into a lower risk category, and 577 subjects were inappropriately reclassified into a higher risk category among subjects who had not developed DM.

### Table 2 Characteristics of the subjects divided by levels of ALT and GGT at baseline

|                  | L-ALT/L-GGT | L-ALT/H-GGT | H-ALT/L-GGT | H-ALT/H-GGT | \( p \) |
|------------------|-------------|-------------|-------------|-------------|--------|
| **All**          | 6,434       | 2,338       | 1,442       | 3,705       |        |
| **Male**         | 3,501       | 1,457       | 1,093       | 2,932       |        |
| **Female**       | 2,933       | 881         | 349         | 773         |        |
| **New onset of diabetes mellitus, number (person-years)** | | | | | |
| **All**          | 157 (44,267)| 120 (15,150)| 78 (9,745)  | 415 (24,394) |        |
| **Male**         | 105 (23,999)| 87 (9,402)  | 67 (7,461)  | 358 (19,565) |        |
| **Female**       | 52 (20,268) | 33 (5,748)  | 11 (2,284)  | 57 (4,829)  |        |
| **Cumulative incidence rate, value per 1,000 person-years (95\% confidence interval)** | | | | | |
| **All**          | 36 (31–43)  | 73 (61–87)  | 77 (62–96)  | 163 (148–178) |        |
| **Male**         | 45 (37–55)  | 83 (67–100)| 85 (67–108)| 173 (157–191)|        |
| **Female**       | 26 (20–34)  | 56 (39–79)  | 49 (27–90)  | 115 (88–149) |        |
| **Age, years**   | | | | | |
| **All**          | 47 ± 10     | 51 ± 9      | 48 ± 10     | 48 ± 9      | <0.001 |
| **Male**         | 48 ± 10     | 51 ± 8      | 47 ± 10     | 47 ± 8      | <0.001 |
| **Female**       | 45 ± 10     | 49 ± 9      | 50 ± 10     | 51 ± 9      | <0.001 |
| **FPG, mg/dL**   | | | | | |
| **All**          | 87 ± 8      | 91 ± 10     | 90 ± 9      | 93 ± 10     | <0.001 |
| **Male**         | 89 ± 8      | 93 ± 9      | 90 ± 8      | 94 ± 10     | <0.001 |
| **Female**       | 84 ± 7      | 87 ± 8      | 86 ± 8      | 89 ± 9      | <0.001 |
| **Hemoglobin A1c, %** | | | | | |
| **All**          | 5.2 ± 0.3   | 5.2 ± 0.4   | 5.3 ± 0.4   | 5.3 ± 0.4   | <0.001 |
| **Male**         | 5.2 ± 0.4   | 5.2 ± 0.4   | 5.3 ± 0.4   | 5.3 ± 0.4   | <0.001 |
| **Female**       | 5.1 ± 0.3   | 5.2 ± 0.4   | 5.2 ± 0.4   | 5.3 ± 0.4   | <0.001 |

Variables are expressed as number or means ± SD. ALT, alanine aminotransferase; FPG, fasting plasma glucose; GGT, γ-glutamyl transpeptidase; H-, high; L-, low.
Discussion

The present study focused on the significance of a combination of high levels of ALT and GGT for predicting the development of DM during a 10-year follow-up period in a general population including both male and female subjects. Multivariable Cox proportional hazard models with a restricted cubic spline showed that HRs of DM development increased with higher levels of ALT and GGT at baseline in both sexes after adjustment of confounding factors. However, the adjusted risk of ALT alone and that of GGT alone for the development of DM were not significant in female subjects in the present study. Therefore, we further investigated the adjusted HR for the combination of ALT and GGT using cutoff values shown by ROC analyses. The adjusted HR in the H-ALT/H-GGT group was significantly higher than HR in the L-ALT/L-GGT group as the reference in males (HR: 1.73, 95% CI: 1.36–2.20, \(p < 0.001\)) but was not significantly higher in females (HR: 1.50, 95% CI: 0.97–2.33, \(p = 0.065\)) (Table 3), though there was no significant interaction between sex and the combination of ALT/GGT.

Fig. 4  Cumulative incidences of new onset of diabetes mellitus in subgroups of high and low levels of ALT and GGT at baseline.

A–C. Kaplan-Meier survival curves for new onset of diabetes mellitus (DM) in the four groups according to high (H-) and low (L-) levels of alanine aminotransferase (ALT) and \(\gamma\)‐glutamyl transferase (GGT) in all of the subjects (A) as well as in male subjects (B) and female subjects (C). Survival curves were compared by the log‐rank test. Black line (L-ALT/L-GGT), red line (L-ALT/H-GGT), green line (H-ALT/L-GGT) and blue line (H-ALT/H-GGT).
The relatively small number of female subjects may have caused a type 2 error since the rate of cumulative incidence of new onset of DM was lower in females than in males (46 [95% CI: 39–54] vs. 99 [92–107] per 1,000 person-years).

To the best of our knowledge, there have been 3 studies on the association of DM with a combination of levels of ALT and GGT [17, 27, 28]. In a cross-sectional study using 8,863 Korean subjects (male/female: 3,408/5,455), the odds ratio for prevalence of DM (male/female: 3,408/5,455) was significantly higher in the H-ALT (male/female: >23/>20 U/L)/H-GGT (male/female: >47/>23 U/L) group than in the L-ALT/L-GGT group [27]. In a case-control study using 571 patients with type 2 DM (male/female: 236/335) and 571 control subjects (male/female: 236/335) in Singapore, the risk for DM development in the H-ALT (≥21 U/L)/H-GGT (≥23 U/L) group was also significantly higher than in the L-ALT/L-GGT group.

Table 3  Cox proportional hazard analyses for new onset of DM in the ALT/GGT groups divided by sex

| Category                        | Male (n = 8,983) | Female (n = 4,936) |
|---------------------------------|-----------------|-------------------|
|                                 | n   | HR (95% CI) | p       | n   | HR (95% CI) | p       |
| L-ALT/L-GGT                     | 3,501 | Reference | —       | 2,933 | Reference | —       |
| L-ALT/H-GGT                     | 1,457 | 1.18 (0.87–1.59) | 0.277 | 881 | 1.20 (0.75–1.91) | 0.443 |
| H-ALT/L-GGT                     | 1,093 | 1.36 (0.99–1.87) | 0.056 | 349 | 0.90 (0.45–1.83) | 0.791 |
| H-ALT/H-GGT                     | 2,932 | 1.73 (1.36–2.20) | <0.001 | 773 | 1.50 (0.97–2.33) | 0.065 |
| Age (per 1 year)                | 1.01 (1.00–1.02) | 0.002 | 1.03 (1.01–1.05) | <0.001 |
| Body mass index (per 1)         | 1.09 (1.07–1.12) | <0.001 | 1.08 (1.04–1.13) | <0.001 |
| FPG (per 1 mg/dL)               | 1.13 (1.12–1.14) | <0.001 | 1.11 (1.09–1.12) | <0.001 |
| Current smoking habit           | 1.71 (1.45–2.02) | <0.001 | 1.47 (0.98–2.22) | 0.061 |
| Alcohol drinking habit          | 0.65 (0.54–0.77) | <0.001 | 0.86 (0.57–1.27) | 0.455 |
| Family history of DM            | 0.91 (0.67–1.24) | 0.586 | 0.97 (0.51–1.86) | 0.948 |
| Hypertension                    | 1.01 (0.83–1.22) | 0.930 | 0.98 (0.64–1.49) | 0.936 |
| Dyslipidemia                    | 1.51 (1.14–2.02) | 0.003 | 1.64 (0.90–2.22) | 0.104 |

AIC, Akaike’s information criterion; ALT, alanine aminotransferase; CI, confidence interval; DM, diabetes mellitus, FPG, fasting plasma glucose; HR, hazard ratio; GGT; γ-glutamyl transpeptidase.

Table 4  Discrimination of the addition of FLI into traditional risk factors for diabetes mellitus

| Category                        | AUC Value (95% CI) | p | NRI Value (95% CI) | p | IDI Value (95% CI) | p |
|---------------------------------|-------------------|---|-------------------|---|-------------------|---|
| Traditional model vs. Addition of H-ALT, H-GGT, or H-ALT/H-GGT | Traditional model* 0.864 (0.848–0.880) | — | — | — | — |
|                                | Traditional model* + H-ALT 0.866 (0.850–0.882) | 0.106 | 0.493 (0.413–0.572) | <0.001 | 0.003 (0.001–0.005) | 0.011 |
|                                | Traditional model* + H-GGT 0.867 (0.851–0.883) | 0.018 | 0.492 (0.412–0.567) | <0.001 | 0.002 (0.000–0.004) | 0.045 |
|                                | Traditional model* + H-ALT/H-GGT 0.868 (0.852–0.884) | <0.001 | 0.505 (0.424–0.586) | <0.001 | 0.003 (0.001–0.006) | 0.009 |
| ALT vs. ALT/GGT                 | Traditional model* + H-ALT 0.866 (0.850–0.882) | — | — | — | — |
|                                | [Traditional model* + H-ALT] + H-GGT 0.868 (0.852–0.884) | 0.058 | 0.411 (0.332–0.491) | <0.001 | 0.001 (0.000–0.002) | 0.239 |
| GGT vs. ALT/GGT                 | Traditional model* + H-GGT 0.867 (0.851–0.883) | — | — | — | — |
|                                | [Traditional model* + H-GGT] + H-ALT 0.868 (0.852–0.884) | 0.474 | 0.473 (0.393–0.552) | <0.001 | 0.002 (0.001–0.003) | 0.047 |

* Traditional model includes age, sex, body mass index, fasting plasma glucose, smoking habit, alcohol drinking habit, family history of diabetes mellitus, and diagnosis of hypertension and dyslipidemia.

ALT, alanine aminotransferase; AUC, area under the curve; CI, confidence interval; GGT, γ-glutamyl transpeptidase; H-, high; IDI, integrated discrimination improvement; L-, low; NRI, net reclassification improvement.

category for the risk for development of DM in all of the subjects (Supplementary Table S4). The relatively small number of female subjects may have caused a type 2 error since the rate of cumulative incidence of new onset of DM was lower in females than in males (46 [95% CI: 39–54]) vs. 99 [92–107] per 1,000 person-years).

To the best of our knowledge, there have been 3 studies on the association of DM with a combination of levels of ALT and GGT [17, 27, 28]. In a cross-sectional study using 8,863 Korean subjects (male/female: 3,408/5,455), the odds ratio for prevalence of DM (male/female: 236/335) was significantly higher in the H-ALT (male/female: ≥23/>20 U/L)/H-GGT (male/female: ≥47/>23 U/L) group than in the L-ALT/L-GGT group [27]. In a case-control study using 571 patients with type 2 DM (male/female: 236/335) and 571 control subjects (male/female: 236/335) in Singapore, the risk for DM development in the H-ALT (≥21 U/L)/H-GGT (≥23 U/L)
regardless of sex with the combination of ALT and GGT for predicting new onset of DM, whereas the numbers of correct and inappropriate reclassifications were the same (n = 21 each) in subjects who had developed DM (Table 5). These findings suggest that the traditional model with H-ALT + H-GGT significantly improves the discriminatory capability, especially specificity, for predicting development of DM.

In the present study, AUCs for cutoff points of ALT and GGT (0.64–0.67) in ROC curve analyses were lower than those of FPG and HbA1c (0.80–0.84) (Fig. 2, Supplementary Fig. S1). In a previous study using 9,748 Japanese subjects, AUCs for cutoff points of ALT and GGT for predicting new onset of DM were 0.71 (95% CI: 0.68–0.74) and 0.67 (95% CI: 0.64–0.69), respectively [29], which were comparable to the results in the present study. Furthermore, AUCs for cutoff points of other markers, including AST (0.64), alkaline phosphatase (0.53), total bilirubin (0.52), fibrosis-4 index (0.51) and AST to platelet ratio index (0.58), were lower than those of ALT (0.71) and GGT (0.67) [29]. As risk factors, but not markers of diagnosis for DM, the AUCs for ALT and GGT (0.64–0.67) in the present study would be appropriate.

Oxidative stress and increased inflammation are implicated in insulin resistance and impaired insulin secretion [30]. Level of ALT is a specific marker of liver dysfunction, and elevated ALT level is strongly correlated with the accumulation of liver fat [12]. Lipid accumulation in the liver may lead to compensatory hyperinsulinemia and secondary pancreatic β-cell failure with progressive hyperglycemia [14]. Furthermore, ALT, known as a gluconeogenic enzyme, has been proposed to be a marker of impaired insulin signaling independent of liver injury [13]. It has also been reported that ALT level is strongly correlated with plasma activity of xanthine oxidoreductase (XOR), which is abundantly expressed in the liver and can increase reactive oxygen species by generating hydrogen peroxide and superoxide [31-33]. On the other hand, GGT is a sensitive, but nonspecific, biomarker of liver dysfunction [11]. GGT is expressed in the surface of cells and mediates intracellular antioxidant defenses via the uptake of glutathione [34]. It has been reported that GGT is directly involved in the generation of reactive oxygen species [15] and it is considered to be a marker of oxidative stress [35]. GGT level has also been reported to be strongly associated with several levels of ALT and GGT, a study with continuous values of ALT and GGT using only male subjects [17] and a case-control study with low and high ALT/GGT categories [28], also showed the improvement of discriminatory capability for predicting new onset of DM in NRI and IDI. In NRI analysis, the number of correct reclassification (n = 852) was higher than that of inappropriate reclassification (n = 577) in subjects had not developed DM, whereas the numbers of correct and inappropriate reclassifications were the same (n = 21 each) in subjects who had developed DM (Table 5). These findings suggest that the traditional model with H-ALT + H-GGT significantly improves the discriminatory capability, especially specificity, for predicting development of DM.

### Table 5: Reclassification for the absolute risk for the development of diabetes mellitus

| Number of subjects who developed diabetes mellitus | Traditional model + H-ALT/H-GGT |
|---------------------------------------------------|---------------------------------|
|                                                   | <1%   | 1–2%  | 2–5%  | >5%   |
| Traditional model                                 |       |       |       |       |
| <1%                                               | 17    | 3     | 0     | 0     |
| 1–2%                                              | 0     | 24    | 5     | 0     |
| 2–5%                                              | 0     | 8     | 49    | 13    |
| >5%                                               | 0     | 0     | 13    | 478   |

| Number of subjects who did not develop diabetes mellitus | Traditional model + H-ALT/H-GGT |
|--------------------------------------------------------|---------------------------------|
|                                                        | <1%   | 1–2%  | 2–5%  | >5%   |
| Traditional model                                      |       |       |       |       |
| <1%                                                    | 2,619 | 136   | 0     | 0     |
| 1–2%                                                   | 277   | 2,233 | 236   | 0     |
| 2–5%                                                   | 0     | 331   | 2,169 | 205   |
| >5%                                                    | 0     | 0     | 244   | 2,040 |

Traditional model includes age, sex, body mass index, fasting plasma glucose, smoking habit, alcohol drinking habit, family history of diabetes mellitus, and diagnosis of hypertension and dyslipidemia. ALT, alanine aminotransferase; GGT, γ-glutamyl transpeptidase; H-, high; L-, low.
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