BMJ Open Diabetes Research & Care

Association between liver enzymes and incident type 2 diabetes in Singapore Chinese men and women

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ABSTRACT

Aims: To assess the association between liver enzymes and the risk of type 2 diabetes (T2D) in a Chinese population.

Methods: A nested case–control study comprising 571 T2D cases and 571 matched controls was conducted within the Singapore Chinese Health Study. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were quantified in baseline plasma collected from them, while γ-glutamyltransferase (GGT) was assayed among 255 T2D cases with baseline hemoglobin A1c <6.5% and 255 matched controls. Participants were free of diagnosed diabetes, cardiovascular disease, and cancer at blood collections (1999–2004). Incident self-reported T2D cases were identified at follow-up II interview (2006–2010). Controls were matched to cases on age, sex, dialect group, and date of blood collection.

Results: Higher levels of ALT and GGT were significantly associated with increased risk of T2D (p for trend <0.001 for ALT, p for trend=0.03 for GGT), and the ORs (95% CIs) comparing highest versus lowest tertiles of ALT and GGT were 2.00 (1.01 to 3.96) and 2.38 (1.21 to 4.66), respectively. A null association was observed for AST, ALP, and LDH with T2D risk. Adding GGT (<23 vs ≥23 IU/L) or ALT (<21 vs ≥21 IU/L) to a prediction model resulted in significant gain in net reclassification improvement and integrated discrimination improvement of T2D prediction (all p<0.001).

Conclusions: Higher levels of GGT and ALT are associated with increased T2D risk. GGT ≥23 IU/L and ALT ≥21 IU/L may identify people at higher risk of developing T2D in this Chinese population.

Key messages

- Increased levels of γ-glutamyltransferase (GGT) and alanine aminotransferase (ALT), even within the normal range, are associated with increased risk of incident type 2 diabetes independent of other diabetes risk factors in this Chinese population.
- GGT and ALT are useful markers for identifying people at higher risk of T2D, and the best cut-offs were 23 and 21 IU/L in this Chinese population.
- The other liver enzymes (aspartate aminotransferase, alkaline phosphatase, and lactate dehydrogenase) were not significantly associated with diabetes risk in the Chinese adults.

the liver, and associated with hepatic insulin resistance[2] and type 2 diabetes (T2D) risk.[3] ALT, found predominately in the liver, has been considered the most specific marker for liver injury,[4] while GGT is present on the surface of most cell types and highly active in liver, kidney, and pancreas.[5] GGT is responsible for catabolism of extracellular glutathione and may be linked to oxidative stress[6] and chronic inflammation,[7] which are also important pathways for T2D development. Thus, ALT and GGT may be underlying biological markers linking between liver disease and T2D. Previous studies on ALT/GGT and T2D risk were conducted in Western[2] [8–15] and Asian populations such as Japanese,[16–18] South Koreans,[19] [20] Thai,[21] and Iranians,[22] but not in a Chinese population.

The measurement of GGT and ALT involves well-standardized, simple, inexpensive, and routine tests with no requirement for fasting prior to venipuncture;[23] therefore, it is of clinical interest to explore whether these convenient biomarkers can help identify people at higher risk of developing T2D. However, existing studies found inconsistent findings: some studies showed that GGT and/or ALT improved T2D prediction significantly,[16] [24–27] while others did not.[22] [28–30] Additionally, a recent study in a white and African-American

INTRODUCTION

Liver, a vital organ in metabolism, plays an important role in maintaining glucose homeostasis.[1] Markers of liver injury, such as alanine aminotransferase (ALT) and γ-glutamyltransferase (GGT), have been shown to be good surrogate measures of non-alcoholic fatty liver disease (NAFLD), the most common chronic liver conditions characterized by excess deposition of fat in

population found that ALT ≥26 IU/L increased diabetes prediction substantially;[27] while a Japanese study has identified a much lower cut-off value for ALT (13 IU/L).[16] Since Asians develop T2D at lower body mass index (BMI) compared to western populations, the observed difference indicates that liver may play a more important role in T2D development in relatively lean Asian populations. However, evidence is limited to confirm this assumption. Moreover, to the best of our knowledge, no study has examined the association of GGT, ALT with T2D risk, and their potential cut-off levels for T2D prediction in a Chinese population yet.

To address these issues, we conducted a nested case–control study within the Singapore Chinese Health Study (SCHS) to examine the relationship between liver enzymes and incident T2D risk in Chinese adults. Moreover, we evaluated the predictive utility of liver enzymes and identified appropriate cut-off values for T2D prediction.

RESEARCH AND METHODS
Study population
The design of SCHS was described previously.[31] Briefly, SCHS was established between 1993 and 1998, and recruited 63 257 Chinese adults aged 45–74 years. At recruitment, an in-person interview was conducted using a structured questionnaire to collect health-related information. Follow-up I (1999–2004) was conducted via telephone to update selected lifestyle habit and medical history. We recontacted 52 322 participants successfully, and among them, 32 535 participants donated their biospecimens during the follow-up I visit. Follow-up II (2006–2010) was conducted via telephone, and 39 528 participants were recontacted successfully. The study protocol was approved by the Institutional Review Boards at the National University of Singapore and the University of Pittsburgh.

Ascertainment of diabetes
History of physician-diagnosed T2D risk was asked at baseline and both follow-ups by the question: "Have you been told by a doctor that you have diabetes?" If the answer was 'yes', participants were also asked for the age at which they were first diagnosed. The robustness and accuracy of the self-reported diabetes data was confirmed in a validation study: among 1651 cohort participants who reported prior diagnosis of diabetes either at baseline or at follow-up I interview, 98.9% were confirmed by medical records (hospital-based discharge summary database) or a supplementary questionnaire about symptoms, diagnostic tests, and hypoglycemic therapy during a telephone interview. Some participants (n=619) with self-reported diabetes refused or were not available for the validation study; however, their main characteristics (age, sex, BMI, etc) were similar with those in the validation study.[32]

Case and control selection
For the current analysis, we established a case–control study nested within SCHS of 571 cases and 571 matched controls. Cases and controls were free of physician-diagnosed diabetes, cardiovascular disease, and cancer at baseline interview and blood collection (1999–2004). Cases were those who reported to be diagnosed with T2D during follow-up II (2006–2010). Controls were selected from the remaining participants who were free of T2D at follow-up II, and were matched for age (±3 years), date (±6 months) of blood collection, sex, and dialect group with cases on a 1:1 ratio. Moreover, the selected controls were screened for the presence of undiagnosed T2D at the time of blood donation by measuring hemoglobin A1c (HbA1c) level. Controls with HbA1c ≥42 mmol/mol (6.0%) were ineligible for the study and excluded, and a replacement control with the same matching criteria was randomly chosen among the remaining eligible controls.

Laboratory procedures
Twenty milliliters of peripheral blood were collected from consenting participants, and transported to the laboratory immediately. All specimens were separated into plasma, serum, red blood cells, and buffy coat, and stored in −80°C freezers. For the current study, one aliquot of frozen plasma for each of study subjects were retrieved from the bioreposity. The plasma samples of matched case–control pairs were randomly placed next to each other with the case/control status blinded to the laboratory personnel, and processed and tested in the same batch. Plasma concentrations of ALT, aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), and high-sensitivity C reactive protein (CRP) were measured via colorimetric method on a chemistry analyzer (AU5800 Analyzer, Beckman Coulter, Brea, California, USA). Adiponectin levels were measured by ELISA (Bio-Rad Laboratories, Hercules, California, USA). HbA1c levels were measured by HPLC method using Bio-Rad Variant II System (Bio-Rad Laboratories) in red blood cells. All biochemical measurements were conducted at the National University Hospital Reference Laboratory.

Among 571 case–control pairs, although 279 cases had baseline HbA1c ≥48 mmol/mol (6.5%) and may be considered to have T2D according to current diagnostic criteria,[33] they were not considered to have T2D because this was not used as criterion for diagnosis during the period of blood collection (1999–2004) and follow-up (2006–2010). Serum GGT was measured in another project focusing on cases with baseline HbA1c <48 mmol/mol (6.5%) among 255 case–controls pairs with available serum samples (37 cases or controls had insufficient serum samples for the measurement). GGT was measured via the same colorimetric method mentioned above. For all liver enzymes, the within-assay

coefficients of variation were <8% and the between-assay coefficients of variation were all below 12%.

Statistical analysis

ALT, AST, ALP, LDH, and GGT were divided into tertiles according to the distribution in the control samples and the lowest tertile served as the reference group. We used conditional logistic regression to model their associations with T2D adjusting for age (continuous), smoking status (never, past, and current smoker), alcohol intake (never, weekly, or daily), weekly moderate-to-vigorous activity levels (<0.5, 0.5–3.9, and ≥4.0 hours/week), education levels (no, primary school, secondary or above), history of hypertension (yes, no), fasting status (yes, no), and BMI (continuous). TG, HDL-C, CRP, and adiponectin levels (tertiles) were further adjusted in a separate model. We repeated the analyses in 292 case–control pairs (GGT: 255 pairs) with baseline HbA1c <48 mmol/mol (6.5%). Moreover, we tested potential interactions with sex, BMI categories (<23 vs ≥23 kg/m^2), or fasting status. In the stratified analysis by BMI or fasting status, we used unconditional logistic regression models with additional adjustment for sex and dialect group.

Since GGT and ALT were associated with T2D, we further classified participants according to their GGT and ALT levels for T2D risk stratification. Receiver-operating characteristic (ROC) analysis and Youden index were used to derive the optimal cut-off values.[34] To assess the predictive utility of dichotomized GGT and ALT, we established a parsimonious logistic regression model, including education level, physical activity, history of hypertension, BMI, TG, and HDL-C, using a forward selection procedure (p<0.05). The improvement in discrimination was examined by comparing area under ROC curve (AUC) between the parsimonious model and the model plus GGT and/or ALT using DeLong's method.[35] Moreover, due to the limitation of AUC such as its insensitivity to model improvement,[36] we also used the category-free net reclassification improvement (NRI) and integrated discrimination improvement (IDI) statistics recommended by Pencina et al.[37 38] Furthermore, the goodness of fit of all models was assessed by Akaike information criteria (AIC), where lower AICs indicate better model fit. Analyses were performed with Stata software, V.11.0 (Stata Corp, College Station, Texas, USA). Two-sided p values <0.05 were considered to be statistically significant.

RESULTS

Among T2D cases, the mean age of diagnosis (SD) was 63.2 (6.4) years and the mean duration (SD) between blood donation and diagnosis of T2D was 4.0 (1.7) years. The baseline characteristics of cases and controls are shown in table 1. Cases had higher BMI, were more likely to have history of hypertension, and had higher levels of HbA1c, CRP, TG, GGT, ALT, AST, and ALP, and lower levels of adiponectin and HDL-C. No significant

differences were found for education level, smoking, alcohol consumption, physical activity levels, and LDH levels.

ALT, AST, and GGT were moderately inter-related (correlation coefficients ranging from 0.50 to 0.67, p<0.001), while their correlations with ALP and LDH were weaker (correlation coefficients ranging from 0.08 to 0.28, p ranges from <0.001 to 0.21) (see online supplementary table S1). The associations between liver enzymes and T2D are presented in table 2. In the whole data set (571 case–control pairs), increased ALT was associated with higher T2D risk (OR comparing extreme tertiles 2.33; 95% CI 1.52 to 3.55) in the final model. However, AST, ALP, and LDH showed no significant association. Moreover, the ALT–T2D association remained similar among 292 case–control pairs with HbA1c <48 mmol/mol (6.5%) (OR comparing highest vs lowest tertiles 2.38; 95% CI 1.21 to 4.66). Higher GGT levels were also positively associated with increased T2D risk among 255 pairs with HbA1c <48 mmol/mol (6.5%) (OR comparing highest versus lowest tertiles 2.00; 95% CI 1.01 to 3.96). Furthermore, no significant interactions were found with sex (see online supplementary table S2), BMI (see online supplementary table S3), and fasting status (see online supplementary table S4).

In this study, the best cut-off predictive values for T2D were 23 IU/L for GGT and 21 IU/L for ALT using Youden index in the ROC analysis. The sensitivities and specificities of the cut-off points were 72% and 48% for GGT, and 74% and 52% for ALT, respectively. A total of 61.6% participants had GGT ≥23 IU/L and 61.2% had ALT ≥21 IU/L. GGT ≥23 IU/L was associated with a 69% greater odds of developing T2D (OR 1.69 (95% CI 1.05 to 2.72)), while ALT ≥21 IU/L was associated with an 88% increased odds of developing T2D (OR 1.88 (95% CI 1.41 to 2.52)). Additionally, compared to those who had GGT <23 IU/L and ALT <21 IU/L, individuals with GGT ≥23 IU/L and ALT ≥21 IU/L were associated with a 2.5-fold odds of developing T2D (OR 2.47 (95% CI 1.27 to 4.79)) (figure 1 and see online supplementary table S5).

The predictive performance of GGT and ALT is presented in table 3 and online supplementary table S6. Adding GGT (<23 vs ≥23 IU/L) improved AUC from 0.76 in the base model to 0.77 (p=0.29). Comparatively, adding ALT (<21 vs ≥21 IU/L) improved AUC from 0.74 to 0.75 (p=0.02). Although the improvements in AUCs were minor, addition of GGT or ALT as binary variables to a prediction model resulted in significant gain in the category-free NRI and IDI of T2D prediction (all p<0.001): 43.5% and 48.9% of T2D cases would be correctly predicted for higher T2D risk after inclusion of GGT or ALT, respectively. Additionally, inclusion of GGT or ALT showed better model fit with decreased AIC values compared to the base model (table 3).

DISCUSSION

In this case–control study nested within the SCHS, higher levels of GGT and ALT were positively associated

Table 1 Baseline characteristics and liver enzymes of diabetes cases and matched controls, the SCHS

	Cases (n=571)	Controls (n=571)	p Value*
Age (years) at blood taken	59.6±6.13	59.7±6.22	–
Gender (female)	335 (58.7)	335 (58.7)	–
Dialect (%)			
Cantonese	287 (50.3)	287 (50.3)	
Hokkien	284 (49.7)	284 (49.7)	
BMI (kg/m^2)	24.8±3.62	22.8±3.28	<0.001
Level of education (%)			0.66
No formal education	104 (18.2)	99 (17.3)	
Primary school	255 (44.7)	233 (40.8)	
Secondary and above	212 (37.1)	239 (41.9)	
History of hypertension (%)	265 (46.4)	148 (25.9)	<0.001
Cigarette smoking (%)			0.23
Never smokers	410 (71.8)	425 (74.4)	
Former smokers	63 (11.0)	71 (12.4)	
Current smokers	98 (17.2)	75 (13.1)	
Weekly moderate-to-vigorous activity (%)			0.89
<0.5 hours/week	456 (79.9)	454 (79.5)	
0.5–3.9 hours/week	82 (14.4)	68 (11.9)	
≥4 hours/week	33 (5.8)	49 (8.6)	
Alcohol intake (%)			0.93
Abstainers	498 (87.2)	497 (87.0)	
Weekly drinkers	55 (9.6)	59 (10.3)	
Daily drinkers	18 (3.2)	15 (2.6)	
Fasting status (yes)	178 (31.2)	156 (27.3)	0.15
GGT† (IU/L)	30 (21–46)	23 (17–35)	<0.001
ALT (IU/L)	27 (20–37)	20 (15–27)	<0.001
AST (IU/L)	26 (21–32)	24 (21–29)	<0.001
ALP (IU/L)	84 (68–101)	77 (66–92)	<0.001
LDH (IU/L)	400±73	403±77	0.94
TC (mmol/L)	5.31±0.95	5.20±0.85	0.05
HDL-C (mmol/L)	1.08±0.24	1.23±0.32	<0.001
TG (mmol/L)	2.15 (1.45–2.97)	1.54 (1.07–2.23)	<0.001
Adiponectin (μg/mL)	7.00±2.70	9.07±3.76	<0.001
C-reactive protein (mg/L)	1.8 (1.0–3.5)	1.2 (0.6–2.3)	<0.001
HbA1c (mmol/mol)	51	38	<0.001
HbA1c (%)	6.83±1.44	5.55±0.27	<0.001

Data are expressed as mean±SD for continuous variables (normally distributed) and median (IQR) for continuous variables (skewed distributed), and n (percentage) for categorical variables.
Cases and controls are matched on age (±3 years), date of blood draw (±6 months), gender, and dialect group (Cantonese, Hokkien).
*p values based on the McNemar's χ^2 test for categorical variables, paired Student's t-test for normally distributed continuous variables, and Wilcoxon signed-rank test for skewed continuous variables.
†GGT concentrations were measured in 510 participants (255 case–control pairs with cases having baseline HbA1c <48 mmol/mol (6.5%)).
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyl transferase; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; LDH, lactate dehydrogenase; SCHS, Singapore Chinese Health Study; TC, total cholesterol; TG, triglycerides.

with the incident T2D risk independent of established T2D risk factors, including BMI, blood lipids, CRP, and adiponectin. Additionally, the best cut-off values for GGT and ALT were 23 and 21 IU/L in this Chinese population, and dichotomized GGT and ALT significantly improved T2D risk prediction.

Our finding of positive relations of GGT and ALT with incident T2D was largely consistent with previous studies across populations: a meta-analysis of 24 prospective studies reported a pooled relative risk of 1.34 (95% CI 1.27 to 1.42) comparing highest versus lowest tertiles of GGT levels,[39] and 1.66 (95% CI 1.31 to 2.09; 17 studies) for ALT,[23] although some included studies with relatively small sample sizes, ranging from 36 to 208 for T2D cases, did not observe the associations. A recent Mendelian randomization study further provided causal evidence for the association between GGT and insulin resistance.[40] In the present study, we did not observe associations between AST and ALP with T2D, which was consistent with previous studies,[14 15 21 23 41] and this may be due to their lack of specificity for liver diseases. Additionally, no association was observed between LDH and T2D risk in the current study. Although increased LDH expression has affected glucose metabolism in a mechanistic study,[42] few prospective human studies have assessed the LDH–T2D association.

Table 2 ORs (95% CIs) of T2D associated with different levels of liver enzymes, the SCHS

	Tertiles of liver enzymes			
Variables	T1	T2	T3	p for trend*
Whole dataset				
ALT				
Median (range)	14 (6–17)	21 (18–24)	33 (25–130)	
Cases/controls	93/218	152/163	326/190	
Model 1	1.00	1.98 (1.34–2.92)	3.73 (2.56–5.44)	<0.001
Model 2	1.00	1.56 (1.02–2.40)	2.33 (1.52–3.55)	<0.001
AST				
Median (range)	20 (12–22)	25 (23–27)	33 (28–135)	
Cases/controls	173/212	153/181	245/178	
Model 1	1.00	0.96 (0.69–1.33)	1.39 (1.01–1.91)	0.03
Model 2	1.00	0.90 (0.63–1.30)	1.10 (0.77–1.58)	0.52
ALP				
Median (range)	61 (23–70)	78 (71–86)	101 (87–401)	
Cases/controls	155/191	168/194	248/186	
Model 1	1.00	1.14 (0.82–1.58)	1.78 (1.28–2.48)	<0.001
Model 2	1.00	1.08 (0.74–1.56)	1.39 (0.96–2.02)	0.07
LDH				
Median (range)	331 (216–366)	394 (367–426)	473 (427–713)	
Cases/controls	205/191	191/191	175/189	
Model 1	1.00	0.85 (0.60–1.20)	0.71 (0.50–1.01)	0.06
Model 2	1.00	0.77 (0.52–1.13)	0.72 (0.49–1.07)	0.12
Limited to cases with baseline HbA1c <48 mmol/mol (6.5%) and their matched controls				
GGT†				
Median (range)	15 (10–18)	24 (19–28)	43 (29–741)	
Cases/controls	48/92	70/80	137/83	
Model 1	1.00	1.53 (0.81–2.87)	2.91 (1.61–5.26)	<0.001
Model 2	1.00	1.28 (0.61–2.68)	2.00 (1.01–3.96)	0.03
ALT				
Median (range)	14 (6–16)	20 (17–23)	33 (24–119)	
Cases/controls	45/98	85/98	162/96	
Model 1	1.00	2.08 (1.15–3.74)	3.85 (2.15–6.90)	<0.001
Model 2	1.00	1.84 (0.94–3.59)	2.38 (1.21–4.66)	0.05
AST				
Median (range)	20 (13–22)	25 (23–27)	33 (28–105)	
Cases/controls	93/104	82/104	117/84	
Model 1	1.00	0.84 (0.51–1.37)	1.38 (0.86–1.37)	0.12
Model 2	1.00	0.75 (0.43–1.30)	1.03 (0.60–1.77)	0.80
ALP				
Median (range)	60 (28–70)	78 (71–86)	101 (87–172)	
Cases/controls	90/101	90/94	112/97	
Model 1	1.00	1.01 (0.65–1.59)	1.33 (0.84–2.10)	0.21
Model 2	1.00	0.94 (0.56–1.57)	0.92 (0.53–1.59)	0.76
LDH				
Median (range)	330 (216–363)	392 (364–425)	472 (427–687)	
Cases/controls	107/98	107/98	78/96	
Model 1	1.00	1.03 (0.62–1.70)	0.67 (0.62–1.70)	0.06
Model 2	1.00	0.94 (0.52–1.68)	0.68 (0.37–1.24)	0.13

ORs of liver enzymes in the whole dataset and among cases with baseline HbA1c <48 mmol/mol (6.5%) and their matched controls were computed using conditional logistic regression models with adjustment for following covariates.
Model 1: adjusted for age at blood taken (continuous), smoking (never, past, and current smoker), alcohol intake (never, weekly, or daily), weekly moderate-to-vigorous activity (<0.5, 0.5–3, and ≥4 hours/week), education level (no, primary school, secondary or above), history of hypertension (yes, no), fasting status (yes, no), and BMI (continuous).
Model 2: model 1 plus adjusted triglycerides (mmol/L), high-density lipoprotein cholesterol (mmol/L), C-reactive protein (mg/L), and adiponectin (μg/mL) (all in tertiles).
*Linear trend was tested using the median level of each tertile of liver enzymes.
†GGT concentrations were measured in 510 participants (255 case–control pairs with cases having baseline HbA1c <48 mmol/mol (6.5%)).
ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyl transferase; HbA1c, hemoglobin A1c; LDH, lactate dehydrogenase; SCHS, Singapore Chinese Health Study; T2D, type 2 diabetes.
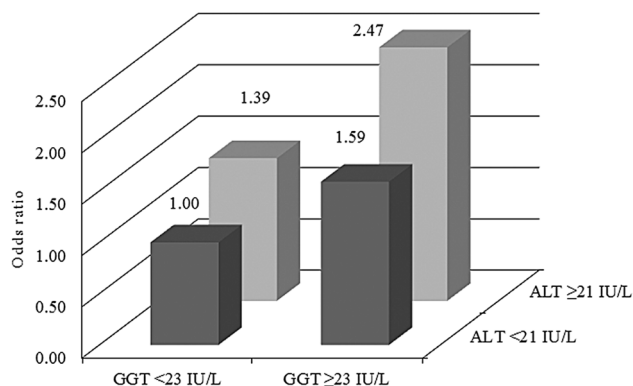
Figure 1 Total T2D risk according to join distribution of GGT and ALT. ALT concentrations were measured in 1142 participants (571 case–control pairs). GGT concentrations were measured in 510 participants (255 case–control pairs with cases having baseline HbA1c <48 mmol/mol (6.5%)). Multivariable model adjusted for age at blood taken (continuous), smoking (never, past, and current smoker), alcohol intake (never, weekly, or daily), weekly moderate-to-vigorous activity (<0.5, 0.5–3, and ≥4 hours/week), education level (no, primary school, secondary or above), history of hypertension (yes, no), fasting status (yes, no), BMI (continuous), plasma tertile concentrations of triglycerides, high-density lipoprotein cholesterol, C-reactive protein, and adiponectin. ALT, alanine aminotransferase; BMI, body mass index; GGT, γ-glutamyl transferase; T2D, type 2 diabetes.

Elevated GGT and ALT levels were linked to T2D development as useful surrogate measures of NAFLD characterized by hepatic fat accumulation.[43] NAFLD may indicate fat deposition in other organs such as skeletal muscle, the myocardium, and the pancreas, which predispose individuals to T2D risk.[43] However, a Korean study showed positive associations between GGT/ALT and T2D risk among participants without fatty liver, and suggested that alternative pathways existed.[20]

Additionally, ALT was linked to T2D via hepatic insulin resistance,[14] and GGT through oxidative stress[6] and inflammation.[7] However, adjustment for CRP (low-grade inflammation marker) had no influence on the association in our study and in other studies,[2 13 15 20] suggesting that GGT may be involved in the pathogenesis of T2D through other mechanisms. Moreover, previous evidence showed that the relations of GGT and ALT with T2D were also independent of other important pathologies in T2D development such as whole-body insulin resistance[2 14] and blood lipids,[2 13 16 19] and the current study further showed that the associations were also independent of adiponectin.

Moreover, our study found that GGT and ALT levels improved T2D risk prediction, and this was supported by several previous studies.[16 24–27] GGT, ALT, glucose, HbA1c, HDL-C, and TG collectively improved the discriminatory power (indicated by AUC) above the basic model in EPIC-Potsdam study.[24] GGT, together with BMI, waist circumference, and TG, showed reasonable discrimination (measured by c-statistics) in D.E.S.I.R study.[25] Moreover, inclusion of HbA1c and GGT in a simple clinical model showed significant improvement in T2D prediction (AUC, NRI, and IDI) in British older men and women.[26] Furthermore, a recent study found that ALT ≥26 IU/L improved T2D prediction (AUC, NRI, and IDI) among white and African-Americans living in the USA.[27] Additionally, a Japanese study has found that GGT (≥18 IU/L) or ALT (≥13 IU/L) had similar discriminatory power with BMI for predicting T2D.[16] We have identified a lower ALT cut-off value (21 IU/L) for T2D prediction compared to the US study, although higher than the Japanese study. As for GGT, some studies observed little improvement in T2D prediction (indicated by NRI) when including GGT and other novel biomarkers to the basic model.[28–30] In the current study, including GGT in the model significantly

Table 3 Summary statistics to assess binary liver enzymes in predicting incident T2D

Variable	Best cut-offs	Multivariable model			
		Discrimination (AUC (95% CI))	Calibration (AIC)	NRI	IDI
Base model		0.74 (0.71 to 0.77)	1394		
ALT	21	0.75 (0.73 to 0.78)*	1370	0.53	0.02
Base model		0.76 (0.72 to 0.80)	613		
GGT	23	0.77 (0.73 to 0.81)†	609	0.41	0.01
Base model		0.76 (0.72 to 0.80)	613		
ALT+GGT	–	0.77 (0.73 to 0.81)†	609	–	–

Base model included education level (no, primary school, secondary and above), weekly moderate-to-vigorous activity (<0.5, 0.5–3.9, and ≥4 hours/week), history of hypertension (yes, no), plasma concentrations of triglycerides (mmol/L) (tertiles) and high-density lipoprotein cholesterol (mmol/L) (tertiles), and BMI (continuous).
Multivariable model adjusted for all the variables included in the base model.
*ALT concentrations were measured in 1142 participants (571 case–control pairs). Compared with the base model, the increment in AUC value was statistically significant (p<0.05).
†GGT concentrations were measured in 510 participants (255 case–control pairs with cases having baseline HbA1c <48 mmol/mol (6.5%)). Compared with the base model (AUC, 0.76 (0.72–0.80)), increment in AUC value by adding GGT to the base model was not significant (p=0.36). Additionally, increment in AUC value by adding GGT together with ALT to the base model was not significant either (p=0.20).
AIC, Akaike information criterion; ALT, alanine aminotransferase; AUC, area under ROC curve; BMI, body mass index; GGT, γ-glutamyl transferase; IDI, integrated discrimination improvement; NRI, net reclassification improvement; T2D, type 2 diabetes.

improved NRI, but not AUC, and the cut-off value (≥23 IU/L) is slightly higher than the one identified in a Japanese study.[16]

The strength of the current study included adjustment for well-established diabetes risk factors (including BMI, CRP, lipids, and adiponectin), and using comprehensive statistical methods (AUC, NRI, and IDI) to explore the predictive utility of liver enzymes. However, there are some limitations. First, we measured liver enzymes only once and may not represent long-term profile. Previous studies have shown that the within-person coefficient of variation of GGT ranged between 12.2% and 13.8% in repeated measures up to 1 year.[44 45] In addition, the impact of single measurement of GGT in the current study would most likely lead to non-differential misclassification and underestimate the GGT–T2D association. For example, a previous study in the D.E.S.I.R. cohort found that correction for the within-person variation of GGT slightly strengthened the association between GGT and T2D risk.[46] Second, incident diabetes was obtained from self-reported information, thus undiagnosed diabetes may exist. However, we have measured HbA1c, which was updated as a diagnosis criterion of diabetes in 2010 by the American Diabetes Association.[33] Similar associations were found when analyzing the data in the total samples or in those cases with HbA1c ≤48 mmol/mol (6.5%) at baseline. Third, although we did not observe any interaction with ALT or GGT, our sample size may be small and thus underpowered to detect the interaction. Fourth, we did not measure hepatitis B and C infection, which could result in elevated liver enzymes. Moreover, we did not have data on insulin resistance; however, the association remained in previous studies adjusting for direct measurement of insulin resistance.[2 14] Last but not least, HbA1c was a selection criterion for controls in the current study; therefore, we could not include it in our prediction model. A study from the Netherlands showed that liver function tests led to small but significant improvement in T2D prediction above basic model; however, the improvement disappeared when HbA1c was included in the basic model.[29]

CONCLUSION
Increased levels of GGT and ALT are associated with increased risks of incident T2D in this Chinese population. Additionally, GGT and ALT are useful markers for identifying people at higher risk of T2D, and the best cut-offs were 23 and 21 IU/L in this Chinese population. Further researches are needed to validate the findings, particularly the predictive utility of liver enzymes for T2D, and investigate the biological mechanisms for the associations.

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