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LPA kringle IV type 2 is associated with type 2 diabetes in a Chinese population with very high cardiovascular risk

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Abstract The connection between lipoprotein (a) [Lp(a)] levels and the risks of cardiovascular disease and diabetes remains poorly understood. Lp(a) is encoded by the *LPA* gene, and evidence suggests that the kringle IV type 2 (KIV-2) variant is particularly important to Lp(a) isoform size. A large isoform size, represented as a high number of KIV-2 repeats in *LPA*, is associated with low serum Lp(a) concentrations and an increased risk of type 2 diabetes. We investigated the associations among Lp(a) concentrations, *LPA* KIV-2 repeats, and type 2 diabetes in a Chinese population of 1,863 consecutive patients with very high cardiovascular risk, as identified by coronary angiography. Individuals with Lp(a) levels in the top tertile [67.86 (35.34–318.50) mg/dl] had a lower risk of diabetes compared with those in the bottom tertile [7.38 (0.60–12.91) mg/dl]. There was an inverse association between the number of KIV-2 repeats and serum Lp(a) concentrations. This study demonstrated that a high number of *LPA* KIV-2 repeats are associated with increased risk of type 2 diabetes in a Chinese population with very high cardiovascular risk, which suggests that large Lp(a) isoform size, associated with low Lp(a) concentration, has a causal effect on type 2 diabetes.—Mu-Han-Ha-Li, D-L-D-E., T-Y. Zhai, Y. Lin, and X. Gao. **LPA kringle IV type 2 is associated with type 2 diabetes in a Chinese population with very high cardiovascular risk. *J. Lipid Res.* 2018.** 59: **884–891.**

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Type 2 diabetes and cardiovascular disease may have common genetic and environmental antecedents according to the well-known "common soil hypothesis" (1). The description of metabolic syndrome largely bolstered the development of the common soil hypothesis (1, 2). Obesity represents a key driver for the occurrence of metabolic syndrome (2, 3). Increased visceral and ectopic fat deposition releases excess fatty acids and a variety of adipokines that elicit metabolic risk factors, which plays a major role in the development of insulin resistance and predisposition to both type 2 diabetes and cardiovascular disease (2, 3). However, lipoprotein (a) [Lp(a)] may be an exception based on its opposite effects on cardiovascular disease and type 2 diabetes. Lp(a) is a LDL-like particle mainly produced by the liver, consisting of an apoB100 molecule covalently bonded to an apo(a) (4). Prospective epidemiological studies have demonstrated that elevated concentrations of Lp(a) are associated with increased risk of ischemic cardiovascular disease (5–7). On the contrary, observational studies found that increased Lp(a) levels are associated with lower risk of type 2 diabetes (8, 9), although inconsistent results were also reported in early small sample studies (10, 11). The first prospective study of Lp(a) levels and the risk of type 2 diabetes is the Women's Health Study, which reported an inverse relationship between Lp(a) levels and incident diabetes in US women (8). Then this finding was replicated in a Danish general population study (8).

Observational studies may suffer from many potential biases, including confounding and reverse causation, which limit them to identify causal associations robustly (12). Genetic studies of polymorphisms affecting Lp(a) levels can be used to substantiate the causality. The serum Lp(a) concentrations were found to be genetically determined to a large extent via variations in the *LPA* gene, which encodes apo(a) (13). Among the variants, the *LPA* kringle IV type 2 (KIV-2) repeat polymorphism is particularly important, which is defined by a 5.5 kb repeat that exists in multiple copy numbers from 1 to more than 40 copies (13). Therefore, the KIV-2 repeat polymorphism determines the number of kringle structures in apo(a), which

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Abbreviations: CHD, coronary heart disease; C_T, cycle threshold; FPG, fasting plasma glucose; 2 h PPG, 2 h postprandial plasma glucose; HbA1c, glycosylated hemoglobin; HDL-C, HDL cholesterol; KIV-2, kringle IV type 2; LDL-C, LDL cholesterol; Lp(a), lipoprotein (a); OR, odds ratio; qPCR, quantitative real-time PCR.

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affects the size of the protein (13). As the size of apo(a) inversely correlates with its hepatic production rate, small Lp(a) isoforms are associated with higher Lp(a) concentrations, while large Lp(a) isoforms are associated with lower concentrations (13). SNPs at the *LPA* locus, like rs10455872 and rs3798220, were also associated with an increased level of Lp(a) (13, 14). Several studies using Mendelian randomization approaches have demonstrated that *LPA* variants were associated with the risk of coronary heart disease (CHD) and myocardial infarction (14–17), which supports a causal role of Lp(a) in ischemic cardiovascular disease.

Similarly, Mendelian randomization studies were used to assess the causality for an association between Lp(a) and type 2 diabetes. In a study of the Danish general population, a high KIV-2 sum of repeats was shown to be associated with increased risk of type 2 diabetes, but no association was observed between rs10455872 and diabetes (18). In another study, rs10455872, which explained 26.8% of the variability in Lp(a) levels, was not associated with risk of type 2 diabetes (19). Therefore, the causality between Lp(a) and type 2 diabetes may not be mediated by the concentrations of Lp(a) per se, but a causal role of large Lp(a) isoform size cannot be excluded. Until recently, one study using a novel genetic approach confirmed that it is a high number of KIV-2 repeats that is causally associated with increased risk of type 2 diabetes, and not low Lp(a) concentrations per se (20).

Previous studies have demonstrated that elevated Lp(a) levels and *LPA* polymorphisms are associated with CHD in the Chinese populations (21–24). To date, evidence regarding the association between Lp(a) levels and type 2 diabetes in the Chinese is rather limited. Recently, two studies investigated the relationship between serum Lp(a) and prevalent type 2 diabetes in large Chinese populations, but the results turned out to be inconsistent (9, 25). The association of *LPA* polymorphisms with type 2 diabetes in the Chinese is unclear. The objective of this study was to investigate the associations among Lp(a) concentrations, *LPA* KIV-2 repeat polymorphism, and type 2 diabetes in a Chinese population with very high cardiovascular risk. By using Mendelian randomization approaches, we aimed to get a better understanding of the nature of associations in the Chinese population.

MATERIALS AND METHODS

Study population

The study participants were recruited between March 2013 and November 2013 in the Cardiology Department of Zhongshan Hospital affiliated with Fudan University, Shanghai, China. Consecutive patients who underwent coronary angiography for suspected CHD were enrolled. All patients had chest symptoms, such as chest pain or dyspnea, and were transferred to the Cardiology Department of Zhongshan Hospital to make a definite diagnosis. In the outpatient department, the patients were first evaluated by one or two tests, such as routine or dynamic electrocardiogram, exercise treadmill test, stress myocardial perfusion imaging, and coronary computed tomography angiography. If one of the above tests was positive, the patient was hospitalized to receive coronary angiography. Patients with missing data were excluded. Finally, 1,863 patients were included in the current study. The study was approved by the Ethics Committee of Zhongshan Hospital, and all participants gave written informed consent.

Clinical assessment

Baseline information about medical history and health-related behaviors was collected. The smoking or alcohol-drinking state was defined as never smoking or drinking, current smokers or drinkers (smoked or consumed alcohol regularly in the past 6 months), or ever smokers or drinkers (cessation of smoking or alcohol drinking for more than 6 months). BMI was calculated as weight divided by height squared (kg/m^2). Waist circumference was measured midway between the lower rib margin and the iliac crest with patients standing properly. Diabetes mellitus was defined as one or more of the following criteria: *1*) diagnosis of diabetes made previously by a physician; *2*) use of insulin or oral hypoglycemic agents; *3*) a fasting plasma glucose (FPG) $\geq$7 mmol/l; *4*) a 2 h postprandial plasma glucose (2 h PPG) $\geq$11.1 mmol/l; and/or *5*) a glycosylated hemoglobin (HbA1c) $\geq$6.5%. Hypertension was defined as one or more of the following criteria: *1*) diagnosis of hypertension made previously by a physician; *2*) a systolic blood pressure $\geq$140 mmHg; *3*) a diastolic blood pressure $\geq$90 mmHg; and/or *4*) treatment with antihypertensive medications.

Laboratory assays

Venous blood samples were obtained on the morning after an overnight fast of at least 12 h before coronary angiography. FPG, 2 h PPG, triglyceride, total cholesterol, HDL cholesterol (HDL-C), apoA-I, and apoB were measured by enzymatic methods (Roche Diagnostics, Basel, Switzerland) using a Hitachi 7600 biochemistry autoanalyzer (Hitachi High-Technologies Corp., Tokyo, Japan). LDL cholesterol (LDL-C) was calculated using the Friedewald formula. Serum Lp(a) levels were measured by using goat monoclonal antibody (DiaSys Diagnostic Systems GmbH, Germany) by particle-enhanced immune transmission turbidimetry in a Hitachi 7600 biochemistry autoanalyzer (Hitachi High-Technologies Corp.). For the laboratory test of serum Lp(a) concentrations, the coefficient of variation within group was 6–8%, and the coefficient of variation between groups was 8–10%. HbA1c was determined by high-performance liquid chromatography using the Bio-Rad Variant II analyzer (Bio-Rad Laboratories, Hercules, CA).

Genotyping

Patients with proper quality DNA samples (n = 1,487) were genotyped for KIV-2 repeat polymorphism. Genomic DNA was extracted from peripheral leukocytes according to established protocols. The number of KIV-2 repeats in *LPA* from genomic DNA was determined by quantitative real-time PCR (qPCR), as reported by Lanktree et al. (26). Briefly, multiplexed qPCR reactions were carried out in the Applied Biosystems ViiA7 real-time PCR system. Primers and probes for the exons 4 and 5 of *LPA* KIV-2 were designed using Applied Biosystems FileBuilder 3.1 (sequences are given in supplemental Table S1). qPCR reactions were also carried out for RNase P (*RNAP*), an endogenous single-copy control gene. The number of KIV-2 repeats, as determined by qPCR, was calculated as the difference in cycle thresholds (C_T) between target and control probes (ΔC_T). The ΔC_T was calculated for the exon 4 ($\Delta C_T 4$) and exon 5 ($\Delta C_T 5$) probes separately for every patient. Then the average difference between $\Delta C_T 4$ and $\Delta C_T 5$ ($\Delta\Delta C_T$) was calculated for all patients, and individuals whose $\Delta\Delta C_T$ was greater than two SDs from the mean were excluded from the analysis. Finally, the average of $\Delta C_T 4$ and $\Delta C_T 5$ ($\overline{\Delta C_T}$) was then used as the relative KIV-2 repeat number for further analysis.

Angiographic analysis

Two experienced cardiologists who were blinded to the study protocol performed the coronary angiography and reviewed the angiographic findings. Then a percentage stenosis was given to the major epicardial arteries and their sub-branches. Finally, patients with a ⩾50% stenosis in one or more coronary vessels were diagnosed as significant coronary stenosis.

Statistical analysis

Continuous variables were expressed as mean ± SD or median (interquartile range) and categorical variables as percentages. The one-way ANOVA and the χ^2 test were used to compare differences of continuous variables and categorical variables between groups, respectively. Pearson correlation analysis was used to examine the relationship between Lp(a) levels and the number of KIV-2 repeats in *LPA*. We divided the distribution of Lp(a) levels or the number of KIV-2 repeats into tertiles. Logistic regression analysis was used to investigate the independent association of Lp(a) or the number of KIV-2 repeats with type 2 diabetes, and the adjusted odds ratios (ORs) were calculated in relation to each tertile increase of Lp(a) concentrations or each tertile decrease of KIV-2 repeat numbers. Linear regression analysis was used to examine the association of the number of KIV-2 repeats in *LPA* with serum Lp(a) levels. Non-normally distributed values were natural log-transformed before analysis.

We performed a Mendelian randomization analysis (two-stage regression) to examine the causal association of serum Lp(a) levels with type 2 diabetes. An instrumental variable method was used. The instrumental variable, the number of KIV-2 repeats in *LPA*, is expected to act as a nonconfounded and unbiased marker for Lp(a) levels, and is used to estimate the causal effects of Lp(a) levels on type 2 diabetes. In stage 1, the association of the number of KIV-2 repeats in *LPA* with Lp(a) levels was analyzed using linear regression analysis and the β-coefficient was documented. The F-statistic from the linear regression analysis of Lp(a) on this polymorphism was obtained, and an F-statistic >10 suggests that potential bias due to weak instruments should not be substantial. In stage 2, the association of genetically predicted Lp(a) levels (calculated according to the equation from stage 1) with type 2 diabetes was analyzed using logistic regression. Three models were performed in stage 2. Model 1 and model 2 were unadjusted and adjusted for age, sex, and BMI, respectively. Model 3 was additionally adjusted for smoking status, drinking status, systolic blood pressure, diastolic blood pressure, antihypertensive medication, significant coronary stenosis, total cholesterol, triglyceride, LDL-C, HDL-C, apoA-I, apoB, and lipid-lowering drugs.

Mendelian randomization analyses were performed using R software version 3.4.2. Other analyses were performed using SPSS software version 19.0. Statistical tests were two-tailed and *P* values <0.05 were considered statistical significant.

RESULTS

Among 1,863 participants, 75.4% were men and 24.6% were women, with a mean age of 62.7 years. The mean BMI was 24.8 kg/m^2. Median Lp(a) was 21.41 mg/dl (interquartile range 9.66–46.44 mg/dl). There were 685 participants with type 2 diabetes, and the prevalence was 36.8%. One thousand five hundred and thirty participants (82.1%) were diagnosed as having significant coronary stenosis; 1,301 participants (69.8%) had hypertension; and 950 participants (51.0%) were using lipid-lowering drugs. **Table 1** shows the baseline characteristics of the study participants by tertiles of serum Lp(a). Serum Lp(a) levels were inversely associated with FPG, 2 h PPG, HbA1c, diabetes, and triglyceride, and positively associated with total cholesterol, LDL-C, apoB, and significant coronary stenosis.

Association of serum Lp(a) levels with type 2 diabetes

In a comparison of individuals with serum Lp(a) values in the top tertile versus the bottom tertile, the crude OR for type 2 diabetes was 0.76 (model 1; 95% CI 0.60–0.95; *P* for trend = 0.02) (**Fig. 1**). The association was similar after adjustment for age, sex, and BMI (model 2; OR = 0.77, 95% CI 0.61–0.97; *P* for trend = 0.02). The inverse association was more pronounced after additional adjustment for smoking and drinking status, blood pressure levels, antihypertensive medication, and significant coronary stenosis (model 3; OR = 0.72, 95% CI 0.57–0.92; *P* for trend = 0.007). Further adjustment for lipid parameters and lipid-lowering drugs made no material difference (model 4; OR = 0.74, 95% CI 0.58–0.95; *P* for trend = 0.02). A per-tertile increase in serum Lp(a) levels was associated with a 14% reduction in risk of diabetes, with an OR of 0.86 (95% CI 0.76–0.98) per tertile (model 4).

Association of the number of KIV-2 repeats in *LPA* with serum Lp(a) levels

One thousand four hundred and eighty-seven participants with proper quality DNA samples were genotyped for the *LPA* KIV-2 repeat polymorphism. Fifty-nine individuals whose $\Delta\Delta C_T$ was greater than two SDs from the mean were excluded from the analysis. Finally, 1,428 participants were included in genetic analysis. The characteristics of individuals who didn't have the *LPA* KIV-2 repeat polymorphism genotyped were not significantly different from those who were genotyped.

There was an inverse correlation between the number of KIV-2 repeats ($\overline{\Delta C_T}$) and the natural-log transformed Lp(a) ($r = -0.26$; $P < 0.001$) (**Fig. 2A**). The Lp(a) level decreased significantly across the tertiles of the number of KIV-2 repeats ($\overline{\Delta C_T}$) ($P < 0.001$) (Fig. 2B).The number of KIV-2 repeats ($\overline{\Delta C_T}$) were significantly associated with serum Lp(a) levels in linear regression with natural-log transformed Lp(a) as the dependent variable (model 1; β = −0.54, 95% CI −0.64 to −0.43; $P < 0.001$) (**Table 2**). The inverse association remained similar after adjustment for age, sex, and BMI (model 2; β = −0.53, 95% CI −0.64 to −0.43; $P < 0.001$). The association was more pronounced after additional adjustment for smoking and drinking status, blood pressure levels, antihypertensive medication, significant coronary stenosis, glucose levels, and antidiabetic medication (model 3; β = −0.56, 95% CI −0.68 to −0.45; $P < 0.001$). Further adjustment for lipid parameters and lipid-lowering drugs made no significant change (model 4; β = −0.55, 95% CI −0.67 to −0.44; $P < 0.001$).

Association of the number of KIV-2 repeats with type 2 diabetes

In a comparison of individuals with the number of KIV-2 repeat ($\overline{\Delta C_T}$) values in the bottom tertile versus the top tertile, the crude OR for type 2 diabetes was 0.75 (model 1;

TABLE 1. Baseline characteristics of study participants by tertiles of serum Lp(a)

	Tertile 1 (n = 622)	Tertile 2 (n = 620)	Tertile 3 (n = 621)	*P*
Lp(a) (mg/dl), median (range)	7.38 (0.60–12.91)	21.42 (12.95–35.27)	67.86 (35.34–318.50)	—
Male (%)	74.76	75.97	75.36	0.89
Age (years)	62.50 ± 10.23	63.12 ± 9.71	62.63 ± 10.31	0.52
BMI (kg/m^2)	25.00 ± 2.93	24.76 ± 3.10	24.61 ± 3.11	0.08
WC (cm)	88.04 ± 9.20	87.44 ± 9.63	87.01 ± 8.97	0.15
FPG (mmol/l)	5.94 ± 1.80	5.61 ± 1.59	5.59 ± 1.39	<0.001
2 h PPG (mmol/l)	9.66 ± 3.94	9.02 ± 3.60	9.08 ± 3.68	0.01
HbA1c (%)	6.30 ± 1.18	6.14 ± 1.05	6.15 ± 1.06	0.01
Diabetes (%)	41.64	33.55	35.10	0.007
Antidiabetic medication (%)	23.15	16.61	20.29	0.02
SBP (mmHg)	129.24 ± 13.79	129.09 ± 13.76	128.66 ± 13.75	0.75
DBP (mmHg)	77.50 ± 7.92	77.58 ± 8.61	77.18 ± 8.31	0.67
Hypertension (%)	68.97	70.81	69.73	0.78
Antihypertensive medication (%)	58.84	60.65	60.06	0.80
Total cholesterol (mmol/l)	3.83 ± 1.03	3.95 ± 0.99	3.99 ± 0.95	0.01
Triglyceride (mmol/l)	1.54 (1.05-2.28)	1.35 (0.99-1.97)	1.35 (0.99-1.94)	<0.001
LDL-C (mmol/l)	1.84 ± 0.79	2.07 ± 0.83	2.12 ± 0.81	<0.001
HDL-C (mmol/l)	1.15 ± 0.31	1.17 ± 0.32	1.16 ± 0.32	0.51
apoA-I (g/l)	1.22 ± 0.24	1.22 ± 0.25	1.20 ± 0.26	0.30
apoB (g/l)	0.67 ± 0.21	0.71 ± 0.21	0.72 ± 0.21	<0.001
Lipid-lowering drugs (%)	49.84	50.00	53.14	0.42
Smoking status				
Non-smokers (%)	50.97	50.81	49.03	0.63
Ex-smokers (%)	6.77	8.87	8.23	—
Current smokers (%)	42.26	40.32	42.74	—
Drinking status				
Non-drinkers (%)	75.08	74.84	72.95	0.85
Ex-drinkers (%)	4.02	3.39	4.19	—
Current drinkers (%)	20.90	21.77	22.87	—
Significant coronary stenosis ⩾50% (%)	79.26	82.58	84.54	0.05

Continuous data are expressed as mean ± SD or median (interquartile range). WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure.

95% CI 0.57–0.97; *P* for trend = 0.03) (**Fig. 3**). The association became slightly more pronounced after adjustment for age, sex, and BMI (model 2; OR = 0.73, 95% CI 0.56–0.99; *P* for trend = 0.02). After additional adjustment for smoking and drinking status, blood pressure levels, antihypertensive medication, and significant coronary stenosis, the association remained significant, and even more pronounced (model 3; OR = 0.70, 95% CI 0.54–0.93; *P* for trend = 0.01). Further adjustment for lipid parameters and lipid-lowering drugs made the association more pronounced (model 4; OR = 0.67, 95% CI 0.51–0.90; *P* for trend = 0.006). Interestingly, the association remained the same when serum Lp(a) was adjusted in the model (model 5; OR = 0.67, 95% CI 0.50–0.89; *P* for trend = 0.006). A per-tertile decrease in was $\overline{\Delta C_T}$ associated with an 18% reduction in risk of diabetes, with an OR of 0.82 (95% CI 0.71–0.94) per tertile (model 5).

Association of genetically elevated Lp(a) levels with type 2 diabetes

The observational OR for type 2 diabetes was decreased across the tertiles of serum Lp(a) in the crude model, the model adjusted for age, sex, and BMI, and the multifactorially adjusted model (**Fig. 4**). In the instrumental variable analysis, the estimated causal OR for type 2 diabetes was decreased for genetically elevated Lp(a) levels (Fig. 4). In

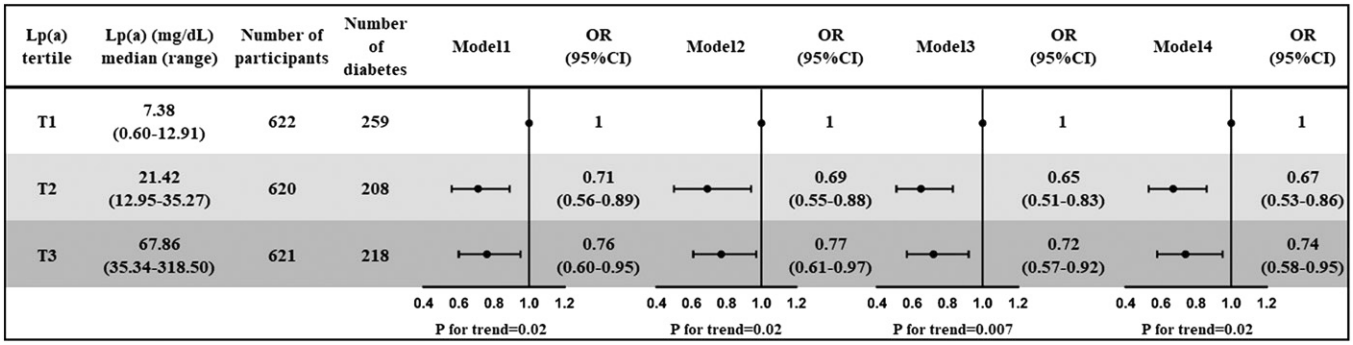

Fig. 1. Association of Lp(a) levels with prevalent type 2 diabetes. Data are shown as OR (95% CI). *P* for trend is from logistic regression model with tertiles. Model 1, unadjusted; model 2, adjusted for age, sex, and BMI; model 3, as in model 2 and additionally adjusted for smoking status, drinking status, systolic blood pressure, diastolic blood pressure, antihypertensive medication, and significant coronary stenosis (⩾50%); model 4, as in model 3 and additionally adjusted for total cholesterol, triglyceride, LDL-C, HDL-C, apoA-I, apoB, and lipid-lowering drugs.

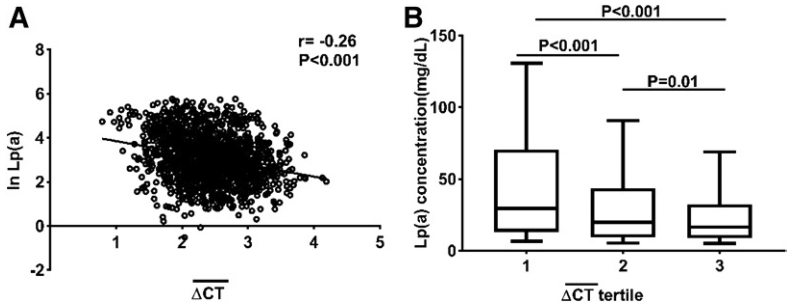

Fig. 2. Association of Lp(a) levels with the number of KIV-2 repeats in *LPA*. A: The inverse correlation between the number of KIV-2 repeats, as measured by ΔC_T, and serum Lp(a) concentrations (natural-log transformed) in the study population (n = 1,428). B: Serum Lp(a) concentrations by the tertiles of ΔC_T in the study population (n = 1,428). The upper, middle, and lower lines represent the 90th percentile, median, and 10th percentile of serum Lp(a) levels, respectively. The bottom and the top of the box represent the 25th and 75th percentiles of serum Lp(a) levels, respectively.

a comparison of individuals with genetically predicted Lp(a) values in the top tertile versus the bottom tertile, the crude OR for type 2 diabetes was 0.59 (95% CI 0.45–0.79; *P* for trend < 0.001) (Fig. 4). The association remained the same after adjustment for age, sex, and BMI (OR = 0.59, 95% CI 0.44–0.79; *P* for trend < 0.001). After further adjustment for smoking and drinking status, blood pressure levels, antihypertensive medication, significant coronary stenosis, lipid parameters, and lipid-lowering drugs, the association was more pronounced (OR = 0.43, 95% CI 0.30–0.61; *P* for trend < 0.001).

DISCUSSION

We observed a strong inverse association between serum Lp(a) levels and type 2 diabetes in a Chinese population with very high cardiovascular risk. We demonstrated that the number of KIV-2 repeats in *LPA* was inversely associated with serum Lp(a) levels. An association between the number of KIV-2 repeats in *LPA* and type 2 diabetes was also demonstrated. Moreover, in Mendelian randomization analyses, genetically elevated Lp(a) levels were associated with a lower risk of type 2 diabetes.

Although our observations were consistent with the previous studies, the study population was very different from those studies (8, 9, 18, 19), which were conducted in general populations. It is the first time that an inverse association between Lp(a) levels and type 2 diabetes was observed in a population with very high cardiovascular risk, where 82.1% of the study participants had significant coronary stenosis (≥50%). In a study of a large Chinese general population, the ORs of prevalent type 2 diabetes for quartiles 2–4 of serum Lp(a) versus quartile 1 were 0.86 (0.73–1.01), 0.88 (0.75–1.04), and 0.76 (0.64–0.90). Compared with this observation of a Chinese general population, the association of serum Lp(a) with diabetes was not attenuated, but even more pronounced in our population with very high cardiovascular risk. Although there was a positive association between Lp(a) levels and CHD in cohort studies (5–7), and previous Mendelian randomization studies indicated a causal role of Lp(a) levels in CHD (14–17), we still observed a strong inverse association between Lp(a) levels and type 2 diabetes in a population with a high proportion of angiography-confirmed CHD. It seems to be contradictory, as lowering Lp(a) concentrations can prevent myocardial infarction and cardiovascular mortality in our population with very high cardiovascular risk, but it may lead to an increased risk of type 2 diabetes.

TABLE 2. Association of the number of KIV-2 repeats in *LPA* with serum Lp(a) levels

	β	95% CI	*P*
Model 1	−0.54	−0.64 to −0.43	<0.001
Model 2	−0.53	−0.64 to −0.43	<0.001
Model 3	−0.56	−0.68 to −0.45	<0.001
Model 4	−0.55	−0.67 to −0.44	<0.001

Calculated using multivariate linear regression in 1,428 participants. The dependent Lp(a) is natural log-transformed. Model 1, unadjusted; model 2, adjusted for age, sex, and BMI; model 3, as in model 2 and additionally adjusted for smoking status, drinking status, systolic blood pressure, diastolic blood pressure, antihypertensive medication, significant coronary stenosis (≥50%), fasting glucose, 2 h postprandial glucose, antidiabetic medication; model 4, as in model 3 and additionally adjusted for total cholesterol, triglyceride, LDL-C, HDL-C, apoA-I, apoB, and lipid-lowering drugs.

To better understand the nature of association between Lp(a) levels and type 2 diabetes, we investigated the association of the number of KIV-2 repeats in *LPA* with type 2 diabetes. We first demonstrated a significant inverse association between the number of KIV-2 repeats and serum Lp(a) levels. Then a significant association between the number of KIV-2 repeats and type 2 diabetes was also demonstrated, which further supports an effect of Lp(a) on the risk of type 2 diabetes. However, it should be noted that the significant association of the number of KIV-2 repeats with diabetes remained unchanged after additional adjustment of Lp(a) levels, suggesting that the effect of Lp(a) on diabetes may not be mediated through the concentrations of Lp(a) per se. The number of KIV-2 repeats are not only inversely correlated with Lp(a) concentrations, but also determine the Lp(a) isoform size, with increasing number of KIV-2 repeats translating into apo(a) isoforms of increasing size. In the prospective EPIC-Norfolk study, there was an inverse association between Lp(a) levels and type 2 diabetes (19). However, in Mendelian randomization analyses, the *LPA* rs10455872, which explained 26.8% of the variability in Lp(a) levels, was not associated with risk of type 2 diabetes (19). In another study of the Danish general population, the number of KIV-2 repeats was causally associated with type 2 diabetes in Mendelian randomization analyses, but rs10455872 did not affect the risk of diabetes (18). In our study, the Mendelian randomization analyses using KIV-2 repeat polymorphism as an instrument variable also suggests a causal effect of Lp(a) on type 2 diabetes. These studies, including ours, indicate that low Lp(a) concentrations alone may not be causally associated with type 2 diabetes, but there may be a causal relationship

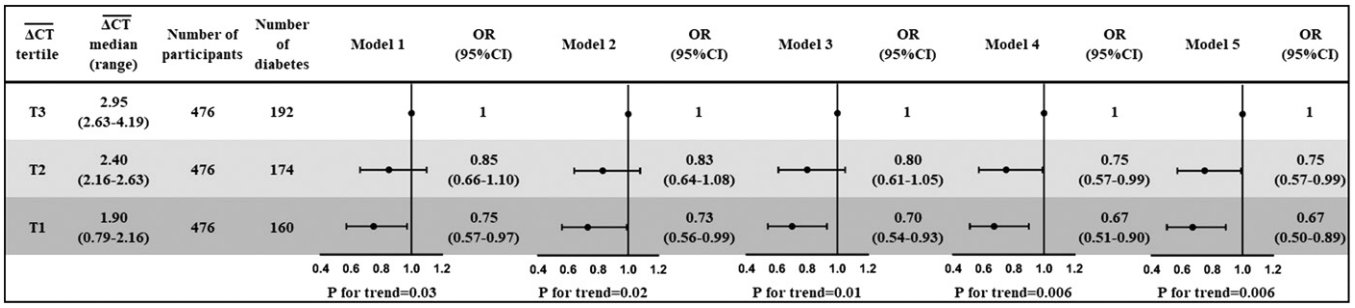

Fig. 3. Association of the number of KIV-2 repeats in LPA with prevalent type 2 diabetes. Data are shown as OR (95% CI). *P* for trend is from logistic regression model with tertiles. Model 1, unadjusted; model 2, adjusted for age, sex, and BMI; model 3, as in model 2 and additionally adjusted for smoking status, drinking status, systolic blood pressure, diastolic blood pressure, antihypertensive medication, and significant coronary stenosis (⩾50%); model 4, as in model 3 and additionally adjusted for total cholesterol, triglyceride, LDL-C, HDL-C, apoA-I, apoB, and lipid-lowering drugs; model 5, as in model 4 and additionally adjusted for Lp(a).

between large Lp(a) isoform size and type 2 diabetes. Recently, by using a novel genetic approach, one study found that SNPs that were associated selectively with the number of KIV-2 repeats were associated with type 2 diabetes, but SNPs that were associated selectively with Lp(a) concentrations were not associated with type 2 diabetes (20). These results reassure that it is a high number of KIV-2 repeats that is causally associated with increased risk of type 2 diabetes, and not low Lp(a) concentrations per se. However, the underlying pathophysiological mechanism for the impact of Lp(a) isoform size on type 2 diabetes warrants future research. Previous studies have demonstrated an inverse relationship between serum Lp(a) concentrations and insulin resistance (9, 27). It is possible that large Lp(a)

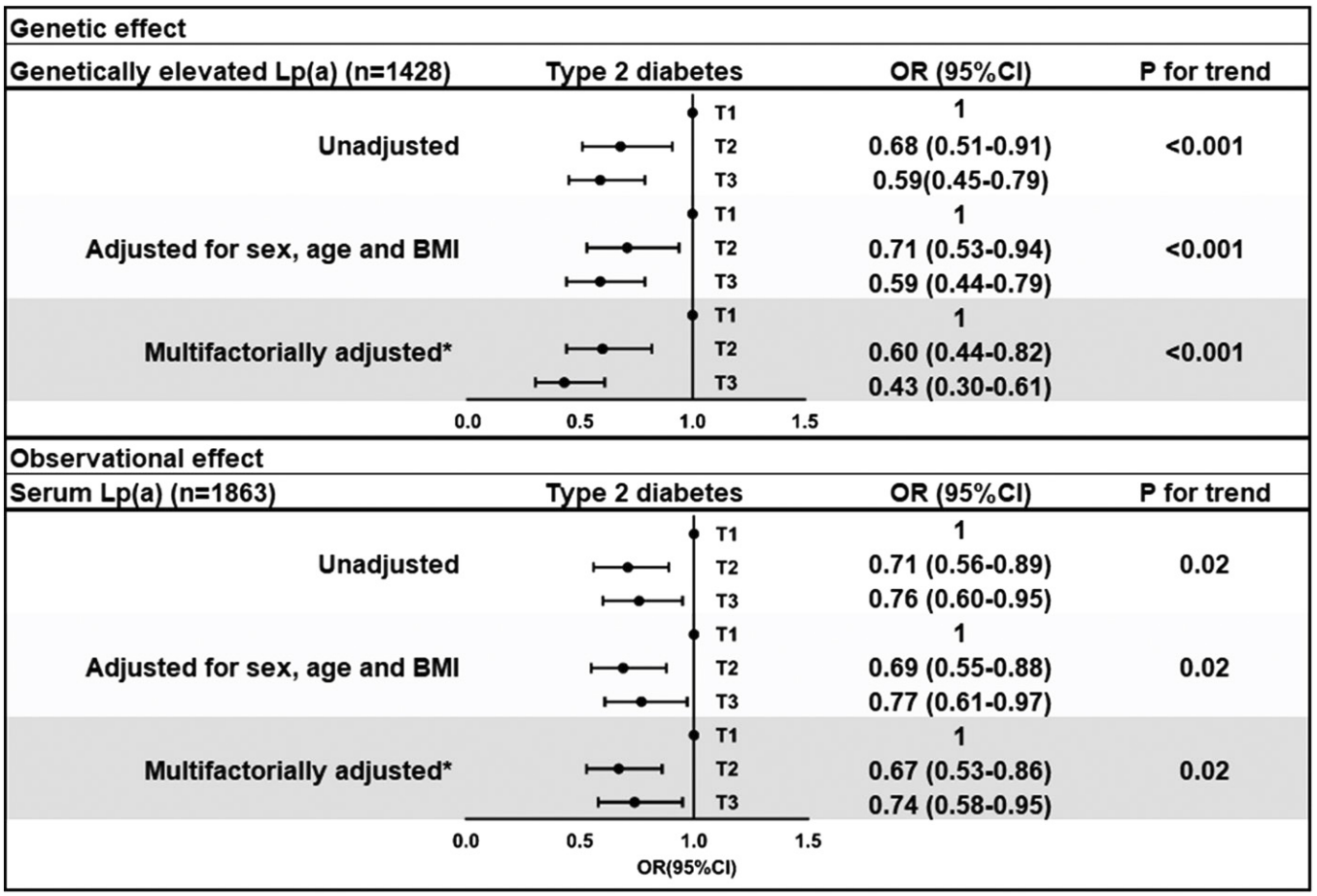

Fig. 4. Instrumental variable analysis estimate of the association of genetically elevated Lp(a) levels with risk of type 2 diabetes using the number of KIV-2 repeats in *LPA* as an instrument. Risk estimates for the tertiles of Lp(a) levels were calculated by logistic regression analyses using the first tertile as the reference. T1, the first tertile; T2, the second tertile; T3, the third tertile. *The multifactorial analysis adjusted for age, sex, BMI, smoking status, drinking status, systolic blood pressure, diastolic blood pressure, antihypertensive medication, significant coronary stenosis, total cholesterol, triglyceride, LDL-C, HDL-C, apoA-I, apoB, and lipid-lowering drugs.

particles represented as low Lp(a) levels induce insulin resistance, which finally causes type 2 diabetes.

Although the inverse association of Lp(a) levels with type 2 diabetes in Chinese populations has been reported previously, ours is the first study to investigate the causality of this observed association. As disease status cannot alter genotype, and genotype-disease associations are not likely to be confounded by lifestyle factors, we are able to demonstrate a causal role of Lp(a) in type 2 diabetes in a Chinese population by using Mendelian randomization analyses with the *LPA* KIV-2 repeat polymorphism as the instrumental variable. Consistent with the observational effect, the genetically predicted Lp(a) levels were inversely associated with type 2 diabetes in the current study. Furthermore, the genetic effect was stronger than the observed effect, with a 57% risk reduction comparing individuals with genetically predicted Lp(a) values in the top tertile versus the bottom tertile. It should be noted that the individuals in the second tertile of Lp(a) levels are at lower risk than those in the third tertile as an observational effect, as shown in Fig. 4. Although confounders were adjusted as much as possible in our study, it is possible that our observations still suffered from some residual confounding due to the inability to adjust the model for all potential confounders. In Mendelian randomization analysis, we computed the nonconfounded genetically predicted Lp(a) levels. The risk of type 2 diabetes decreased gradually with increasing genetically predicted Lp(a) levels as expected, as shown in Fig. 4. We believe that the real relationship between Lp(a) levels and type 2 diabetes was presented by using genetic approaches. Although a Mendelian randomization study may be considered nature's own randomized intervention trial, profiting from a random assortment of alleles from parents to offspring, the possibility of reverse causality still cannot be totally excluded. Therefore, final proof of causality between Lp(a) and type 2 diabetes still requires future randomized clinical trials demonstrating reduced risk of diabetes when targeting large Lp(a) isoforms.

Our findings have some implications for prevention and treatment of type 2 diabetes and CHD. Identification of large Lp(a) isoform size represented as a high number of *LPA* KIV-2 repeats may help to prevent type 2 diabetes in a population with very high cardiovascular risk. Use of Lp(a)-lowering drugs to reduce cardiovascular risk in such a population is feasible, as these therapies are unlikely to increase the number of KIV-2 repeats, which is determined by genetics. Our results do not support a differential management of Lp(a) levels for the prevention of type 2 diabetes and CHD.

Our study has some limitations. Our study indicates that it is the isoform size of Lp(a) that is causally associated with the risk of type 2 diabetes. However, as Lp(a) isoforms were not measured directly in our study, we are unable to provide specific information about the association of Lp(a) isoform size with type 2 diabetes. Further, we measured the number of KIV-2 repeats with qPCR, which cannot estimate the number of KIV-2 repeats in an allele-specific manner. The obtained value is the sum of the number of repeats at the two different *LPA* alleles, which represents an average particle size. As it is the large Lp(a) isoform that causes diabetes and not the average particle size, our measurements may introduce some biases to the results. Also, our assay may be biased because of its inability to account for nonexpression of alleles. However, the current gold standard method, like electrophoresis with immunoblotting, is technically challenging, laborious, and time consuming, which restricts its application in genetic studies. Therefore, the limitations of the qPCR method are balanced by the fact that it provides a fast and cost-effective method of evaluation of Lp(a) isoform size from genomic DNA in genetic epidemiology studies. The new genetic approach (20) using SNPs associated selectively with Lp(a) concentrations or with KIV-2 repeats has its advantages, and is worth being applied in future studies of Chinese populations to confirm that it is a high number of KIV-2 repeats that are causally associated with increased risk of type 2 diabetes.

In conclusion, our study demonstrated that a high number of *LPA* KIV-2 repeats are associated with increased risk of type 2 diabetes in a Chinese population with very high cardiovascular risk. Our results indicate that large Lp(a) isoform size, which is associated with low Lp(a) concentrations, has a causal effect on type 2 diabetes. More studies in Chinese populations are needed to confirm our findings, and studies to understand the pathophysiological mechanism underlying the causal association between Lp(a) isoform size and type 2 diabetes are also warranted.

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