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Circulating YKL-40 levels but not *CHI3L1* or *TRIB1* gene variants predict long-term outcomes in patients with angiographically confirmed multivessel coronary artery disease

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YKL-40 is significantly associated with the prevalence and severity of coronary artery disease (CAD). YKL-40 levels are significantly associated with variations in the *CHI3L1* and *TRIB1* genes. We investigated candidate genes for YKL-40 levels and evaluated the prognostic value of this biomarker and corresponding variants for long-term outcomes in patients with CAD. We included 4664 and 521 participants from the Taiwan Biobank (TWB) and CAD cohorts, respectively. Candidate variants for circulating YKL-40 levels were investigated using genome-wide association study (GWAS) data from the TWB cohort, and the results were validated in the CAD cohort. The primary endpoint was all-cause mortality. The secondary endpoint was major adverse cardiac events (MACEs), which included the composite endpoints of all-cause mortality, nonfatal acute coronary syndrome, hospitalization for heart failure, and nonfatal stroke. According to the GWAS data from the TWB cohort, three *CHI3L1* variants (rs4950928, rs10399931, and rs872129) and one *TRIB1* variant (rs6982502) were independently associated with YKL-40 levels. These findings were validated in the CAD cohort. The combined *CHI3L1* and *TRIB1* weighted genetic risk scores (WGRSs) were not associated with the long-term outcomes (median follow-up period of 3.7 years) in patients with CAD. Conversely, patients with YKL-40 levels in the upper tertile had the highest rates of all-cause mortality and MACEs (log-rank $p = 9.58 \times 10^{-8}$ for all-cause mortality and 1.34×10^{-7} for MACEs). Furthermore, YKL-40 levels predicted poor clinical outcomes only in patients with multivessel CAD (log-rank $p = 3.0 \times 10^{-6}$ for all-cause mortality and 1.10×10^{-5} for MACEs) and not in patients with single-vessel CAD. This study revealed that YKL-40 levels but not the combined *CHI3L1* and *TRIB1* WGRSs were found to be independent predictors of poor clinical outcomes in patients with multivessel CAD.

Keywords YKL-40, *CHI3L1*, *TRIB1*, Weighted genetic risk score, Taiwan Biobank, Coronary artery disease

Despite improvements in guideline-directed medical therapy and coronary revascularization, coronary artery disease (CAD) remains the leading cause of disease burden and mortality and has resulted in rising health-care costs from 1990 to 2019[1,2]. To provide more effective prevention and treatment strategies, identifying individuals at high risk of developing CAD is crucial. Large cohort studies have demonstrated that most patients with CAD exhibit at least one of the four major conventional risk factors: a smoking habit, diabetes, hypertension, or

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hypercholesterolemia[3,4]. However, at least 10% to 15% of patients with CAD, especially older patients, do not meet the criteria for traditional CAD risk factors, emphasizing the importance of research on nontraditional risk factors and genetic causes of heart disease[4]. Among nontraditional risk factors, low-grade inflammation, such as elevated levels of C-reactive protein (CRP) or other proinflammatory cytokines, is well known to be crucial in the pathogenesis of CAD and to predict poor long-term outcomes[5–7]. Consistent with those findings, our previous research demonstrated that higher circulating resistin and soluble suppression of tumorigenesis-2 (sST2) levels predict poor clinical outcomes in patients with CAD[8]. The combination of resistin and sST2 levels with the genetic risk scores of their corresponding genetic variants synergistically affected CAD outcomes.

YKL-40, or chitinase-3 like-1 (CH3L1), is an evolving inflammatory biomarker secreted by numerous types of human cells, including activated macrophages, neutrophils, and vascular smooth muscle cells[9–11]. Elevated YKL-40 is observed in patients with diseases characterized by inflammation and ongoing tissue remodelling, such as cancer[12,13], insulin resistance and diabetes[14,15], asthma[16], and rheumatoid arthritis[17]. YKL-40 is also involved in angiogenesis and participates in the progression of atherosclerosis during its early stages[10,13,18,19]. Circulating YKL-40 levels correlate with the prevalence and disease severity of CAD[20–24] and with incident thromboembolic stroke[25]. Additionally, in the Effect of Clarithromycin on Mortality and Morbidity in Patients with Ischaemic Heart Disease (CLARICOR) trial, greater circulating YKL-40 was associated with an increased risk of adverse cardiovascular outcomes and mortality in patients with CAD[26]. However, the addition of YKL-40 did not improve risk prediction in patients with stable CAD.

The *CHI3L1* gene is located at chromosomal region 1q31–1q32 and is responsible for YKL-40 expression in humans. Several genetic variants of *CHI3L1* are significantly associated with plasma YKL-40 levels[25,27–31]. Consequently, genetic variation in *CHI3L1* is closely linked to the incidence and prognosis of various diseases, such as asthma[28,31], liver fibrosis[32], and neoplastic disease[33]. Several studies have revealed no significant correlations between *CHI3L1* genetic variants and the prevalence or outcomes of CAD[21,22,25]. In our previous investigation, we also reported that circulating YKL-40 levels but not *CHI3L1* genetic variants were associated with the risk of peripheral artery disease in Taiwanese individuals[30]. However, few common genetic variants of *CHI3L1* were detected in those studies, and genome-wide association studies (GWASs) examining the prevalence and outcomes of CAD are scarce.

The *TRIB1 (Tribbles 1)* gene is located at chromosome 8q24 and encodes the TRIB1 protein, which may play a key role in plasma lipid homeostasis[34]. Since dyslipidaemia is one of the most critical risk factors for CAD, it is speculated that *TRIB1* genetic variants may increase the risk of CAD. Accordingly, GWASs and meta-analyses have revealed significant associations of genetic variants of the *TRIB1* gene with dyslipidaemia and the risk of coronary artery disease[35,36]. Furthermore, the genetic variant of *TRIB1,* rs28601761, also influences the level of YKL-40 in the circulation[37]. However, the associations between genetic variants of *TRIB1* that affect YKL-40 levels and the prognosis of CAD have not been evaluated.

The Taiwan Biobank (TWB) conducted a large-scale population-based cohort study of 30–70-year-old volunteers with no history of cancer[38]. Genetic information was available for all participants. In the present study, we investigated candidate genetic variants associated with plasma YKL-40 levels in the Taiwanese population using GWAS data from TWB. The candidate genetic variants were validated in patients with angiographically confirmed CAD (CAD cohort). We also investigated whether the weighted genetic risk scores (WGRSs) of these candidate genes and circulating YKL-40 levels predict long-term clinical outcomes in patients with CAD and compared the impacts of YKL-40 levels on CAD outcomes between patients with single-vessel CAD and those with multivessel CAD.

Methods

Study participants and design

The present study included data from two independent cohorts: the TWB cohort and the CAD cohort.

Participants from the TWB cohort were recruited from recruitment centres across Taiwan between 2008 and 2015[38]. TWB is a national resource containing genetic and baseline information, blood and urine test data, and questionnaire data regarding the lifestyle factors of Taiwanese adults aged 30–70 years. The participants provided written informed consent prior to data collection. Participants without a history of cancer were enrolled, and all participants self-reported having Han Chinese ethnicity. A total of 5000 participants with available GWAS data and blood samples were recruited for the current study. After excluding participants who withdrew informed consent after participation (2), fasted for < 6 h (96), had relative pairs of second-degree relatives or closer by descent (IBD) > 0.187 (227), and were missing *CHI3L1* or *TRIB1* single-nucleotide polymorphism (SNP; 11) data, data for 4664 participants were included in the final analysis (Supplementary Fig. S1).

In the CAD cohort, 565 patients who underwent coronary angiography and had at least 50% stenosis of one major coronary artery between July 2010 and September 2013 were recruited from National Taiwan University Hospital. Blood samples for DNA and biomarker analysis were available from all of the participants. After the exclusion of patients with significant haemolysis in blood samples (29), missing YKL-40 data (6), missing follow-up data (2), and missing *CHI3L1* or *TRIB1* SNP data (7), 521 patients were included in the analysis (Supplementary Fig. S1). All clinical data were obtained from patient medical records. The primary endpoint was all-cause mortality. The secondary endpoint was major adverse cardiac events (MACEs), which included the composite endpoints of all-cause mortality, nonfatal acute coronary syndrome (unstable angina, non-ST elevation myocardial infarction and ST elevation myocardial infarction), hospitalization for heart failure, and nonfatal stroke. Seven patients were lost to regular follow-up after enrolment and were contacted by telephone to obtain survival and disease status data before the end of the study. Three of those patients had died, and the causes of death were provided by relatives.

Laboratory examination

Clinical phenotypes, including body height, body weight, body mass index, and blood pressure, were examined. Biochemical and haematological data such as fasting plasma glucose levels, total cholesterol levels, high-density lipoprotein (HDL) cholesterol levels, low-density lipoprotein (LDL) cholesterol levels, triglyceride levels, serum creatinine levels, the estimated glomerular filtration rate (eGFR), uric acid levels, white blood cell counts, platelet counts, and haematocrit values were also collected. The definitions of hypertension, diabetes mellitus, hyperlipidaemia, and current smoking status are presented in Supplementary Table S1. Commercially available enzyme-linked immunosorbent assay kits (R&D, Minneapolis, MN, USA) were used for the measurement of YKL-40 levels. Circulating CRP levels were calculated using a particle-enhanced turbidimetric immunoassay (Siemens Healthcare Diagnostics, Camberley, UK). The intra- and interassay coefficients of variation were 6.7% and 7.7%, respectively, for YKL-40 and 7.1% and 9.5%, respectively, for CRP.

Genomic DNA extraction, genotyping, and GWAS analysis

Detailed descriptions of the genomic DNA extraction, genotyping, and GWAS analysis methods used are provided in our previous study[8]. Genotyping for the *CHI3L1* rs4950928, rs10399931, and rs872129 genotypes and for *TRIB1* rs6982502 in participants with CAD was performed using TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). Approximately 10% of the samples were regenotyped blindly for quality control, and identical results were obtained. GWAS analysis was performed using the Axiom Genome-Wide CHB1 Array Plate (Affymetrix) designed by the Taiwan Biomarker Study Group, which comprised 642,832 SNPs. With 1,000 Genomes Project Phase 3 East Asian population as a reference panel, genome-wide genotype imputation was carried out using SHAPEIT and IMPUTE2. Following imputation, quality control was performed by filtering SNPs with an imputation quality score of IMPUTE2 more than 0.3. Indels were removed using VCF tools. There were 16,537,709 SNPs obtained after imputation. For SNP quality control, we excluded samples with a SNP call rate < 3% (1705 SNPs), a minor allele frequency < 0.01 (9,197,156 SNPs) and a violation of Hardy–Weinberg equilibrium ($P < 10^{-6}$) (295 SNPs) from subsequent analyses. In total, 4664 participants and 7,338,553 SNPs were included in the GWAS analysis after quality control.

Statistical analysis

To adhere to normality assumptions, YKL-40 levels were logarithmically transformed before analysis. Analysis of YKL-40 levels in relation to the investigated genotypes and confounders was performed using a generalized linear model. Genome-wide scans were conducted using the software package PLINK (version 1.07, Cambridge, MA, USA), and a P value of $< 5 \times 10^{-8}$ indicated genome-wide significance. For the GWAS, a conditional analysis was conducted to assess the residual associations of the remaining SNPs after adjustment for the most strongly associated SNP at a locus by adding the SNP as a covariate into the regression model.

Patients in the CAD cohort were stratified according to tertiles of YKL-40. The baseline characteristics and laboratory data of the participants were compared using analysis of variance for continuous variables and the chi-square test for categorical variables. Bonferroni correction was used for the analysis of variance to adjust for multiple comparisons. The genetic risk score was calculated using the weighted method, which assumes that each SNP is independently associated with YKL-40 levels (i.e., that there are no interactions among the SNPs)[39]. We applied linear weighting of 0, 1, or 2 to genotypes containing a corresponding number of risk alleles. Assuming an additive effect of risk alleles for each SNP, WGRSs were calculated as the sum of the estimated beta coefficient (adjusted for age, sex, current smoking status and body mass index) of each SNP multiplied by the number of corresponding risk alleles (0, 1, or 2). Kaplan–Meier curves were calculated and the log-rank test was conducted to compare the rates of freedom from primary and secondary endpoints in patients with CAD among the YKL-40 tertiles and to bisect the WGRSs of the candidate variants. The impact of circulating YKL-40 levels on clinical outcomes in relation to single-vessel CAD and multivessel CAD was analysed. Cox regression was conducted to determine the hazard ratios (HRs) of the primary and secondary endpoints among the three YKL-40 tertiles, adjusting for patient baseline characteristics, conventional cardiovascular risk factors, CRP levels and eGFR. Harrell's C analysis for Cox regression model was calculated to examine the predictive ability of long-term outcomes before and after adding the YKL-40 tertiles to conventional CV risk factors and CRP levels. A receiver-operating characteristic curve was used to calculate the values most strongly associated with the primary and secondary endpoints in patients with multivessel CAD. We used IBM SPSS Statistics version 24.0 (IBM, Armonk, NY, USA) and R software version 4.2.3 (Foundation for Statistical Computing, Vienna, Austria) to perform all calculations. A two-sided p value of < 0.05 was considered to indicate statistical significance.

Ethics approval and consent to participates

The Research Ethics Committee of Taipei Tzu Chi Hospital (approval number: 05-X04-007), Buddhist Tzu Chi Medical Foundation had approved the present study. The TWB study cohort had been approved by Ethics and Governance Council of the Taiwan Biobank (approval number: TWBR10507-02 and TWBR10611-03). The CAD study cohort had been approved by the Research Ethics Committee of National Taiwan University Hospital. Written informed consent was obtained from all participants before participation. All procedures were performed in accordance with the Declaration of Helsinki and the ethical standards of the Committee on Human Experimentation.

Results

GWAS and replication genotyping results for YKL-40

GWAS analysis of the TWB genotype with imputation for YKL-40 levels was performed using the data of the 4664 participants from the TWB. A linear regression model for genotype trend effects was applied with adjustment for age, sex, BMI, smoking status and 3 principal components. GWAS analysis demonstrated that the

genome-wide significance threshold was exceeded for chromosome 1q32.1, where the *CHI3L1* gene is located, and for chromosome 8q24.13, where the *TRIB1* gene is located (Fig. 1a), with rs4950928 ($p = 1.81 \times 10^{-118}$) as the lead SNP for the *CHI3L1* gene (Fig. 1b) and rs6982502 ($p = 9.60 \times 10^{-18}$) as the lead SNP for the *TRIB1* gene (Fig. 1c). In addition, four SNPs were found to show genome-wide significant association with YKL-40 levels (Fig. 1a, Supplementary Table S2). However, the Manhattan plot showed that each chromosome locus had only one variant that exhibited significant association with YKL-40 levels for these additional SNPs. This finding was unusual for a common variant. Furthermore, the *p* values for Hardy–Weinberg equilibrium of these SNPs were between 1.0×10^{-4} to 1.0×10^{-5}, suggesting a trend toward a violation of Hardy–Weinberg equilibrium. Although these SNPs might be candidate variants for YKL-40 levels, the possibility of false discovery could not be excluded. Since these SNPs only explained little variation in YKL-40 levels in the TWB cohort (0.2% to 0.8%), they were excluded from further genetic analysis in the CAD cohort.

To clarify whether other *CHI3L1* SNPs were associated with YKL-40 levels independent of the lead SNP, stepwise conditional analysis was performed. After adjustment for the rs4950928 genotype, rs10399931 in the regional plot at the *CHI3L1* locus exhibited a stronger association with YKL-40 levels ($p = 4.01 \times 10^{-19}$; Fig. 1d). After adjustment for both the rs4950928 and rs10399931 genotypes, rs872129 was significantly associated with YKL-40 levels ($p = 9.72 \times 10^{-25}$; Fig. 1e). After adjustment for the three SNPs in the GWAS, no other SNPs in the regional plot near the *CHI3L1* locus exhibited genome-wide significance, indicating that in this chromosome region, variances in YKL-40 levels were mainly explained by these three signals. For the *TRIB1* locus, no

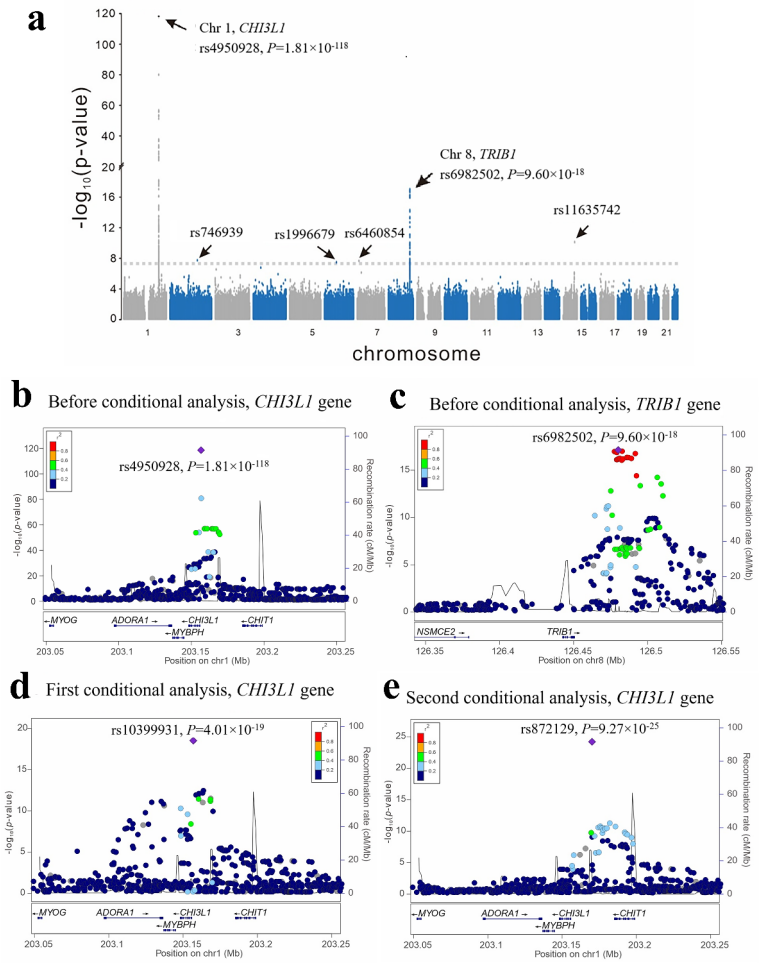

Fig. 1. Conditional analysis of candidate single-nucleotide polymorphisms (SNPs) for YKL-40 levels using genome-wide association study (GWAS) data from the Taiwan Biobank (TWB) cohort. (**a**) Manhattan plots for YKL-40 levels from a GWAS analysis with imputation of 4664 TWB participants. Two peaks exceeded the genome-wide significance level. One peak was located at chromosome 1q32.1, where the *CHI3L1* gene is located. Another peak was located at chromosome 8q24.13, where the *TRIB1* gene is located. Additionally, four SNPs with genome-wide significant association with YKL-40 levels were also demonstrated. (**b**, **c**) Before conditional analysis, regional association plots for YKL-40 levels showed rs4950928 as the lead SNP surrounding the *CHI3L1* locus and rs6982502 as the lead SNP surrounding the *TRIB1* locus. (**d**) After the first conditional analysis adjusted for the rs4950928 genotype, rs10399931 was significantly associated with YKL-40 levels at the *CHI3L1* locus. (**e**) After the second conditional analysis adjusted for both rs4950928 and rs10399931, rs872129 was significantly associated with YKL-40 levels.

significant SNP remained after adjustment for rs6982502. The associations of the four candidate SNPs and combined *CHI3L1* and *TRIB1* WGRSs with YKL-40 levels in the TWB cohort are provided in Supplementary Table S3.

The associations of *CHI3L1* and *TRIB1* SNPs with YKL-40 levels in the CAD cohort were also evaluated (Supplementary Table S3). Only rs4950928 and rs10399931 in the *CHI3L1* locus and rs6982502 in the *TRIB1* locus exhibited significant associations with YKL-40 levels ($p = 1.36 \times 10^{-10}$ for rs4950928, $p = 1.41 \times 10^{-7}$ for rs10399931, $p = 0.041$ for rs6982502). The combined *CHI3L1* and *TRIB1* WGRSs (calculated on the basis of the four SNPs) correlated strongly with YKL-40 levels in the CAD cohort ($p = 1.65 \times 10^{-13}$). The three lead *CHI3L1* SNPs and the lead *TRIB1* SNP explained 14.4% of the variation in YKL-40 levels in the TWB cohort and 9.2% of the variation in YKL-40 levels in the CAD cohort (Supplementary Table S4). The SNP table of *CHI3L1* and *TRIB1* is provided in Supplementary Table S5. Correlations of the candidate SNPs and combined WGRSs with baseline characteristics of patients with CAD is shown in Supplementary Table S6.

Baseline characteristics of patients with CAD stratified by tertiles of YKL-40

The baseline characteristics of patients with CAD are presented in Supplementary Table S7. Participants were stratified according to tertiles of YKL-40, and their baseline characteristics were compared. Patients in the upper YKL-40 tertile were older and more likely to be women, had multivessel CAD, and had diabetes. The triglyceride levels differed, but the fasting glucose, total cholesterol, HDL cholesterol, and LDL cholesterol levels were similar among the three tertiles; these results may have been biased because most of the patients with hyperlipidaemia were receiving lipid-lowering agents, and most of the patients with diabetes were receiving hypoglycaemic agents. Patients in the upper YKL-40 tertile also exhibited poor renal function, elevated leukocyte counts, and lower haematocrit and CRP levels.

Predictions of YKL-40 levels and combined CHI3L1 and TRIB1 WGRSs on long-term outcomes in patients with CAD

In the CAD cohort, the follow-up duration was 1347 ± 420 days; 46 patients died, and 92 patients developed MACEs. Cox regression analysis revealed that YKL-40 levels strongly predicted long-term outcomes in patients with CAD ($p = 1.67 \times 10^{-11}$ for all-cause mortality, $p = 8.20 \times 10^{-12}$ for MACEs). The patients were further stratified according to the tertile of YKL-40 levels. Kaplan–Meier survival analysis revealed that patients in the upper YKL-40 tertile exhibited the poorest clinical outcomes, with significantly greater rates of all-cause mortality and MACEs (Fig. 2a and 2b, $p = 9.58 \times 10^{-8}$ for all-cause mortality, $p = 1.34 \times 10^{-7}$ for MACEs). A comparison of all-cause mortality and MACEs in patients with CAD between each of the two groups of YKL-40 tertiles is provided in Supplementary Fig. S2. In contrast, the combined *CHI3L1* and *TRIB1* WGRSs did not predict all-cause mortality or MACEs during long-term follow-up in patients with CAD (Fig. 2c and 2d, $p = 0.104$ for all-cause mortality, $p = 0.917$ for MACEs).

Results of the Cox regression of all-cause mortality and MACEs between the three YKL-40 tertiles are provided in Table 1. After adjustment for baseline characteristics and conventional cardiovascular risk factors, the all-cause mortality and MACEs were significantly higher for the patients in the upper YKL-40 tertile than for those in the lowest tertile (HR: 5.98, 95% CI [confidence interval]: 1.77 to 20.25, $p = 0.04$ for all-cause mortality; HR: 2.94, 95% CI: 1.55 to 5.59, $p = 0.001$ for MACEs). After further adjustment for CRP levels or eGFR, the results were mildly attenuated but remained significant.

Predictions of the effects of YKL-40 levels on long-term outcomes in patients with single-vessel or multivessel CAD

We investigated the ability of YKL-40 levels to predict long-term outcomes in patients with CAD with single-vessel disease or multivessel disease (i.e., double- or triple-vessel disease; Fig. 3). For patients with single-vessel CAD, all-cause mortality and MACEs did not significantly differ between patients with lower and higher YKL-40 levels (Fig. 3a and b; $p = 0.163$ for all-cause mortality; $p = 0.054$ for MACEs). Conversely, higher YKL-40 levels predicted poorer clinical outcomes in patients with multivessel CAD after long-term follow-up, and patients in the upper YKL-40 tertile exhibited the highest rates of all-cause mortality and MACEs (Fig. 3c and d, $p = 3.0 \times 10^{-6}$ for all-cause mortality, $p = 1.10 \times 10^{-5}$ for MACEs).

Cox regression of all-cause mortality and MACEs between patients with single-vessel or multivessel CAD were compared among the YKL-40 tertiles. In patients with single-vessel CAD, all-cause mortality and MACEs were similar among the three patient tertiles (Supplementary Table S8). Conversely, YKL-40 levels strongly influenced long-term outcomes in patients with multivessel CAD (Table 2). Patients in the upper YKL-40 tertile had significantly greater rates of all-cause mortality and MACEs after adjustment for baseline characteristics and conventional cardiovascular risk factors (HR: 6.36, 95% CI: 1.46 to 27.64, $p = 0.014$ for all-cause mortality; HR: 2.81, 95% CI: 1.37 to 5.76, $p = 0.005$ for MACEs). After further adjustment for CRP levels or eGFR, this predictive power was mildly attenuated but remained significant. Comparison of predictive ability of long-term outcomes in patients with multi-vessel CAD with and without YKL-40 tertiles is presented in Supplementary Table S9. Addition of YKL-40 tertiles to conventional CV risk factors and CRP levels significantly improved risk prediction of all-cause mortality (Harrell's C 0.802 and 0.870 respectively, $p = 0.036$ for difference) and MACEs (Harrell's C 0.678 and 0.742 respectively, $p = 0.017$ for difference) in patients with multi-vessel CAD. We calculated receiver-operating characteristic curves to determine the values most strongly associated with all-cause mortality and MACEs in patients with multivessel CAD (Supplementary Fig. S3). The cut-off YKL-40 level for predicting all-cause mortality and MACEs in patients with multivessel CAD was 130.50 ng/mL (sensitivity: 65.9%, specificity: 74.9% for all-cause mortality; sensitivity: 51.3%, specificity: 76.3% for MACEs).

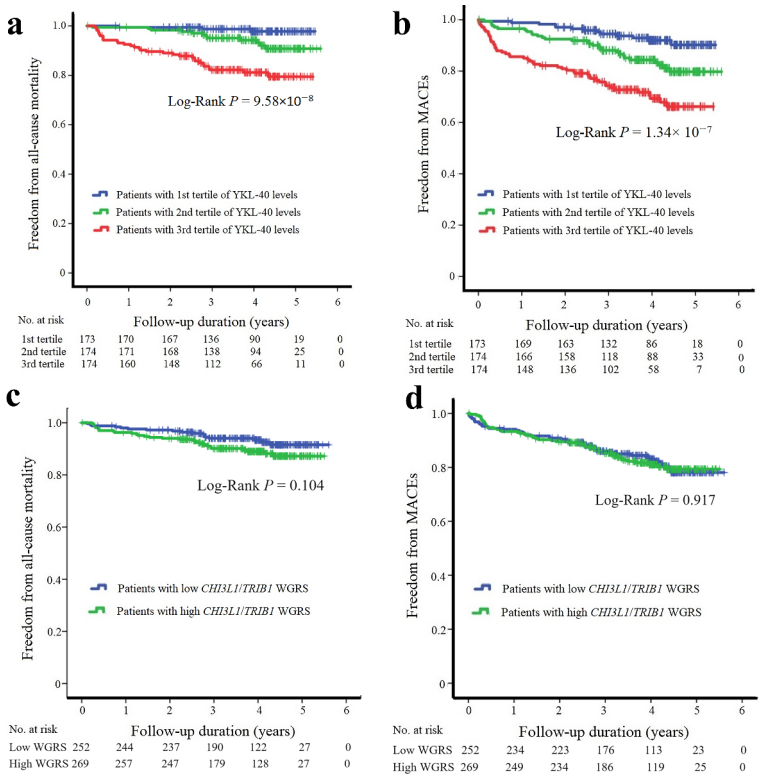

Fig. 2. Kaplan–Meier curve analysis of YKL-40 levels and the combined *CHI3L1* and *TRIB1* WGRSs for long-term outcomes in patients with CAD. (**a**, **b**) YKL-40 levels predict poorer clinical outcomes. Patients in the upper YKL-40 tertile had significantly lower rates of freedom from all-cause mortality and MACEs. (**c**, **d**) *CHI3L1* and *TRIB1* WGRSs did not predict all-cause mortality or MACEs during long-term follow-up. MACEs, major adverse cardiac events; WGRSs, weighted genetic risk scores.

Discussion

In this study, we performed a GWAS analysis with imputation of 4664 participants in the TWB cohort to determine the genetic basis of YKL-40 levels in Han Chinese individuals. Three *CHI3L1* variants—rs4850928, rs10399931, and rs872129—and one *TRIB1* variant—rs6982502—were independently associated with YKL-40 levels. These results were validated in the CAD cohort, except for the association of rs872129 in the *CHI3L1* locus. In the CAD cohort, the combined *CHI3L1* and *TRIB1* WGRSs did not influence the long-term outcomes of patients with CAD. Conversely, YKL-40 levels strongly predicted poorer clinical outcomes in these patients, and patients in the upper YKL-40 tertile had the highest rates of all-cause mortality and MACEs after a median of 3.7 years of follow-up. The ability of YKL-40 levels to predict long-term outcomes was significant in patients with multivessel disease but not in patients with single-vessel CAD. The prognostic value of YKL-40 levels for all-cause mortality and MACEs remained significant after adjustment for baseline characteristics, conventional cardiovascular risk factors, CRP levels or eGFR, suggesting that YKL-40 levels independently affect long-term outcomes in patients with multivessel CAD.

Genetic determinants of YKL-40 levels in the Chinese population

In humans, the major gene encoding YKL-40 is *CHI3L1*, which is located on chromosomes 1q31–1q32. Genetic variants of *CHI3L1*, especially rs4950928, are strongly associated with circulating YKL-40 levels[25,27–30]. Genetic variation in *CHI3L1* is also associated with asthma susceptibility and severity[28,31]. Another genetic variation in the *TRIB1* gene on chromosome 8, rs28601761, is strongly associated with YKL-40 levels[37]. However, comprehensive analyses of the genetic determinants of YKL-40 levels using GWAS data are rare. In the present study, we used GWAS analysis with imputation from TWB to determine candidate genes for circulating YKL-40 levels. Three SNPs in the *CHI3L1* gene (rs4950928, rs10399931, and rs872129) and one SNP in the *TRIB1* gene (rs6982502) were significantly associated with circulating YKL-40 levels after stepwise conditional analysis. The associations of these SNPs with circulating YKL-40 levels were validated in the CAD cohort, except for that of rs872129 in the *CHI3L1* gene. The association between rs6982506 in the *TRIB1* gene and YKL-40 levels has not been reported in the literature. According to the TWB GWAS data, rs6982502 and rs28601761 exhibited low linkage disequilibrium (0.0864), indicating that rs6982502 is a novel SNP in the *TRIB1* gene associated with circulating YKL-40 levels in Chinese populations. These results suggest genetic variations in YKL-40 levels according to ethnicity.

	1st tertile (N = 173)	2nd tertile (N = 174)		3rd tertile (N = 174)	
All-cause mortality					
Number of events	**3**	**12**		**31**	
		HR (95% CI)	***p* value**	**HR (95% CI)**	***p* value**
Model 1	Reference	3.93 (1.11–13.91)	0.034	11.78 (3.60–38.55)	4.50×10^{-5}
Model 2	Reference	3.05 (0.86–10.90)	0.085	6.78 (2.02–22.78)	0.002
Model 3	Reference	2.90 (0.81–10.90)	0.102	5.98 (1.77–20.25)	0.004
Model 4	Reference	2.81 (0.79–10.07)	0.112	4.31 (1.25–14.88)	0.021
Model 5	Reference	2.52 (0.70–9.14)	0.159	4.20 (1.20–14.70)	0.025
MACEs					
Number of events	**13**	**29**		**50**	
		HR (95% CI)	***p* value**	**HR (95% CI)**	***p* value**
Model 1	Reference	2.25 (1.17–4.33)	0.015	4.65 (0.53–8.57)	8.07×10^{-7}
Model 2	Reference	1.93 (0.99–3.74)	0.051	3.42 (1.81–6.47)	1.54×10^{-4}
Model 3	Reference	1.78 (0.92–3.41)	0.088	2.94 (1.55–5.59)	0.001
Model 4	Reference	1.77 (0.91–3.45)	0.092	2.57 (1.34–4.95)	0.005
Model 5	Reference	1.61 (0.82–3.15)	0.169	2.39 (1.22–4.68)	0.011

Table 1. Cox regression of all-cause mortality and MACEs between YKL-40 level tertiles. Model 1: Unadjusted. Model 2: Adjusted for age, sex, BMI, and smoking status. Model 3: Adjusted for age, sex, BMI, smoking status, diabetes mellitus, hypertension, and dyslipidaemia. Model 4: Adjusted for age, sex, BMI, smoking status, diabetes mellitus, hypertension, dyslipidaemia, and CRP levels. Model 5: Adjusted for age, sex, BMI, smoking status, diabetes mellitus, hypertension, dyslipidaemia, and eGFR. HR, hazard ratio; CI, confidence interval; MACEs, major adverse cardiac events; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

Associations of CHI3L1 and TRIB1 genetic variants with CAD outcomes

We calculated the WGRSs of the four candidate SNPs (rs4950928, rs10399931, rs872129, and rs6982502) in the CAD cohort and evaluated the prognostic value of the combined *CHI3L1* and *TRIB1* WGRSs for long-term outcomes. The combined *CHI3L1* and *TRIB1* WGRSs did not affect all-cause mortality or MACEs in patients with CAD. These results were consistent with reports indicating that genetic variants of *CHI3L1* were significantly associated with circulating YKL-40 levels but not with the prevalence or severity of CAD[21,22] and did not predict future cardiovascular events in healthy individuals[25]. In the present study, the four lead SNPs of the *CHI3L1* and *TRIB1* genes explained 14.4% of the variation in YKL-40 levels in the TWB cohort, while these SNPs explained only 9.2% of the variation in YKL-40 levels in the CAD cohort (Supplementary Table S4). It is reasonable that the impact of these candidate variants on circulating YKL-40 levels is not strong enough to translate into poorer long-term outcomes in patients with CAD, despite their significant association with YKL-40 levels. Furthermore, the diminished effect of these candidate variants on circulating YKL-40 levels in the CAD cohort may also explain why circulating YKL-40 levels, but not the lead SNPs *CHI3L1* and *TRIB1*, predict the long-term outcome of angiographically confirmed CAD. This was the first study to illustrate the null impact of genetic variants of *CHI3L1* and *TRIB1* on long-term outcomes in patients with CAD.

Association of YKL-40 levels with CAD outcomes

As an inflammatory biomarker, YKL-40 is involved in vascular smooth muscle cell differentiation, migration, and proliferation[10,18]; promotes chemotaxis and migration of vascular endothelial cells; and contributes to atherosclerotic plaque formation and vascular occlusion[13,19]. Elevated circulating YKL-40 levels are linked to the presence of CAD[20–22], lesion progression[40], and disease severity in patients with CAD[23,24]. In the CLARICOR trial, Schroder et al. identified an independent association between elevated YKL-40 levels and the risk of adverse cardiovascular outcomes and all-cause mortality in patients with stable CAD after 10 years of follow-up[26]. This association remained significant after adjustment for CRP levels and baseline cardiovascular risk factors according to Cox proportional hazards regression models. Our results agree with those of the CLARICOR trial, which demonstrated poorer clinical outcomes and higher event rates in patients in the upper tertile of YKL-40 levels. The prognostic effect of YKL-40 levels on the long-term outcomes of patients with CAD was also independent of patient baseline characteristics, conventional cardiovascular risk factors, CRP levels or eGFR. Kaplan–Meier survival curve analysis was also conducted to assess the prognostic effect of YKL-40 levels on long-term outcomes, and the results demonstrated a lower rate of freedom from all-cause mortality and MACEs in patients with CAD in the upper YKL-40 tertile.

The prognostic effect of YKL-40 on clinical outcomes was evident only in patients with multivessel CAD, and participants in the upper YKL-40 tertile had the worst prognosis during long-term follow-up (4.41 times greater risk of all-cause mortality and 2.39 times greater risk of MACEs, compared with patients in the bottom tertile). This difference in prognosis remained significant after adjustment for patient baseline characteristics, conventional cardiovascular risk factors, CRP levels or eGFR. Unlike the CLARICOR trial, which showed no improvement of risk prediction after addition of YKL-40 in patients with stable CAD, our results demonstrated

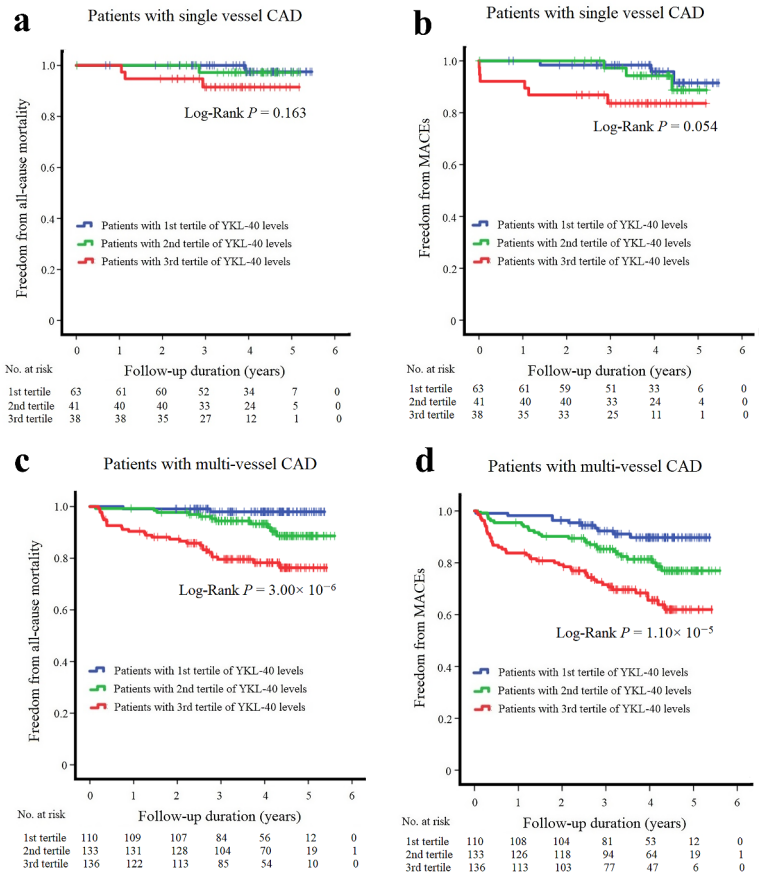

Fig. 3. Kaplan–Meier curve analysis of YKL-40 levels and long-term outcomes in patients with single-vessel and multivessel coronary artery disease (CAD). (**a**, **b**) For patients with single-vessel CAD, all-cause mortality and major adverse cardiac event rates do not differ significantly among individuals with different tertiles of YKL-40. (**c**, **d**) For patients with multivessel CAD, higher levels of YKL-40 significantly predicted poorer clinical outcomes.

a significant improvement of prediction power on long-term outcomes in patients with multivessel CAD after addition of YKL-40 tertiles to conventional CV risk factors and CRP levels. The CLARICOR trial enrolled stable patients with clinical diagnosis of myocardial infarction or angina pectoris using ICD codes. Patients with myocardial infarction or unstable angina within 3 months, patients having percutaneous coronary intervention or coronary bypass surgery within 6 months, and patients with impaired renal or hepatic function were excluded from the study. Contrast to the CLARICOR trial, our study enrolled more complex patients with advanced disease, and all patients had angiographically confirmed CAD. Our results showed YKL-40 tertile only improved the prediction power of long-term outcomes in patients with multivessel CAD, which might not be seen in relative stable patients with less disease severity enrolled in the CLARICOR trial. The cut-off YKL-40 level for predicting all-cause mortality and MACEs in patients with multivessel CAD was 130.50 ng/mL. In contrast, YKL-40 did not affect long-term outcomes in patients with single-vessel CAD. In these patients, long-term outcomes were more favourable, with lower event rates for all-cause mortality and MACEs than those of patients with multivessel CAD ($p = 0.009$ for all-cause mortality, $p = 0.001$ for MACEs; Supplementary Fig. S4). YKL-40 levels were also significantly lower in this group (median level 67.02 ng/mL for single-vessel CAD vs. 83.98 ng/mL for multivessel CAD, $p = 0.007$). With the improvement in guideline-directed medical therapy, the impact of YKL-40 on long-term outcomes may have diminished or been obscured in patients with single-vessel CAD, which has lower event rates.

Limitations

Although a large sample of individuals with available GWAS data was recruited for the TWB cohort, the sample in the CAD cohort was small. However, the candidate variants in the *CHI3L1* and *TRIB1* genes that were associated with YKL-40 levels were consistent between the two groups, providing strong evidence of the relevance of these *CHI3L1* and *TRIB1* genetic variants in the Han Chinese population. The event rates of individual MACE endpoints were relatively low, which can be attributed to the small sample size in the CAD cohort and advancements in guideline-directed medical therapy. This precluded significant analysis of the prognostic value of YKL-40 levels for each individual endpoint. However, YKL-40 levels significantly predicted all-cause mortality and MACEs during long-term follow-up in patients with CAD. This was especially true in

	1st tertile (N = 110)	2nd tertile (N = 133)		3rd tertile (N = 136)	
All-cause mortality					
Number of events	**2**	**11**		**28**	
		HR (95% CI)	***p* value**	**HR (95% CI)**	***p* value**
Model 1	Reference	4.49 (0.99–20.25)	0.051	12.97 (3.09–54.44)	4.65×10^{-4}
Model 2	Reference	3.43 (0.76–15.57)	0.111	7.04 (1.63–50.33)	0.009
Model 3	Reference	3.32 (0.73–15.11)	0.121	6.36 (1.46–27.64)	0.014
Model 4	Reference	3.20 (0.70–14.54)	0.133	4.41 (1.00–19.54)	0.050
Model 5	Reference	3.10 (0.68–3.15)	0.169	4.57 (1.02–20.47)	0.047
MACEs					
Number of events	**10**	**26**		**44**	
		HR (95% CI)	***p* value**	**HR (95% CI)**	***p* value**
Model 1	Reference	2.21 (1.07–4.59)	0.033	4.33 (2.18–8.60)	2.90×10^{-5}
Model 2	Reference	1.92 (0.92–4.00)	0.083	3.17 (1.55–6.48)	0.002
Model 3	Reference	1.81 (0.87–3.80)	0.115	2.81 (1.37–5.76)	0.005
Model 4	Reference	1.80 (0.86–3.77)	0.121	2.39 (1.15–4.98)	0.020
Model 5	Reference	1.65 (0.78–3.47)	0.190	2.22 (1.05–4.71)	0.037

Table 2. Cox regression of all-cause mortality and MACEs between YKL-40 level tertiles in patients with multivessel CAD. Model 1: Unadjusted. Model 2: Adjusted for age, sex, BMI, and smoking status. Model 3: Adjusted for age, sex, BMI, smoking status, diabetes mellitus, hypertension, and dyslipidaemia. Model 4: Adjusted for age, sex, BMI, smoking status, diabetes mellitus, hypertension, dyslipidaemia, and CRP levels. Model 5: Adjusted for age, sex, BMI, smoking status, diabetes mellitus, hypertension, dyslipidaemia, and eGFR. CAD, coronary artery disease; HR, hazard ratio; CI, confidence interval; MACEs, major adverse cardiac events; BMI, body mass index; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate.

patients with multivessel CAD. Nevertheless, scientific studies enrolling large samples of patients with CAD are necessary to verify these findings. Finally, because we included only individuals of Han Chinese ethnicity, our results cannot be generalized to other ethnic groups.

Conclusion

In this study, we demonstrated that three *CHI3L1* lead SNPs (rs4950928, rs10399931, and rs872129) and one *TRIB1* lead SNP (rs6982502) were strongly associated with YKL-40 levels in Han Chinese individuals. These findings were confirmed in the CAD cohort, except for the association between rs872129 at the *CHI3L1* gene and YKL-40 levels. The combined *CHI3L1* and *TRIB1* WGRSs did not influence clinical outcomes in patients with CAD. Conversely, circulating YKL-40 levels were a strong predictor of poor clinical outcomes in patients with CAD, and participants in the upper tertile of YKL-40 levels had the highest rates of all-cause mortality and MACEs after long-term follow-up. YKL-40 levels predicted poorer clinical outcomes in patients with multivessel CAD but not in patients with single-vessel CAD. This difference in prognosis remained significant after further adjustment for traditional cardiovascular risk factors, CRP levels or eGFR, suggesting that YKL-40 levels independently affect long-term outcomes in patients with CAD. This study provides further insight into the effects of biomarkers and corresponding genetic variants on the prognostication of clinical outcomes in patients with CAD. Studies including more patients with CAD of different ethnicities are necessary to verify these findings.

Data availability

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

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Author contributions
HHC and YLK conceived and designed the study. JMJ, FTC and YLK were responsible for data extraction. MST and SW carried out the experiments. HHC, IST and YLK participated in data analysis. HHC and YLK wrote the manuscript and obtained funding. All authors have read and approved the final version of the manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

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