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LncRNA-TUG1 as a potential diagnostic biomarker for coronary atherosclerotic heart disease

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Abstract

Objectives: Accumulating evidence suggests that people living in cold regions have a higher risk of developing coronary atherosclerotic heart disease (CHD). Long non-coding RNAs (lncRNAs) have been implicated in the pathogenesis and treatment of a variety of diseases. The present study aimed to investigate the serum level of lncRNA-taurine upregulated gene 1 (TUG1) in patients with CHD and assess its potential as a diagnostic biomarker. This study aimes to investigate the serum level of lncRNA-TUG1 in patients with CHD and assess its potential as a diagnostic biomarker. **Methods:** The Gene Expression Omnibus (GEO) database was employed to identify the potential lncRNAs serving as biomarkers for CHD. To validate lncRNA-TUG1, 232 subjects were enrolled in both test and diagnostic cohorts. Serum lncRNA-TUG1 levels were measured by RT-qPCR. The association between lncRNA-TUG1 levels and CHD severity was analyzed using Pearson's correlation test. Diagnostic value was assessed by receiver operating characteristic (ROC) curve analysis and compared with established cardiac biomarkers. **Results:** LncRNA-TUG1 was identified in the GEO database as a potential biomarker for CHD. Serum lncRNA-TUG1 levels were significantly higher in CHD patients compared with healthy controls and non-CHD patients. CRP levels also differed between CHD and non-CHD groups, while other biomarkers showed no significant differences. ROC curve analysis demonstrated that lncRNA-TUG1 could distinguish CHD from non-CHD patients, with an area under the curve (AUC) of 0.8916, which was higher than that of conventional biomarkers such as cTnI. At a cut-off value of 2.311, the sensitivity and specificity of lncRNA-TUG1 were 61.63% and 97.67%, respectively, surpassing the diagnostic performance of cTnI. Furthermore, lncRNA-TUG1 levels in CHD patients were positively correlated with SYNTAX scores from coronary angiography and increased with the severity of vascular stenosis. **Conclusion:** Elevated serum lncRNA-TUG1 levels in CHD patients suggest that lncRNA-TUG1 may serve as a novel and valuable diagnostic biomarker for CHD, with potential utility in differentiating CHD from other cardiac diseases.

Keywords

coronary atherosclerotic heart disease; long non-coding RNAs-taurine upregulated gene 1; biomarker

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1 Introduction

Numerous studies have demonstrated that long-term exposure to low ambient temperatures increases the risk of plaque rupture, thrombosis, myocardial ischemia, and even sudden death, ultimately leading to adverse cardiovascular events such as coronary atherosclerotic heart disease (CHD). CHD is one of the most prevalent cardiovascular diseases and remains a leading cause of mortality worldwide[1]. Its occurrence is closely associated with risk factors including hypertension, diets rich in fat and sugar, smoking, and other lifestyle or environmental contributors[2]. Despite advances in both pharmacological therapies and surgical interventions, the prognosis of CHD remains unsatisfactory, with persistently high mortality. One key reason for this is the lack of highly sensitive, specific, and convenient biomarkers for early diagnosis. Currently, coronary angiography is considered the gold standard for diagnosing CHD; however, its invasiveness and high cost limit its routine clinical application. Thus, there is an urgent

need to identify non-invasive and highly effective biomarkers for early detection and risk stratification of CHD.

Emerging evidence indicates that long non-coding RNAs (lncRNAs) circulating in blood are involved in the onset and progression of numerous diseases, including cardiovascular disorders[3-4]. LncRNAs are a class of non-coding RNAs longer than 200 nucleotides that are relatively stable in the bloodstream and play regulatory roles in diverse pathological processes[5]. Taurine upregulated gene 1 (TUG1), located on chromosome 22q12, has been reported to regulate the occurrence and development of tumors[6], osteoporosis[7], acute lung injury, and other diseases[8]. Recent studies further suggest that lncRNA-TUG1 participates in cardiovascular pathophysiology, including overexpression in atherosclerotic endothelial cells and exerting protective effects against ischemic myocardial injury[9-10]. Collectively, these findings highlight the important role of lncRNA-TUG1 in cardiovascular disease.

In the present study, we identified lncRNA-TUG1 as a potential diagnostic biomarker for CHD using the gene expression omnibus (GEO) database. We then evaluated serum lncRNA-TUG1 levels in CHD patients and assessed its diagnostic value, proposing lncRNA-TUG1 as a promising non-invasive biomarker for CHD.

2 Material and methods

2.1 Patient selection and data collection

This study was approved by the Ethics Committee of the Fourth Affiliated Hospital of Harbin Medical University (2023-Ethical Review-49), and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participant. Patients over 18 years of age who were prospectively admitted to the Department of Cardiology at the Fourth Affiliated Hospital of Harbin Medical University between March 2019 and May 2023 were considered for inclusion. Exclusion criteria included recent or active acute/chronic infectious disease, pulmonary disease, diabetes mellitus, hepatic or renal disease, malignancy, and autoimmune disorders

Before initiation of any treatment, venous blood samples were collected. Laboratory measurements included lactate dehydrogenase (LDH), creatine kinase (CK), creatine kinase-MB (CK-MB), alpha-hydroxybutyrate dehydrogenase (HBDH), aspartate aminotransferase (AST), cardiac troponin I (cTnI), N-terminal pro-B-type natriuretic peptide precursor (NT-proBNP), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-c), homocysteine (HCY), and C-reactive protein (CRP). For RNA extraction, 750 μL of TRIzol LS reagent was added to each 250 μL serum sample, which was then stored at -80 °C until analysis. Coronary angiography was performed for diagnostic confirmation.

By May 2023, a total of 60 subjects had been included in the test cohort, consisting of 30 patients with CHD and 30 healthy controls[11]. An additional 172 subjects were included in the diagnostic cohort, comprising 86 patients with CHD and 86 non-CHD patients, as confirmed by coronary angiography[12] (Fig. 1). The diagnostic cohort sample size was determined by calculating diagnostic sensitivity and specificity in the test cohort[13].

2.2 Coronary angiography and SYNTAX score

Coronary angiography was performed *via* radial artery access using the Judkins technique. Angiographic results were independently evaluated by two experienced interventional cardiologists, each blinded to patient information and to the other's assessments. The diagnosis of CHD was made according to American College of Cardiology (ACC) and American Heart Association (AHA) guidelines, defined as > 50% stenosis in at least one major coronary artery (left main trunk, left anterior descending branch, circumflex artery, and right coronary artery). The SYNTAX score was calculated using the SYNTAX score 2.28 calculator (available at www.SYNTAXscore.com).

2.3 Clinical parameter

Demographic and clinical characteristics included age, sex, and coronary risk factors such as hypertension, diabetes mellitus, hyperlipidemia, and smoking status. Hypertension was defined as blood pressure ≥ 140/90 mmHg or current use of antihypertensive medication. Diabetes mellitus was defined as fasting blood glucose ≥ 7.0 mmol/L or a physician's diagnosis requiring dietary modification or antidiabetic therapy. Hyperlipidemia was defined as TC ≥ 6.2 mmol/L and/or TG ≥ 2.3 mmol/L and/or LDL-c ≥ 4.1 mmol/L, or current use of lipid-lowering therapy. Smoking was defined as current or prior consumption of ≥ 10 cigarettes per day for at least 2 years.

Serum samples were collected for biochemical analyses. Concentrations of cTnI and NT-proBNP were measured using an automated biochemical analyzer with original reagents (Vitros® 5600, Johnson Ltd., New Jersey, USA). LDH, CK, CK-MB, HBDH, AST, TC, TG, HCY, and LDL-c were measured with an automated biochemical analyzer (AU5821, Beckman Ltd., CA, USA) and original reagents. CRP levels were determined using an automated hematology analyzer (BC-760CS, Mindray, Shenzhen, China) with original reagents.

2.4 RNA isolation

Fasting venous blood (5 mL) was collected from each subject. After standing for 30 min, samples were centrifuged at 3000 rpm for 8 min. Serum supernatant was separated, and 750 μL of

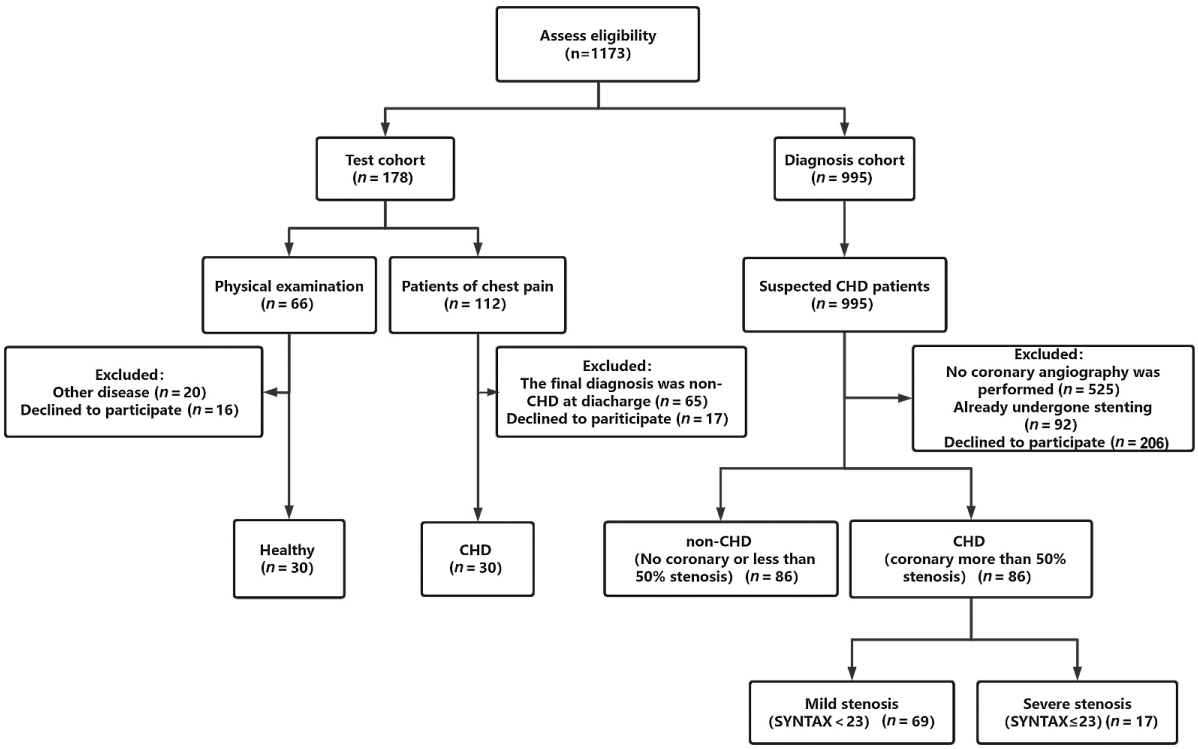

Fig. 1 Flow diagram illustrating the identification and enrollment of study participants

From March 2019 to May 2023, patients admitted to the Department of Cardiology at the Fourth Affiliated Hospital of Harbin Medical University were screened according to inclusion and exclusion criteria. A total of 60 subjects were included in the test cohort (30 coronary atherosclerotic heart disease patients and 30 healthy controls). An additional 172 subjects were enrolled in the diagnostic cohort, comprising 86 CHD patients and 86 non-CHD patients confirmed by coronary angiography. CHD, coronary atherosclerotic heart disease.

TRIzol LS reagent (Invitrogen, Carlsbad, CA, USA) was added to 250 μL of serum to extract total RNA according to the manufacturer's instructions. RNA purity and concentration were assessed with a NanoReady spectrophotometer (FC-1100, Suizhen, Hangzhou, China).

2.5 Reverse transcription (RT) and quantitative (q) PCR

Complementary DNA (cDNA) was synthesized using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA). qPCR was performed with SYBR Green Master Mix (Roche, Penzberg, Germany) on a LightCycler 480 RT-PCR system (Roche, Penzberg, Germany). U6 was used as an internal control. Relative expression of lncRNA-TUG1 was calculated using the $2^{-\Delta\Delta Ct}$ method. All primers (Table 1) were synthesized by General Biotech (Anhui Province, China).

2.6 Bioinformatics analysis

CHD-related gene expression data were obtained from the GEO database (GSE71226 and GSE142008). Data analysis was conducted using the Sangerbox platform. The structural information of lncRNA-TUG1 was retrieved from the UCSC Genome Browser and Ensembl database.

2.7 Statistical analysis

Statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY, USA) and GraphPad Prism version 9 (GraphPad Software, San Diego, CA, USA). Continuous variables were expressed as mean ± standard deviation (SD), and categorical variables were expressed as frequencies and percentages. Comparisons of continuous variables were performed using the Student's *t*-test, while categorical variables were compared using the Chi-square test. For normally distributed data, one-way ANOVA followed by the least significant difference (LSD) post hoc test was applied. For non-normally distributed data, the Mann-Whitney *U* test was used.

Correlations between serum lncRNA-TUG1 levels and SYNTAX scores were analyzed using Pearson's correlation test. The

diagnostic performance of lncRNA-TUG1 and conventional cardiac biomarkers was assessed by receiver operating characteristic (ROC) curve analysis, with the area under the curve (AUC) calculated to evaluate diagnostic accuracy. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

3 Results

3.1 Screening of lncRNAs as potential diagnostic biomarkers for CHD in the GEO database

To identify reliable diagnostic biomarkers for CHD, we analyzed total RNA expression profiles in peripheral blood samples from healthy individuals and CHD patients using the GEO database (GSE71226). This analysis revealed 2122 differentially expressed genes (Fig. 2A). To further refine potential biomarkers relevant to therapeutic response, we analyzed mRNA expression in peripheral blood mononuclear cells from patients treated with Tongmai Yangxin Pill using another GEO dataset (GSE142008). A total of 1169 differentially expressed genes were identified (Fig. 2B).

After excluding genes without annotated names and integrating the datasets, 156 genes were found to be differentially expressed in both analyses. Among these, TUG1 attracted particular attention (Fig. 2C). Although TUG1 was initially detected as an mRNA, prior studies have demonstrated that it lacks protein-coding potential and instead functions as a lncRNA. With a length greater than 200 nucleotides, TUG1 exerts regulatory roles in transcription, translation, and other cellular processes (Fig. 2D).

Table 1 Sequences of primers

Primers	Forward (5'-3')	Reverse
LncRNA-TUG1	5'-TAGCAGTTCCCCAATCCTTG-3'	5'-CACAAATTCCCATCATTCCC-3'
U6	5'-CTCGCTTCGGCAGCACATATACT-3'	5'-ACGCTTCACGAATTTGCGTGTC-3'

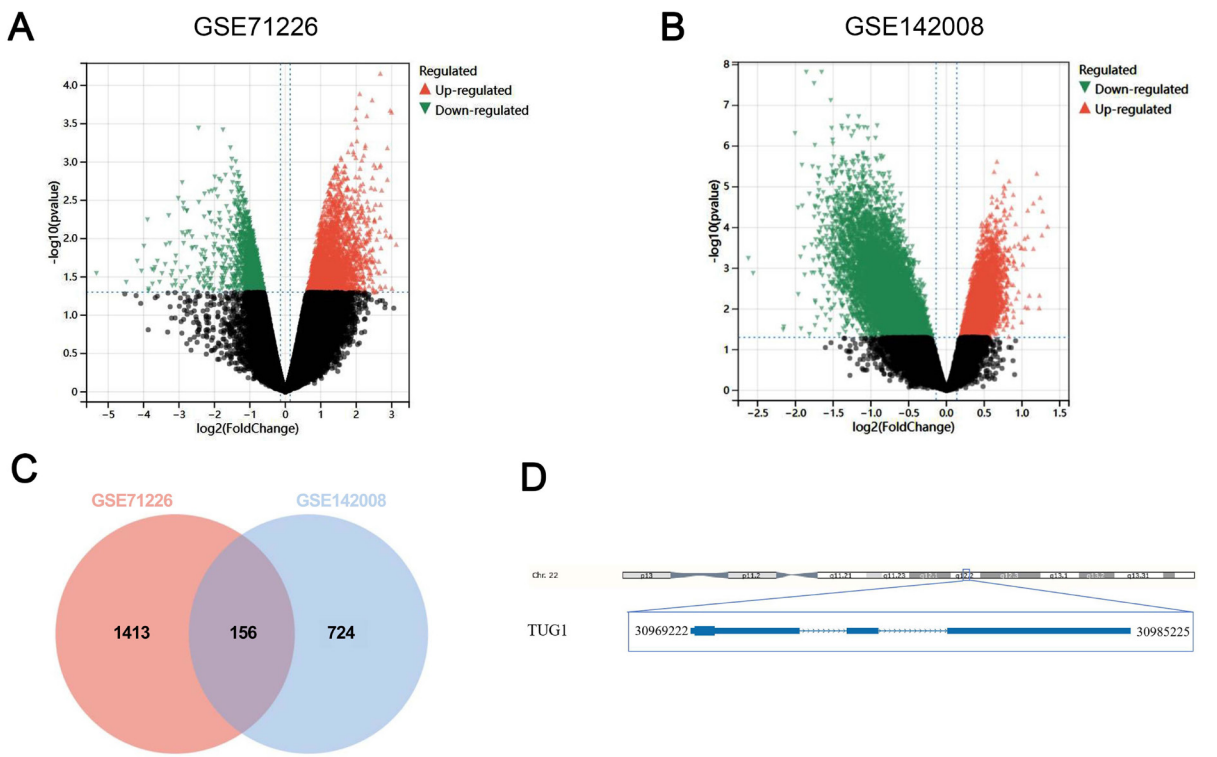

Fig. 2 Long non-coding RNA (lncRNA) screening for coronary atherosclerotic heart disease (CHD) using the Gene Expression Omnibus (GEO) database
(A) Volcano plot of differentially expressed genes in peripheral blood from CHD patients and healthy controls (GSE71226). (B) Volcano plot of differentially expressed genes in peripheral blood mononuclear cells from CHD patients before and after treatment with Tongmai Yangxin Pill (GSE142008). (C) Venn diagram showing 156 overlapping differentially expressed genes between GSE71226 and GSE142008 datasets, highlighting taurine upregulated gene 1 (TUG1). (D) Genomic location of TUG1, identified as a long non-coding RNA with potential diagnostic relevance in CHD.

Compared with other candidate genes, lncRNA-TUG1 possesses advantages such as relatively small molecular weight, structural stability, and previously reported involvement in disease mechanisms. Therefore, we selected lncRNA-TUG1 as a candidate diagnostic biomarker for CHD.

3.2 Analysis of clinical data

The clinical characteristics of all participants were summarized in Table 2. A total of 60 subjects were included in the test cohort. No significant differences were observed between CHD patients and healthy controls in terms of sex (P = 0.6023), smoking status (P = 0.2429), diabetes (P > 0.9999), hypertension (P = 0.0693), or LDL-c levels (P = 0.6691). However, systolic blood pressure (SBP; P = 0.0013), diastolic blood pressure (DBP; P = 0.0008), prevalence of hyperlipidemia (P = 0.0125), TG (P < 0.0001), and TC (P = 0.0319) were significantly higher in the CHD group compared with the healthy group. In the diagnostic cohort, no significant differences were found in age (P = 0.0541), sex (P = 0.3390), SBP (P = 0.9592), DBP (P = 0.6870), smoking (P = 0.0673), hypertension (P > 0.9999), diabetes (P > 0.9999), hyperlipidemia (P = 0.4115), LDL-c (P = 0.2056), TG (P = 0.2996), or TC (P = 0.1190) between CHD and non-CHD patients.

3.3 Diagnostic ability of LncRNA-TUG1 in CHD patients and healthy individuals

To evaluate the diagnostic potential of serum lncRNA-TUG1, we analyzed samples from 30 CHD patients and 30 healthy controls. Expression levels of lncRNA-TUG1 were significantly higher in CHD patients compared with healthy individuals (Fig. 3A). ROC curve analysis demonstrated that lncRNA-TUG1 effectively distinguished CHD patients from healthy controls, with an AUC of 0.9456 (95% CI: 0.8934-0.9976, P < 0.0001) (Fig. 3B). At the optimal cut-off point, the sensitivity and specificity were 90% (95% CI: 74.28%-96.54%) and 86.67% (95% CI: 70.43%-94.69%), respectively. These findings indicate that lncRNA-TUG1 is significantly elevated in CHD patients and may serve as a valuable candidate biomarker for CHD.

3.4 Analysis of LncRNA-TUG1 in CHD and non-CHD patients

To further validate the diagnosis efficiency of lncRNA-TUG1 in CHD patients, we enrolled a diagnostic cohort consisting of 172 subjects, including 86 non-CHD patients and 86 CHD patients. The sample size was determined based on the specificity and sensitivity obtained from the test cohort.

Compare with non-CHD patients, CHD patients exhibited significantly higher serum lncRNA-TUG1 levels (P < 0.0001; Fig. 4A). When CHD patients were stratified according to SYNTAX score, those with SYNTAX score ≥ 23 showed a more pronounced increase in lncRNA-TUG1 expression compared with the SYNTAX < 23 group (P = 0.0141; Fig. 4B). However, no significant differences in lncRNA-TUG1 expression were observed between patients with single-vessel coronary stenosis and those with multi-vessel stenosis (P = 0.6754; Fig. 4C). These findings suggest that lncRNA-TUG1 expression is associated with the presence and severity of coronary stenosis, warranting further analysis of its correlation with angiographic indices.

3.5 Correlation analysis between LncRNA-TUG1 and SYNTAX score in CHD patients

To further explore the relationship between lncRNA-TUG1 expression and the severity of coronary artery disease (CAD), we analyzed correlations with SYNTAX scores obtained from coronary angiography. In CHD patients overall, serum

Table 2 Clinical characteristics of each group

Clinical characteristics	Test cohort			Diagnosis cohort		
	Healthy	CHD	*P* value	Control	CHD	*P* value
Age (year)	58 ± 7	66 ± 10	0.0055	69 ± 13	65 ± 10	0.0541
Gender (M/F)	30 (18/12)	30 (16/14)	0.6023	86 (52/34)	86 (59/27)	0.3390
SBP (mmHg)	120.5 ± 12.5	140.7 ± 17.3	0.0013	138.69 ± 22.16	137.61 ± 18.01	0.9592
DBP (mmHg)	73.5 ± 6.29	83.83 ± 10.0	0.0008	83.40 ± 14.28	82.70 ± 11.16	0.6870
Smoking (%)	10 (30.33)	6 (20.00)	0.2429	20 (23.53)	32 (37.21)	0.0673
Hypertension (%)	13 (43.33)	20 (66.67)	0.0693	45 (52.30)	45 (52.30)	> 0.9999
Diabetes (%)	9 (30.00)	9 (30.00)	> 0.9999	31 (36.05)	32 (37.21)	> 0.9999
Hyperlipidemia (%)	16 (53.33)	25 (83.33)	0.0125	30 (34.88)	24 (27.91)	0.4115
LDL-C (mmol/L)	1.90 ± 0.99	2.28 ± 1.32	0.6691	2.79 ± 1.07	2.56 ± 0.88	0.2056
TG (mmol/L)	1.61 ± 0.49	3.65 ± 1.48	< 0.0001	1.64 ± 0.92	1.75 ± 0.99	0.2996
TC (mmol/L)	3.76 ± 1.34	3.60 ± 1.10	0.0319	4.16 ± 1.49	4.44 ± 1.16	0.1190

CHD, coronary atherosclerotic heart disease; SBP, systolic blood pressure; DBP, diastolic blood pressure; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride.

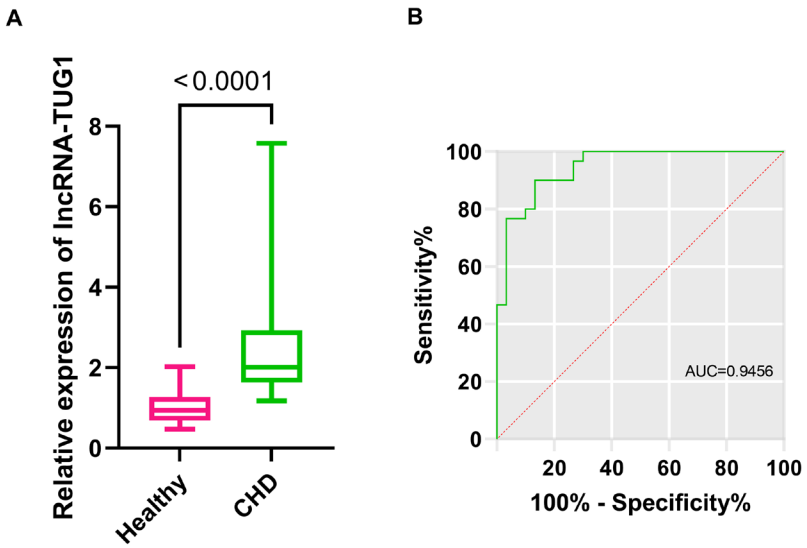

Fig. 3 Diagnostic ability of long non-coding RNA (lncRNA)-taurine upregulated gene 1 (TUG1) in the test cohort

(A) Serum lncRNA-TUG1 expression levels in patients with coronary atherosclerotic heart disease (CHD) compared with healthy controls. (B) Receiver operating characteristic (ROC) curve analysis demonstrating the diagnostic performance of lncRNA-TUG1 for distinguishing CHD patients from healthy individuals.

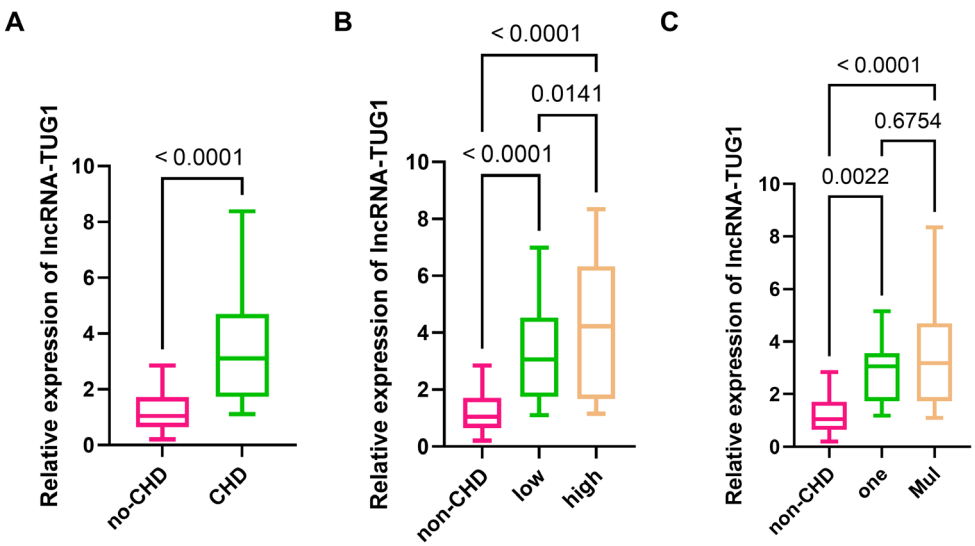

Fig. 4 Analysis of long non-coding RNA (lncRNA)-taurine upregulated gene 1 (TUG1) in the diagnosis cohort

(A) Serum lncRNA-TUG1 levels in non-coronary atherosclerotic heart disease (non-CHD) patients and CHD patients. (B) Serum lncRNA-TUG1 levels in non-CHD patients, CHD patients with mild stenosis (SYNTAX score < 23), and CHD patients with severe stenosis (SYNTAX score ≥ 23). (C) Serum lncRNA-TUG1 levels in non-CHD patients, CHD patients with single-vessel stenosis, and CHD patients with multi-vessel stenosis.

lncRNA-TUG1 levels were significantly correlated with SYNTAX scores (r = 0.4067, P = 0.0001; Fig. 5A). When stratified by stenosis severity, lncRNA-TUG1 levels in patients with mild stenosis (SYNTAX score < 23) showed a moderate positive correlation with SYNTAX scores (r = 0.4558, P < 0.0001; Fig. 5B). In contrast, in patients with severe stenosis (SYNTAX score ≥ 23), the correlation between lncRNA-TUG1 and SYNTAX scores was weak and not statistically significant (r = 0.2077, P = 0.4237; Fig. 5C). These findings suggest that lncRNA-TUG1 may have greater diagnostic value for CHD patients with mild stenosis, whereas its predictive power diminishes in patients with advanced disease.

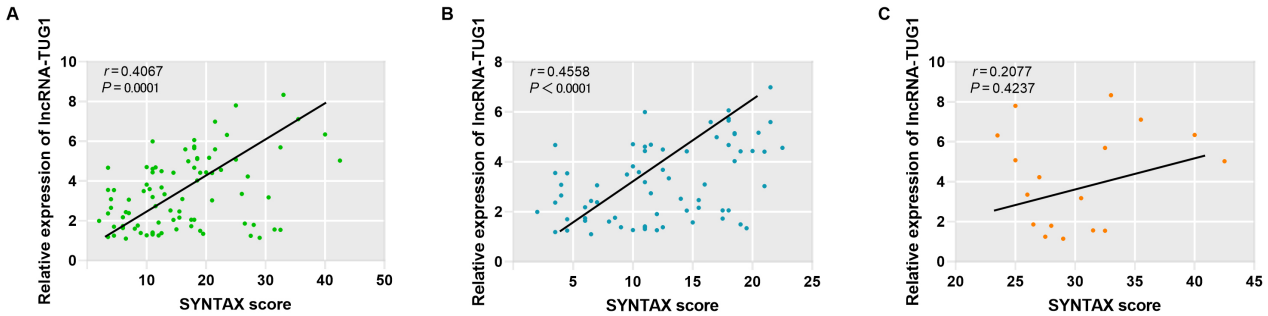

Fig. 5 Correlation analysis between long non-coding RNA (lncRNA)-taurine upregulated gene 1 (TUG1) expression and SYNTAX score in coronary atherosclerotic heart disease (CHD) patients

(A) Correlation between serum lncRNA-TUG1 levels and SYNTAX score in all CHD patients. (B) Correlation between lncRNA-TUG1 expression and SYNTAX score in CHD patients with mild stenosis (SYNTAX score < 23). (C) Correlation between lncRNA-TUG1 expression and SYNTAX score in CHD patients with severe stenosis (SYNTAX score ≥ 23).

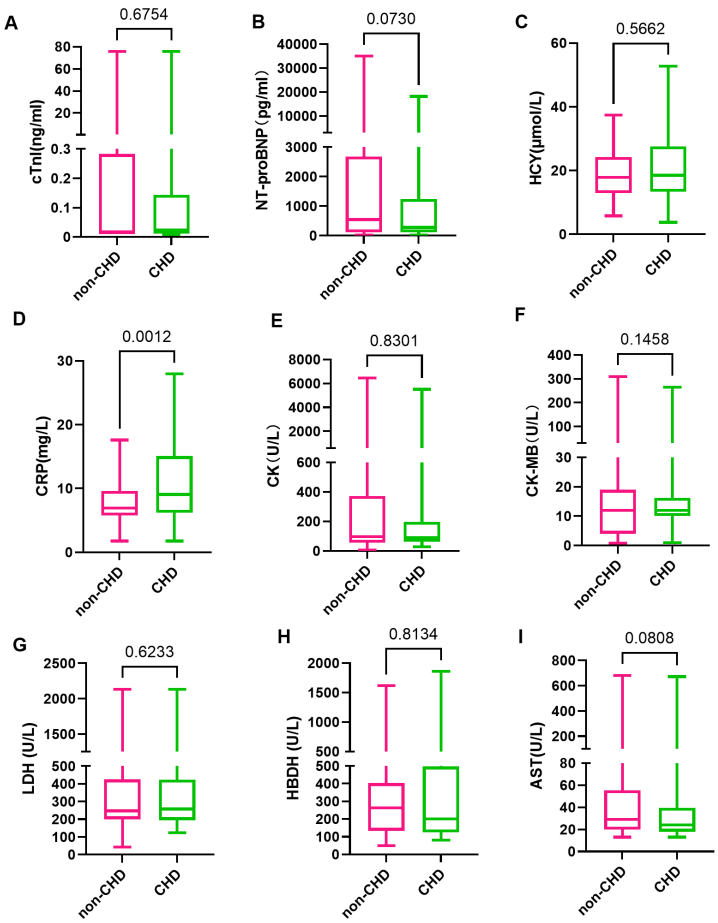

Fig. 6 Serum levels of conventional cardiac disease biomarkers in the diagnostic cohort

Box plots showing the distribution of (A) cTnI, (B) NT-proBNP, (C) HCY, (D) CRP, (E) CK, (F) CK-MB, (G) LDH, (H) HBDH, and (I) AST in patients with CHD compared with non-CHD patients. Among these biomarkers, only CRP was significantly elevated in CHD patients, while no significant differences were observed for the others. cTnI, cardiac troponin I; NT-proBNP, N-terminal pro-B-type natriuretic peptide precursor; HCY, Homocysteine; CRP, C-reactive protein; CK, creatine kinase; LDH, lactate dehydrogenase; HBDH, alpha-hydroxybutyrate dehydrogenase; AST, aspartate aminotransferase; CHD, coronary atherosclerotic heart disease.

3.6 Analysis of diagnostic efficacy of LncRNA-TUG1 for CHD

We compared serum levels of established cardiac biomarkers—including cTnI, NT-proBNP, HCY, CRP, and myocardial enzymes (CK, CK-MB, LDH, HBDH, AST)—between CHD and non-CHD patients. Among these, only CRP was significantly elevated in CHD patients (P = 0.0012; Fig. 6D). No significant differences were observed for cTnI (P = 0.6754; Fig. 6A), NT-proBNP (P = 0.0730; Fig. 6B), HCY (P = 0.5662; Fig. 6C), CK (P = 0.8301; Fig. 6E), CK-MB (P = 0.1458; Fig. 6F), LDH (P = 0.6233; Fig. 6G), HBDH (P = 0.8134; Fig. 6H), or AST (P = 0.0808; Fig. 6I).

ROC curve analysis was then performed to assess the diagnostic performance of lncRNA-TUG1 compared with conventional cardiac biomarkers. LncRNA-TUG1 demonstrated the highest diagnostic accuracy, with an AUC of 0.8916 (95% CI 0.8469-0.9362; P < 0.0001; Fig. 7A). In contrast, the AUC values for cTnI , NT-proBNP, CRP, HCY, LDH, HBDH, CK, AST, CK-MB were lower: 0.5177 (95% CI 0.3876-0.6476; P = 0.7909), 0.5792 (95% CI 0.4935-0.6648; P = 0.0730), 0.6423 (95% CI 0.5600-0.7246; P = 0.0013), 0.5254 (95% CI 0.4385-0.6123; P = 0.5648), 0.5218 (95% CI 0.4349-0.6086; P = 0.6220), 0.5105 (95% CI 0.4232-0.5977; P = 0.8124), 0.5095 (95% CI 0.4221-0.5970; P = 0.8291), 0.5771 (95% CI 0.4918-0.6623; P = 0.0809), 0.564 3 (95% CI 0.4762-0.6524; P = 0.1453), respectively (Fig. 7A).

At the optimal cut-off value of 2.311, lncRNA-TUG1 showed a specificity of 97.67% (95% CI 91.91%-99.59%) and a sensitivity of 61.63% (95% CI 51.06%-71.2%) (Fig. 7B). In comparison, other cardiac biomarkers demonstrated lower sensitivity and specificity at their respective cut-off points (Fig. 7C-K).

Table 3 summarizes the diagnostic characteristics of lncRNA-TUG1 at different thresholds. At the best cut-off (2.311), the sensitivity was 61.63% and the specificity was 97.67% for distinguishing CHD from non-CHD. At a higher cut-off (8.110), lncRNA-TUG1 achieved near-perfect specificity (100%; 95% CI: 95.72%-100%) but very low sensitivity (1.16%; 95% CI: 0.060%-6.300%).

These findings suggest that lncRNA-TUG1 provides superior diagnostic efficacy for CHD compared with conventional cardiac disease biomarkers, particularly when using the optimal cut-off value.

4 Discussion

CHD remains the leading cause of mortality among cardiovascular diseases, particularly in populations exposed to cold temperatures. Early-stage CHD, if undiagnosed or untreated, can progress to myocardial infarction (MI), leading to irreversible myocardial damage, ventricular remodeling, and eventually congestive heart failure (CHF)[14]. Currently available diagnostic tools—including coronary angiography (CAG), routine electrocardiography (ECG), Holter monitoring, treadmill exercise testing (TET), and coronary computed tomography angiography (CTA)—are helpful but limited by invasiveness, cost, or reduced sensitivity[15-17]. Inflammatory biomarkers such as CRP, serum amyloid A, complement, interleukin-6, tumor necrosis factor-α, and myeloperoxidase have been explored as potential diagnostic indicators, but their validity remains debatable[18]. Despite advances in medical technology, there remains an unmet need for non-invasive and convenient biomarkers for the early detection of CHD.

In recent years, molecular biomarkers have attracted attention as non-invasive diagnostic tools. Their relatively small molecular size, high stability, and early release during disease progression make them promising candidates for both diagnosis and therapeutic targeting[19-22]. Several lncRNAs have already been implicated in CHD. For instance, lncRNA-ANRIL regulates cellular proliferation and apoptosis by targeting miR-181b and activating NF-κB signaling, thereby influencing inflammation and CHD progression[23]. Similarly, lncRNA-MALAT1 inhibits autophagy and apoptosis of endothelial progenitor cells *via* activation of the mTOR pathway, thereby promoting cell survival in CHD[24]. Other non-coding RNAs (ncRNAs), such as miR-10a (downregulated), lncRNA-GAS5 (upregulated), and lncRNA-H19 (upregulated), have shown diagnostic potential in CAD, with high AUC values reported[25]. However, many of these studies compared patients with CAD to healthy individuals only, which may overestimate diagnostic performance.

LncRNA-TUG1 has been implicated in multiple cardiovascular processes, including vascular endothelial injury and myocardial ischemia. Experimental evidence shows that lncRNA-TUG1 is upregulated in ox-LDL-treated human umbilical vein endothelial cells (HUVECs) and modulates vascular injury through the Runx2/ANPEP axis[26]. It has also been shown to accelerate atherosclerosis (AS) *via* the miR-141-3p/ROR2 axis[27] and regulate apolipoprotein expression through the miR-92a/FXR1 axis[28]. In animal models, lncRNA-TUG1 aggravated myocardial ischemia/reperfusion injury by promoting HMGB1/Rac1-mediated cardiomyocyte apoptosis[29]. Conversely, metformin may exert protective effects against atherosclerosis *via* activation of the AMPK/mTOR pathway through modulation of lncRNA-TUG1[30]. Collectively, these studies highlight the pivotal role of lncRNA-TUG1 in vascular injury, atheromatous

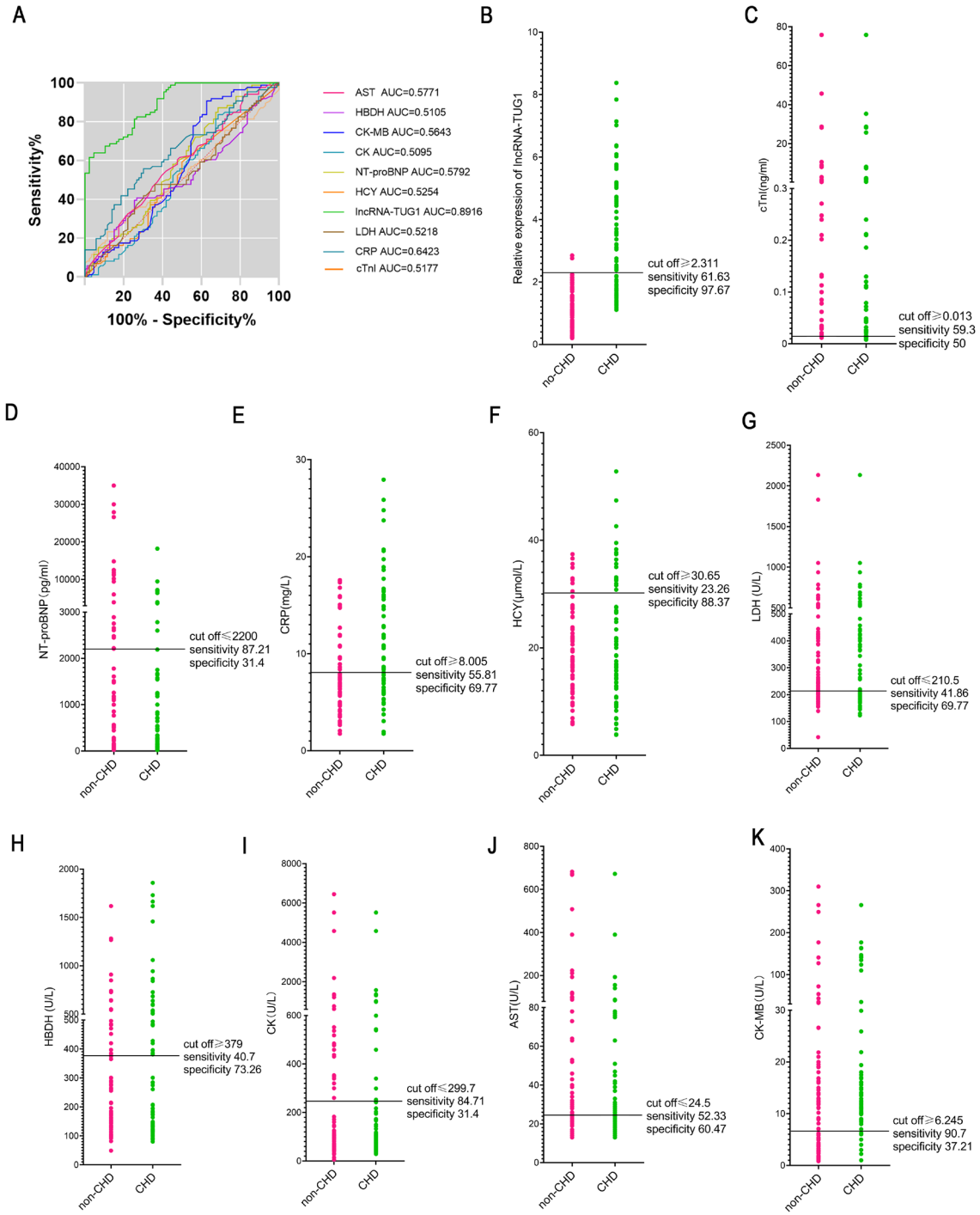

Fig. 7 Diagnostic efficacy of lncRNA TUG1 for CHD

(A) Receiver operating characteristic (ROC) curves comparing the predictive value of lncRNA-TUG1 with conventional cardiac disease biomarkers (cTnI, NT-proBNP, CRP, HCY, CK, CK-MB, LDH, HBDH, and AST). (B-K) Dot plots showing the distribution of lncRNA-TUG1 values in non-CHD and CHD patients, compared with the distribution of conventional cardiac biomarkers across outcome groups. lnc-RNA, long non-coding RNA; TUG1, taurine upregulated gene 1; CHD, coronary atherosclerotic heart disease; cTnI, cardiac troponin I; NT-proBNP, N-terminal pro-B-type natriuretic peptide precursor; HCY, Homocysteine; CRP, C-reactive protein; CK, creatine kinase; LDH, lactate dehydrogenase; HBDH, alpha-hydroxybutyrate dehydrogenase; AST, aspartate aminotransferase.

Table 3 Criterion values and coordinates of the receiver operating characteristic (ROC) curve for Long non-coding RNAs (lncRNAs)-taurine upregulated gene 1 (TUG1)

LncRNA-TUG1	Sensitivity	95%CI	Specificity	95%CI
> 0.217	100	95.720 to 100	1.163	0.060 to 6.296
> 1.548	82.560	73.200 to 89.140	73.260	63.050 to 81.470
> 2.311	61.630	51.060 to 71.200	97.670	91.910 to 99.590
> 8.110	1.163	0.060 to 6.296	100	95.720 to 100

plaque formation, vascular stenosis, and myocardial damage—all key mechanisms in CHD.

In our study, we confirmed that lncRNA-TUG1 expression was significantly elevated in CHD patients compared with both healthy individuals and non-CHD patients. Furthermore, lncRNA-TUG1 levels correlated with SYNTAX scores from coronary angiography, suggesting an association with the severity of coronary stenosis. Notably, conventional cardiac biomarkers such as cTnI and NT-proBNP showed no significant differences between CHD and non-CHD groups, and their ROC curve performance was inferior to that of lncRNA-TUG1. ROC analysis demonstrated that lncRNA-TUG1 exhibited high specificity and sensitivity, and its diagnostic performance could be adjusted by varying cut-off thresholds, making it particularly suitable for screening or differential diagnosis. Unlike conventional biomarkers that are generally elevated in multiple cardiac conditions, lncRNA-TUG1 was more specifically elevated in CHD patients. Combination with other biomarkers may further enhance diagnostic specificity without compromising sensitivity.

Nevertheless, several limitations must be acknowledged. First, this was a single-center study with a relatively small sample size, particularly for patients with severe stenosis, which may explain the lack of significant increase in lncRNA-TUG1 in this subgroup. Second, the diagnostic cohort consisted of patients undergoing coronary angiography, which inherently excluded individuals with mild symptoms deemed not to require invasive testing. This selection bias may have led to overestimation of specificity and underestimation of sensitivity. Third, while we focused on the diagnostic potential of lncRNA-TUG1, its value in monitoring disease progression or prognosis remains unclear. Finally, the underlying mechanisms of lncRNA-TUG1 upregulation in CHD were not explored and warrant further investigation.

In summary, our study demonstrates that lncRNA-TUG1 is significantly elevated in CHD patients and shows superior diagnostic performance compared with conventional cardiac biomarkers. These findings suggest that lncRNA-TUG1 may serve as a novel, non-invasive biomarker for CHD, providing new evidence to improve clinical diagnosis.

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Not applicable.

Research ethics

The study was approved by the Ethics Committee of the Fourth Affiliated Hospital of Harbin Medical University (2023-Ethical Review-49) and was conducted in accordance with ethical standards of the Declaration of Helsinki.

Informed consent

Informed consent was obtained from all the patients involved in this study.

Author contributions

Wang X designed and performed the experiments and drafted the manuscript. Ji X Y designed and performed the experiments and analyzed the data. Zhang S Y performed the experiments. Cai B Z conceived and supervised the study and revised the manuscript. Liu Y conceived and supervised the study and revised the manuscript. All authors read and approved the final manuscript.

Use of large language models, AI and machine learning tools

No large language models, AI or machine learning tool was used for any part of the present study.

Conflicts of Interests

The authors declare that they have no competing interests.

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Data availability

The original contributions presented in the study are included in the article and its supplementary material. Further inquiries can be directed to the corresponding authors.

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