Over-expression of IL1R2 in PBMCs of Patients with Coronary Artery Disease and Its Clinical Significance

ORIGINAL INVESTIGATION

ABSTRACT

Background: IL-1 has been widely explored and played a role in regulating inflammatory and immune responses to various disorders. Nevertheless, the role of interleukin-1 receptor type II, a protein-coding gene of interleukin-1 in coronary artery disease patients with peripheral blood mononuclear cells, persists to be undetermined.

Methods: Our study discovered the IL-1 receptor type II expression through gene expression omnibus (GEO) public repository based on bioinformatics tools and further validation was carried out between coronary artery disease patients and healthy participants using peripheral blood mononuclear cells samples in Second Hospital of Tianjin Medical University. A total of 180 participants, comprising 90 cases of coronary artery disease and 90 samples of healthy control were retrospectively evaluated and the correlation of IL-1 receptor type II was observed between serum levels of oxidized low-density lipoprotein and SYNTAX score. Furthermore, the clinical significance of IL-1 receptor type II was evaluated in peripheral blood mononuclear cells of coronary artery disease patients by the receiver operating curve using the area under the curve.

Results: IL-1 receptor type II was markedly overexpressed in peripheral blood mononuclear cells and severe patients with coronary artery disease compared to the healthy control participants. Meanwhile, a positive correlation of IL-1 receptor type II expression was significantly observed between SYNTAX score and oxidized low-density lipoprotein of coronary artery disease patients. Further, the receiver operating curve achieved a significantly higher area under the curve of 0.813 in coronary artery disease patients with peripheral blood mononuclear cells. Thus, IL-1 receptor type II expressions were not only directly correlated with peripheral blood mononuclear cells but also showed potential significance in coronary artery disease patients.

Conclusion: IL-1 receptor type II might be involved in the immune/inflammatory responses of coronary artery disease accompanied by other cytokine receptor genes.

Keywords: Coronary artery disease, cytokines, IL1R2, PBMCs, receptor

INTRODUCTION

Coronary artery disease (CAD), also known as coronary heart disease (CHD), is the most common type of heart disease reported worldwide.[1] In near future, it is expected to be the topmost cause of death across the world.[2,3] The pathological basis of CAD is myocardial ischemia and hypoxia, which are caused by coronary atherosclerosis. Meanwhile, coronary atherosclerosis has been linked to certain risk factors such as dyslipidemia, hypertension, hyperglycemia, and obesity.[4] Recently, chronic inflammation with low grades is found in CAD patients.[5] Many shreds of evidence showed that inflammation plays a key role in the pathogenesis of CAD.[6] Where chronic inflammation in the arterial walls leads to coronary atherosclerosis,[7] which finally progresses to CAD. The social consequences of CAD are growing, as the exploration of its mechanisms brooks no further delay.

Interleukin-1 receptor type II (IL1R2), a member of the interleukin-1 (IL-1) receptor family, acts as a negative immune regulator of the IL-1 system.[8] In many inflammatory diseases, IL1R2 is abnormally expressed. Likewise, Mora-Buch et al[9] reported that IL-1R2 was upregulated during remission of ulcerative colitis. Alshevskaya et al[10]

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found changes in the expression of IL1R2 in patients with atopic dermatitis. Furthermore, IL1R2 is found to be highly expressed in gastric cancer tissues.[11] Interleukin-1 receptor type II, activated by IL-1β, is upregulated in breast cancer tissues and especially in breast tumor-initiating cells.[12]

In CAD patients, adverse cardiovascular processes are mediated by inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β).[6] On one hand, TNF-α is the major pro-inflammatory cytokine, and on the other hand, interleukin-10 (IL-10) is the major anti-inflammatory cytokine in patients with CAD.[13] Serum levels of IL-37 in patients with CAD are positively connected with serum levels of CRP and IL-6.[14] One latest research showed that IL-32 levels in CAD patients with one major cardiac arterial stenosis were notably increased than those with more major cardiac arterials stenosis.[15] Furthermore, IL-1 played a role in regulating inflammatory and immune responses to various disorders. Nevertheless, the role of IL1R2, a protein-coding gene of IL-1 in CAD patients, persists to be undetermined.

In this study, we investigated the possible role of IL1R2 in peripheral blood mononuclear cells (PBMCs) of CAD patients.

METHODS

Study Design

The present study aims to evaluate IL1R2 receptor coding gene expression using a GEO-based repository and further validated it retrospectively in mild-moderate to severe CAD patients and healthy participants with PBMCs samples from Second Hospital of Tianjin Medical University. Subsequently, IL1R2 expression was intended to compare with SYNTAX score and oxidized low-density lipoprotein (Ox-LDL) of serum in CAD patients. Finally, the significant potential of IL1R2 was achieved in CAD patients.

Patients

A total of 90 cases of CAD and 90 healthy control samples with PBMCs were collected from Second Hospital of Tianjin Medical University. The subjects were recruited from January 2020 to May 2021. All patients signed informed consent during their hospital stay, and the study was authorized by the Ethics Committee of Tianjin Medical University and was conducted following the Declaration of Helsinki guidelines.

All patients met the WHO diagnostic criteria for coronary artery disease based on medical history, physical examination, serum high-sensitivity troponin, electrocardiogram and cardiac ultrasound, and confirmed by coronary angiography. Exclusion criteria: patients with other cardiovascular diseases, combined acute and chronic infections, collagenous connective tissue disease, hematologic diseases, autoimmune diseases, peripheral vascular diseases, malignant tumors, progressive hepatic and renal diseases; taking drugs such as immunosuppressants and nonsteroidal inflammatory inhibitors.

Specimen Collection and PBMC Isolation

All participants collected 5 mL of peripheral blood within 24 hours of admission and were placed in heparin-anticoagulated sterile tubes. The collected heparin-anticoagulated venous blood samples were centrifuged to remove the upper serum layer, diluted with phosphate buffered solution (PBS) and transferred. Peripheral blood mononuclear cells were isolated by fiction density gradient centrifugation, and the cells were resuspended in RPMI 1640 medium containing 10% calf serum and cultivated at 37°C with 5% CO_2.

RNA Extraction

Total RNA was isolated by utilizing the solutions of phenol-chloroform and afterward managing the homogenization by guanidine isothiocyanate (Trizol RNA Preparation kit). RNA concentrations were analyzed by NanoDrop spectrophotometer ND1000 (NanoDrop Technologies Inc, USA).

RT-qPCR

The total RNA in the PBMCs samples was isolated by Trizol reagent (Thermo Fisher Scientific) according to protocols. Thereafter, the total RNA was reverse-transcribed into cDNA by the reverse transcription kit (provided by Shanghai Sangon Biological Engineering Co., LTD). Real-time qPCR was carried out using SYBR Green Master Mix (Applied Biosystems) in a StepOnePlus instrument (Applied Biosystems). qPCR was performed with the following thermocycling conditions: initial denaturation for 10 minutes at 25°C, followed by 10 minutes at 50°C and 5 minutes at 85°C. A mixture of 10 μL SYBR™ Green PCR Master Mix, 7 μL water and 1 μL primer working solution was added to each well containing 2 μL cDNA for each reaction. The primers used are as follows: IL1R2 5′-F: AGGCGCCAGAGGCACCATGGAC-3′, R: 5′-CATCCTGTGCGTTGGCTGCC-3′, GAPDH F: 5′-TGTGGG CATCAATGGATTTGG-3′, R: 5′-ACACCATGTATTCCGGG TCAAT-3′. PCR reaction conditions were followed as (1) pre-denaturation at 95℃ for 10 minutes; (2) denaturation 95℃ for 15 seconds; annealing at 60℃ for 15 seconds, elongation at 72℃ for 20 seconds, a total of 40 cycles; and (3) 72℃ for 15 min. The reaction is terminated at 4℃. GAPDH functioned as an internal control. Three replicates were set for each sample, and $2^{-\triangle\triangle Ct}$[16] was utilized for quantitative analysis of the data.

Datasets and Data Processing

Six microarray datasets with CAD patients and healthy controls were achieved from the GEO database (GSE71226) using GEO queries. The bioinformatics tools with the R package of MetaQC were used to complete the dataset preprocessing stages.[17] Six datasets with expression levels were combined

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HIGHLIGHTS

- IL-1 receptor type II can be discoverable in coronary artery disease patients with peripheral blood mononuclear cells.
- IL-1 receptor type II might be involved in the inflammatory responses of coronary artery disease.
- Discoverable IL-1 receptor type II can act as a gene that may play an effective role in causing coronary artery disease.

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following the symbol of gene and processed out genes which have been observed in less than 5 datasets. Taken together, many of the genes were neither informative nor expressed in vivo whereas 20% of them were excluded due to a decline in false positives. Besides visualizing, the quality of studies in the analysis was achieved by principal component analysis (PCA) biplots using MetaQC. These measures of quality control were shown by 2D space, which determined the relation of each quality criterion coordinates with two principal coordinates.

Statistical and Datasets Analysis

R studios and Graphpad software were utilized for all the statistical analysis of the study and data were expressed as mean ± SD. Bioinformatics tools were used to analyze box plots and PCA biplots from the GEO database. Kolmogorov-Smirnov test, D'Agostino and Pearson omnibus normality test were applied for the normality test. When continuous variables were distributed as normal distribution, t-test was performed for comparing the two groups. Pearson correlation was used for correlation analysis. The clinical significance of IL1R2 was evaluated in PBMCs by the receiver operating curve (ROC) using the area under the curve (AUC). $P < .05$ was considered to be representing statistically significant.

RESULTS

Discovery of IL1R2 expression in CAD

The coronary artery disease patients of six microarray datasets with healthy controls were achieved from the GEO database (GSE71226). After excluding unspecified samples, a total of 90 CAD patients and 90 healthy controls were chosen for subsequent analysis. The clinical information were shown in Table 1. GEO database was used to discover IL1R2 coding gene in between CAD patients and healthy control by bioinformatics tools via PCA biplots and box plot analyses (Figure 1A, 1B, and 1C). These six datasets have represented the expression of IL1R2 individually in Figure 1A. PCA biplots were shown as the quantitative measure of six microarray datasets (Figure 1B). Based on these biplots of PCA, the present study involved all of the six datasets for further analysis. In the box plot, the IL1R2 expressions were significantly higher in CAD patients compared to healthy controls (Figure 1C, $P < .05$).

Validation of IL1R2 in PBMCs of CAD

To validate between PBMCs of IL1R2 expressions were shown by scatter plots in 90 CAD patients and 90 healthy controls. Herein, IL1R2 expressions were markedly over-expressed in PBMCs of CAD patients compared to the healthy control group (Figure 2A, $P < .01$). Herein, the severe CAD patients showed significantly higher expression of IL1R2 in PBMCs compared to mild-moderate CAD (Figure 2B, $P < .01$). Therefore, the coding gene expression of IL1R2 in PBMCs was notably observed not only in severe CAD but also in mild-moderate CAD patients.

Correlation and Significance of IL1R2 in CAD

First, IL1R2 expression was compared to SYNTAX score and observed the serum level of Ox-LDL in CAD patients and healthy controls. Herein, IL1R2 expression was positively correlated to the SYNTAX score in Figure 3A ($r = 0.3447$, $P < .001$). Besides, Ox-LDL expression level in serum was higher in CAD patients compared to healthy controls significantly and IL1R2 was positively correlated to the Ox-LDL (Figure 4(A, B), $r = 0.3956$, $P < .01$). Further, the ROC achieved a significantly higher AUC of 0.813 (Figure 5, $P < .05$) in CAD patients with PBMCs. Thus, IL1R2 expressions were not only directly correlated with PBMCs but also showed potential significance in CAD patients.
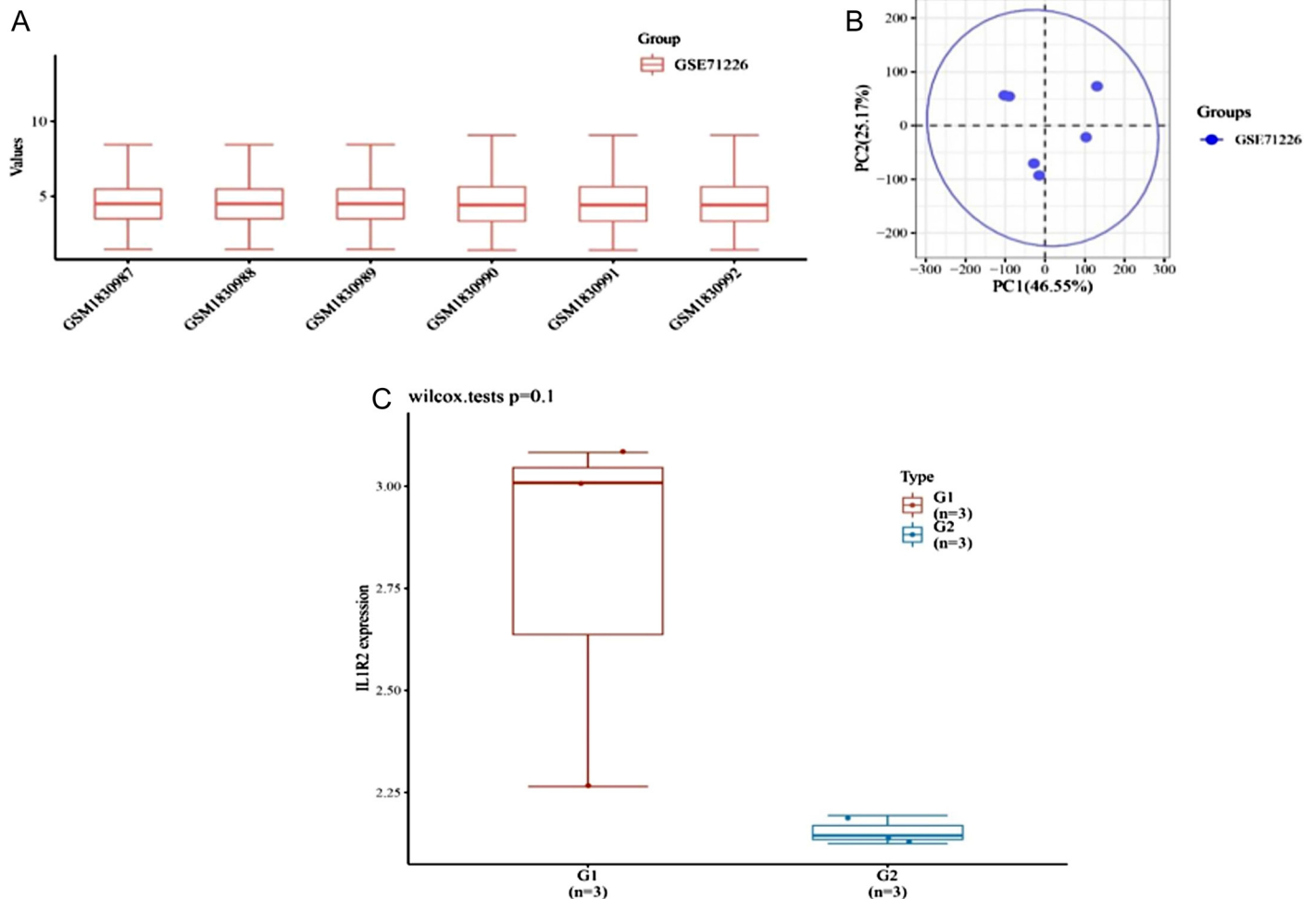
DISCUSSION

Cardiovascular diseases (CVDs) are crucial health problems throughout the world, which demonstrates soaring incidence and case-fatality rates due to its delayed diagnosis and insufficiencies of highly sensitive and specific biomarkers.[18,19] Meanwhile, only a few biomarkers, for instance, troponin, detect disease at later stages.[19] Previous studies have shown the specific cytokines dysregulation and association in the pathogenicity of CVDs and comprehended in immune and inflammatory mechanisms linked to atherogenesis.[20] While, the involvement of SMAD, STAT, and MAPK in cellular and molecular pathways are regulated by cytokines.[21]

At present, the researchers can find out the targeted genes for specific or aimed disorders by utilizing GEO-based

Table 1. Clinical Information of CAD Patients and Controls

	Healthy control (n = 90)	CAD (n = 90)	P
Baseline information			
Age (years)	59.14 ± 7.21	58.87 ± 7.54	.806
Gender (Male/Female)	52/38	55/35	
BMI (kg/m²)	23.79 ± 3.37	24.02 ± 3.61	.659
SBP (mm Hg)	125.14 ± 22.43	128.75 ± 24.27	.302
DBP (mm Hg)	79.52 ± 10.14	81.19 ± 11.36	.300
Laboratory tests			
TG (mmol/L)	1.12 ± 0.37	1.08 ± 0.45	.516
TC (mmol/L)	3.68 ± 1.05	3.83 ± 1.24	.382
LDL-C (mmol/L)	101.26 ± 28.91	105.71 ± 30.89	.320
HDL-C (mmol/L)	1.88 ± 0.52	2.13 ± 0.68	.006**
Comorbidity			
Diabetes mellitus (%)	28/90 (31.11%)		
Hypertension (%)	72/90 (80.00%)		
Obesity (%)	47/90 (52.22%)		
Medication history			
Anti-platelet drug (%)	42/90 (46.67%)		
Beta-blockers (%)	14/90 (15.56%)		
ACEI/ARBs (%)	7/90 (7.78%)		
Coronary-expansion drugs (%)	19/90 (21.11%)		

**$P < .01$.

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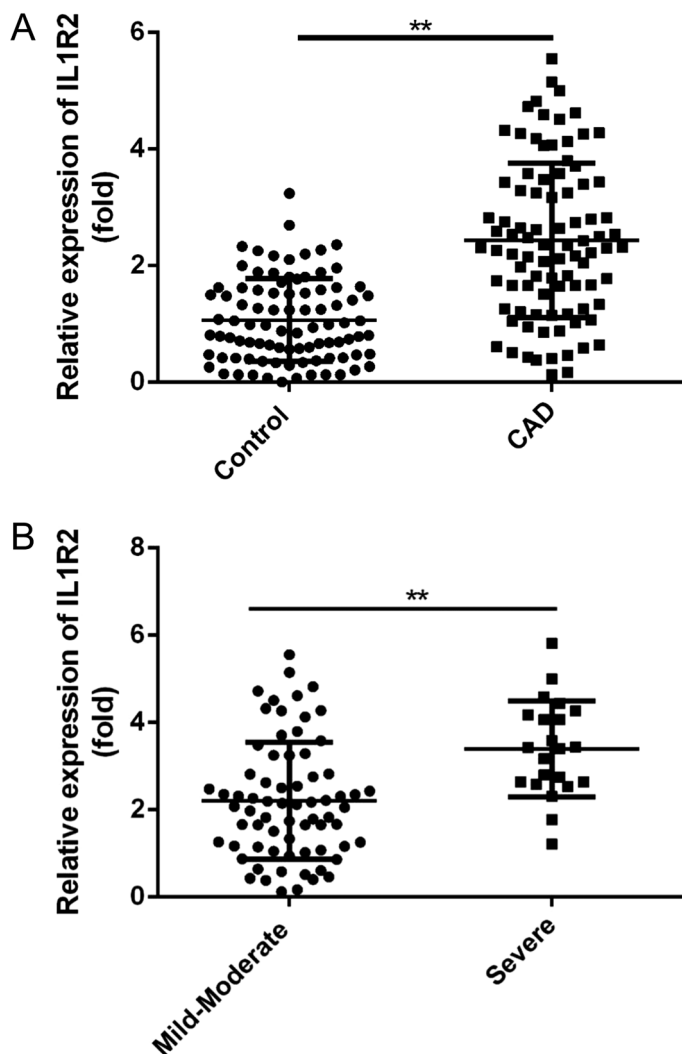Chen et al. Over-expression of IL1R2 in PBMCs

Figure 1. Discovery of IL1R2 expression in CAD patients by analyzing GEO database. (A) The expression value of IL1R2 in CAD patients by each of the 6 microarray datasets of GEO database (GSE71226) in the box plot. (B) PCA biplots of 6 microarray datasets for quality control measures. (C) The comparison of IL1R2 expression between 2 groups by box plot. G1, CAD; G2, Healthy control.

microarray expression data, which can provide the potential direction in the diagnosis and appropriate treatments of the interested diseases. Despite certain benefits, these microarray data may not be able to reproduce or are prone to the slightest disruption of data.[22] Furthermore, more than thousands of probes are scrutinized in a few hundreds of biological specimens, which results in the incremented false positive targets gradually. Thus in the present study, we adjoined six microarray datasets of CAD and explored the IL1R2 gene by combined maximum *P*-value, which was dysregulated expressed in CAD patients and controls. Taken together it urged significant roles of IL1R2 in CAD.

Interleukin-1 is fabricated by various cells of endothelium and smooth muscle and macrophages.[23] Meanwhile, IL-1 is the main significant pro-inflammatory cytokine, which promotes several genes expression connected to immunity and inflammation.[24] Besides, IL1R2, a receptor-coding gene associated with the family of IL-1 receptors has been found to play a role with other cytokines in inflammatory and immune responses as a profound mediator.[25,26] Interleukin-1 receptor type II may not only dominate immune response led by various cytokines but also dominate the metabolism of

cells.[27] Moreover, IL-1-based interfered inflammation leads to the pathogenesis of various diseases comprising systolic heart failure while IL1R2 is been indicated in the pathogenesis of atherosclerosis.[28] In this context, our findings convey that IL1R2 might play a significant role in CAD and acute myocardial infarction (AMI).[29] Similarly, another member of the receptor-associated kinase protein family of IL-1, known as IL-1 receptor associate kinase 3 (IRAK3), played role in Toll-like receptor signaling as a negative regulator and was involved in the response of adaptive immunity and host defense of innate immunity.[30]

The present study has discovered and validated the over-expression of IL1R2 in CAD and healthy controls with PBMCs samples. Meanwhile, IL1R2 expression based on PBMCs was positively correlated to SYNTAX scoring and the serum base Ox-LDL. Here, the serum-based Ox-LDL showed upregulated expression in CAD patients, which was consistent with previous studies.[31,32] Moreover, previous research mentioned that IL1R2 protein expression from autoimmune inner ear disease (AIED) patients in PBMCs might be able to predict the response of individuals to steroid therapy.[33] Furthermore, in the current study, the

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A

B

Figure 2. The validation of IL1R2 expression in PBMCs samples between CAD and healthy control. (A) The scatter plot represented the overall validation of IL1R2 in CAD patients and healthy control. (B) Expression of IL1R2 in mild-moderate and severe CAD patients by scatter-plot.

expression of IL1R2 was significantly upregulated in severe CAD compared to mild-moderate CAD of PBMCs samples while IL1R2 represented a significant potential AUC of 0.81. Taken together, the IL1R2 receptor gene may not only affect the pathogenesis of CAD but also may accurately differentiate and express it in mild-moderate to severe CAD.

Study Limitations

Despite interesting findings, the study has few limitations. First, the discovery of IL1R2 expression was conducted by an online GEO-based database, which could demonstrate biased samples or outcomes. Second, the validation of IL1R2 was carried out by fewer samples. Therefore further studies are needed to validate IL1R2 by larger multi centers cohorts. Third, the IL1R2 was evaluated and compared using only PBMCs samples whereas Ox-LDL was explored using serum samples. Thus, further researches are needed

r = 0.3447
p = 0.0009
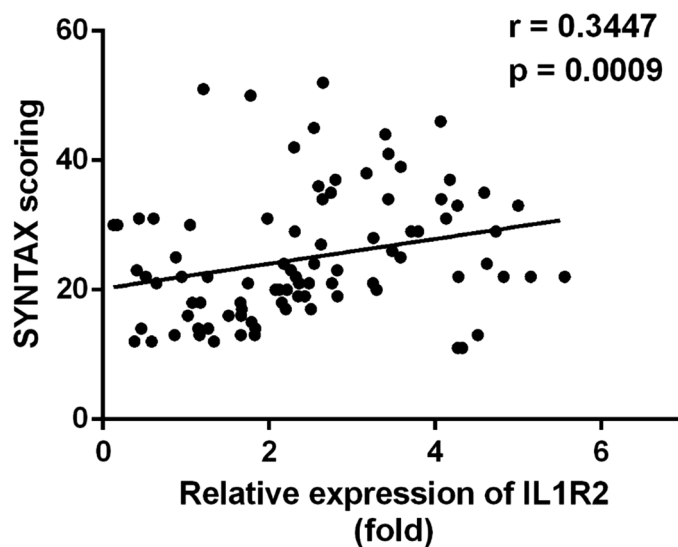
Figure 3. The correlation of IL1R2 with SYNTAX score in CAD patients by scatter plot.

A

B

r = 0.3956
p = 0.0001
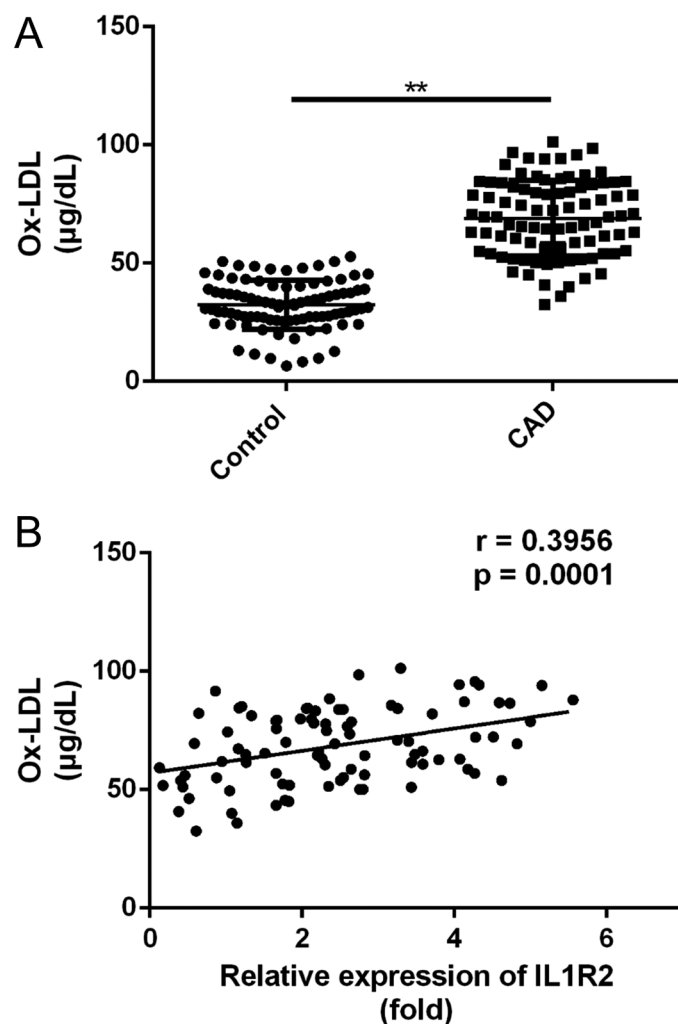
Figure 4. Expression of Ox-LDL in CAD patients and healthy control. (A) Serum levels of Ox-LDL are represented by a scatter plot in between two groups. (B) Correlation-based scatter plot shown between IL1R2 and Ox-LDL.

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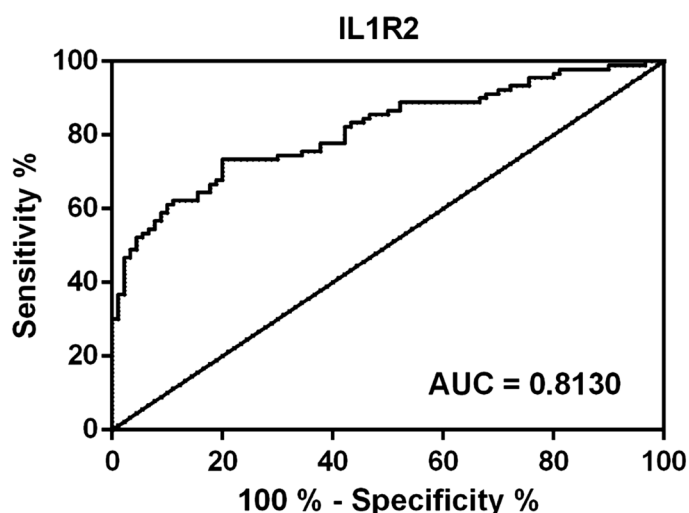
IL1R2

Figure 5. The significance of IL1R2 in PBMCs of CAD patients by ROC curve.

for various exploration of IL1R2 with other cytokine receptor genes, which might play a role in the development and progression of CAD.

CONCLUSION

Interleukin-1 receptor type II can notably be discoverable in CAD patients with PBMCs, which can act as an upregulated protein-coding gene that may play an effective role in causing CAD. Interleukin-1 receptor type II might be involved in the immune/inflammatory responses of CAD accompanied by other cytokine receptor genes.

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