# Correlation between Expression Levels of Early Growth Response Factor 3 and Interleukin-6 and Coronary Atherosclerotic Heart Disease: A Post-print

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## Abstract

Background: Coronary heart disease (CHD) is a leading cause of mortality worldwide. Coronary angiography is commonly employed as an effective diagnostic modality for CHD; however, its utilization rate remains relatively low at the primary care level due to multiple constraints. There is an urgent need to identify more meaningful biomarkers to provide evidence for primary care physicians in the diagnosis and management of CHD. Our research group previously identified that the Early Growth Response 3 (Egr3) gene may represent a susceptibility factor for pathogenic heterogeneity in the pathogenesis of CHD. Currently, few studies have reported on the correlation between CHD and the Egr3 gene in conjunction with inflammatory markers.

Objective: To investigate the correlation between Egr3, interleukin-6 (IL-6) and CHD, and to explore the relationship between their expression levels and coronary artery disease severity, thereby providing valuable laboratory evidence for clinical diagnosis and treatment at the primary care level.

Methods: A total of 110 CHD patients who presented to the Fifth Affiliated Hospital of Xinjiang Medical University between June and December 2021 were enrolled. All participants presented with CHD symptoms and underwent coronary angiography. Based on the coronary angiography findings, confirmed CHD patients were stratified into a mild stenosis group (Group A, $ $52 points, n=50) and a moderate-to-severe stenosis group (Group B, >52 points, n=30) according to the median Gensini score (52 points). Thirty individuals with normal coronary angiography results were selected as the control group (Group C). Serum Egr3 and IL-6 expression levels were measured using enzyme-linked immunosorbent assay (ELISA).

Results: The IL-6 expression level in Group B was significantly higher than those in Group A and Group C (P<0.05). Egr3 expression levels in both Group A and Group B were significantly higher than that in Group C (P<0.05). The area under the ROC curve for CHD diagnosis using Egr3 expression level was 0.648, with a sensitivity of 35.0% and specificity of 93.3%. In CHD patients, IL-6 was positively correlated with Egr3 (r=0.231, P<0.01). Both Egr3 and IL-6 were positively correlated with the Gensini score (r=0.39, 0.317, P<0.01).

Conclusion: Egr3 demonstrates favorable specificity for CHD diagnosis. The expression levels of both Egr3 and IL-6 are positively correlated with the degree of coronary vascular lesions.

## Full Text

# Correlation of Early Growth Response 3 and Interleukin-6 Expression Levels with Coronary Heart Disease

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## Abstract

**Background:** Coronary heart disease (CHD) is the leading cause of death worldwide. While coronary angiography serves as an effective diagnostic modality, its utilization remains limited in primary care settings due to various constraints. Identifying meaningful biomarkers to support CHD diagnosis and management at the primary care level is therefore essential. Our research group previously identified the early growth response 3 (Egr3) gene as a potential susceptibility factor underlying the pathogenic heterogeneity of CHD. However, few studies have examined the correlation between CHD and both Egr3 gene variants and inflammatory markers.

**Objective:** To investigate the correlation between Egr3, interleukin-6 (IL-6) and CHD, and to explore the relationship between their expression levels and coronary artery stenosis severity, thereby providing valuable laboratory evidence for clinical diagnosis and treatment in primary care settings.

**Methods:** We enrolled 110 CHD patients who presented to the Fifth Affiliated Hospital of Xinjiang Medical University between June and December 2021. All participants underwent coronary angiography for suspected CHD. Based on angiographic findings, patients were stratified according to the median Gensini score (52 points) into a mild stenosis group (Group A, $ $52 points, n=50) and a moderate-to-severe stenosis group (Group B, >52 points, n=30). An

additional 30 individuals with normal coronary angiography results served as controls (Group C). Serum Egr3 and IL-6 expression levels were measured using enzyme-linked immunosorbent assay (ELISA).

**Results:** IL-6 expression was significantly higher in Group B compared to Groups A and C ($P<0.05$). Egr3 expression levels in Groups A and B were both elevated relative to Group C ($P<0.05$). The area under the ROC curve for Egr3 in diagnosing CHD was 0.648, with a sensitivity of 35.0% and specificity of 93.3%. In CHD patients, IL-6 and Egr3 showed a positive correlation ($r=0.231$, $P<0.01$). Both Egr3 and IL-6 were positively correlated with Gensini score ($r=0.39$ and 0.317, respectively, $P<0.01$).

**Conclusion:** Egr3 demonstrates good diagnostic specificity for CHD, and the expression levels of both Egr3 and IL-6 are positively associated with the severity of coronary artery disease.

**Keywords:** Coronary disease; Early growth response 3; Intercellular signaling peptides and proteins; Interleukin-6; Gensini score; Coronary stenosis; Correlation analysis

## Introduction

Coronary atherosclerotic heart disease (CHD) represents the leading cause of mortality globally, characterized by the accumulation of obstructive or non-obstructive atherosclerotic plaques in epicardial vessels. Inflammatory responses play a pivotal role in atherogenesis and disease progression, with chronic low-grade inflammation recognized as a key driver of atherosclerotic disease development. Interleukin-6 (IL-6) functions as a pro-atherogenic cytokine, and recent research has demonstrated that inflammatory factors including IL-1$\beta$, IL-6, and C-reactive protein (CRP) activate inflammatory pathways in coronary atherosclerosis.

Early growth response 3 (Egr3), a member of the EGR family, is an essential transcription factor for gene expression that can be activated by various stimuli and inflammatory factors. Previous work by Li et al. identified associations between Egr3 genetic polymorphisms and CHD pathogenesis. Building on this foundation, the present study investigates the relationship between Egr3 and IL-6 expression levels and CHD, aiming to clarify their diagnostic value and correlation with coronary lesion severity to provide evidence for CHD management.

## Methods

### Study Subjects

We enrolled 110 CHD patients who presented to the Fifth Affiliated Hospital of Xinjiang Medical University between June and December 2021 with CHD

symptoms and completed coronary angiography. Based on angiographic findings, confirmed CHD patients were divided using the median Gensini score (52 points) into a mild stenosis group (Group A, $ $52 points, n=50) and a moderate-to-severe stenosis group (Group B, >52 points, n=30). Thirty individuals with normal coronary angiography served as controls (Group C). This study was approved by the hospital' s Medical Ethics Committee (approval number: XYDWFYLSH-2022-042), and all participants provided written informed consent.

### Sample Size Calculation

As a case-control study, sample size for two-group mean comparisons was calculated using the formula $n=(Z\alpha+Z\beta)^2 \cdot 2\sigma^2/\delta^2$, with $\alpha$=0.05 and power of 90%. For multi-group comparisons, PASS 15.0 software was employed. Based on preliminary results showing minimum mean differences and standard deviations, the calculated sample size was 90 cases total, requiring at least 30 cases per group.

### Inclusion and Exclusion Criteria

**Inclusion criteria:** (1) Age $ $18 years; (2) CHD diagnosis confirmed by coronary angiography showing $ $50% diameter stenosis in the left main artery or at least one of the three major branches (or their main subdivisions); (3) Control group comprised hospitalized patients undergoing coronary angiography with normal results.

**Exclusion criteria:** (1) Malignant tumors, schizophrenia, or psychological disorders; (2) Cardiomyopathy of any etiology, acute or chronic endocarditis, or myocarditis; (3) Pulmonary heart disease, congenital heart disease, acute or chronic infection, hematologic disorders, or autoimmune diseases; (4) Non-cooperative patients.

### Baseline Data Collection

Upon admission, the following data were collected: (1) Demographics including sex, age, heart rate, blood pressure, body mass index, and smoking history (defined as >1 cigarette daily); (2) Medical history of hypertension and diabetes; (3) Blood biochemical indicators measured by the hospital laboratory and left ventricular ejection fraction (LVEF) by cardiac ultrasound; (4) Coronary angiography results including lesion location and stenosis severity.

### Serum Egr3 and IL-6 Measurement

Fasting venous blood (2 ml) was collected in the morning and allowed to stand at room temperature for 30 minutes. After centrifugation at 1,500 r/min for 30 minutes (radius 13.5 cm), serum was isolated and stored at -80°C. ELISA kits for Egr3 (catalog: ml622406) and IL-6 (catalog: ml058097) were purchased from

Shanghai Enzyme-linked Biotechnology. All procedures followed manufacturer instructions, and absorbance (OD values) was measured at 450 nm using a microplate reader (Model K6600A, Beijing KaiAo Technology Development Co., Ltd.).

### Assessment of Coronary Stenosis Severity

The Gensini scoring system was used to quantify coronary stenosis severity. This method calculates a total score by multiplying each lesion' s severity score by a weight coefficient reflecting its anatomic importance, providing a comprehensive assessment of disease extent and severity as detailed in .

### Statistical Analysis

Data were analyzed using SPSS 25.0 software, with graphs generated using GraphPad Prism 5.0. Continuous variables are presented as mean±standard deviation ($\bar{x}\pm s$). Multi-group comparisons employed one-way ANOVA, while two-group comparisons used independent samples t-tests. Categorical data are expressed as percentages and compared using $^2$ tests. Pearson correlation analysis examined the relationship between Egr3 and IL-6, while Spearman correlation analysis assessed associations between Egr3, IL-6 and Gensini score. Receiver operating characteristic (ROC) curve analysis evaluated the diagnostic performance of Egr3 for CHD. Statistical significance was defined as $P<0.05$.

## Results

### Comparison of Baseline Characteristics Among Three Groups

Significant differences were observed among the three groups in sex distribution, hypertension history, diastolic blood pressure, and LVEF ($P<0.05$). No statistically significant differences were found in other parameters. Lipid levels were comparable across groups, likely because 80% of CHD patients were receiving lipid-lowering therapy, which facilitated investigation of the inflammatory relationships between Egr3, IL-6 and CHD.

### Comparison of Egr3 and IL-6 Expression Levels

Significant differences in Egr3 and IL-6 expression were detected among the three groups ($P<0.05$). Serum Egr3 levels in Groups A and B were both significantly higher than in Group C ($P<0.05$), with no significant difference between Groups A and B. IL-6 expression in Group B exceeded that in Groups A and C ($P<0.05$), while no significant difference existed between Groups A and C. Detailed data are presented in .

### Diagnostic Value of Serum Egr3 for CHD

The ROC curve analysis yielded an area under the curve of 0.648 for Egr3 expression in diagnosing CHD, with a sensitivity of 35.0% and specificity of

93.3% [Figure 1: see original paper].

### Correlation Between Egr3 and IL-6 in CHD Patients

A positive correlation was observed between IL-6 and Egr3 expression levels in CHD patients (r=0.231, P<0.01) [Figure 2: see original paper].

### Correlation Between Egr3, IL-6 and Gensini Score

Both Egr3 and IL-6 demonstrated positive correlations with Gensini score (r=0.39 and 0.317, respectively, P<0.01) [Figure 3: see original paper]-[Figure 4: see original paper].

### Multivariate Linear Regression Analysis With Egr3 as Dependent Variable

Multivariate linear regression analysis was performed with serum Egr3 expression level as the dependent variable and hypertension history (yes=1, no=0), diastolic blood pressure, LVEF, Gensini score, and IL-6 expression level as independent variables. Results showed that serum IL-6 expression level (b=21.33, t=4.251, P<0.01) and Gensini score (b=0.253, t=0.486, P=0.015) were independently associated with Egr3 expression level .

## Discussion

CHD remains a major cardiovascular disease with established treatment strategies including percutaneous coronary intervention and surgical bypass grafting. However, these approaches have not reduced CHD incidence or cardiovascular event risk. As CHD pathogenesis involves multifactorial genetic and environmental interactions, identifying novel biomarkers and developing targeted therapies represent urgent priorities. Based on our preliminary research, we investigated the relationships between Egr3, IL-6 and CHD severity to provide diagnostic evidence.

Egr3 is a zinc-finger transcription factor activated by mitogenic signals that has been studied primarily in nervous system development, cancer, muscle spindle function, fibrosis regulation, angiogenesis, and immunity. Our findings demonstrate elevated Egr3 in CHD patients compared to controls, with expression levels positively correlating with atherosclerotic severity, consistent with previous reports of Egr3 genetic polymorphisms in CHD pathogenesis and increased Egr3 expression in atherosclerotic plaques of mouse models. The lack of significant difference between mild and severe stenosis groups may reflect the limited sample size in the severe group and potential influence of other regulatory factors, which warrants further investigation.

Numerous studies have established the pro-atherogenic role of IL-6 in cardiovascular disease. Our results showing higher IL-6 in the moderate-to-severe group

and its positive correlation with Gensini score align with previous research suggesting IL-6 as a potential therapeutic target in CHD. The absence of significant difference between mild and control groups may be attributable to insufficient sample size and requires validation in larger cohorts.

The positive correlation between Egr3 and IL-6 (r=0.231, P<0.01) suggests a mechanistic link, as Egr3 functions as a transcription factor critical for inflammatory responses in lymphocyte proliferation. Studies have shown Egr3 activates pro-inflammatory cytokines IL-6 and IL-8 in prostate cancer and promotes inflammation in lung cancer. This indicates that CHD pathogenesis involves close interplay between Egr3 and IL-6, potentially through NF- B pathway interactions where Egr3 forms complexes with p65 subunits to activate inflammatory factor transcription.

Regarding early CHD diagnosis, Egr3 demonstrated an ROC AUC of 0.648 with high specificity (93.3%) but low sensitivity (35.0%), suggesting potential feasibility as a laboratory marker for CHD diagnosis and severity assessment that requires confirmation in larger studies. The positive correlation between Egr3 and Gensini score supports further investigation of Egr3 combined with other inflammatory markers for enhanced diagnostic value.

BARON et al. demonstrated that Egr3 regulates expression of 100 inflammation-related genes mapping to canonical pathways such as NF- B, which modulates inflammatory responses. WIELAND et al. reported that Egr3 and NF- B act as cofactors regulating various inflammatory genes, with Egr3 forming complexes with NF- B p65 subunits to activate transcription of inflammatory factors like IL-2 in prostate tumor cells. Our finding of Egr3-IL-6 correlation in CHD patients suggests Egr3 may influence NF- B signaling to activate inflammatory factor expression, promoting atherosclerosis. Egr3 may thus represent an upstream regulator of IL-6, and our research group will further investigate its specific roles and mechanisms in CHD inflammatory pathways.

In summary, Egr3 and IL-6 expression levels are closely associated with CHD development and correlate with disease severity. Egr3 shows promise for CHD diagnosis and prognostic assessment, potentially aiding primary care management. However, limitations include the small sample size, particularly in the severe stenosis group, and lack of patient follow-up. Future studies should expand sample sizes, incorporate longitudinal follow-up, and investigate additional inflammatory markers to validate these findings.

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## Author Contributions

ZUMURETI Abudukeyimu and LI Xia jointly conceived the overall research objectives and design. ZHU Kairui participated in experimental procedures and data analysis. MA Yanlin integrated research data through statistical and computational analysis. The National Natural Science Foundation and the State Key Laboratory Special Fund provided financial support. LIU Fang and ZHU Kairui participated in data collection. LI Xia supervised and led the research planning and execution. ZUMURETI Abudukeyimu drafted the manuscript. All authors contributed to data analysis, drafting, and revision of the manuscript.

## Conflict of Interest Statement

The authors declare no conflicts of interest.

*Note: Figure translations are in progress. See original paper for figures.*