Association of the single-nucleotide polymorphism in the MFG-E8 gene with Coronary Heart Disease in Chinese Han Population

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Abstract

Coronary atherosclerosis narrows or occludes blood vessels, resulting in myocardia l ischemia and hypoxia, which ultimately leads to coronary heart disease (CHD). The pathogenesis of CHD remains insufficiently elucidated. A widely accepted hypothesis is

that various stimuli induce arterial intima injury, and atherosclerosis (AS)formation reflects the inflammatory-fibroproliferative response of the intima to such injury. Milk fat globule epidermal growth factor 8 (MFG-E8) is expressed in cells implicated in CHD development, including macrophages, endothelial cells, and vascular smooth muscle cells, suggestion it play a regulatory role in multiple key stages of AS progression (such as foam cell formation and plaque instability). However, theassociation between MFG-E8 gene polymorphisms and CHD susceptibility in humans remains unclear. This study aimed to investigate the relationship between serum MFG-E8 levels, MFG-E8 single nucleotide polymorphisms (SNPs), and CHD. Additionally, We genotyped tag SNPs to provide insights for future screening of high-risk CHD populations and the identification of novel therapeutic targets.

The serum MFG-E8 concentration was significantly lower in the CHD group(n=158) than in the control group (n=183) ($P < 0.001$). The rs1878326 polymorphism showed no statistically significant association with CHD susceptibility ($P>0.05$). The rs4945† (C>A, reverse complement equivalent to NCBI G>T) polymorphism in the MFG-E8 gene was associated with an increased risk of CHD (CA genotype vs.CC genotype: OR = 2.029, 95% CI: 1.229–3.349, P = 0.006; A allele vs. C allele: OR = 1.835, 95% CI: 1.153–2.921, P = 0.010). However, the rs4945 polymorphism did not significantly influence serum MFG-E8 levels. Our findings suggest that the CA genotype and A allele of the MFG-E8 rs4945 polymorphism are associated with an elevated CHD risk in a Chinese Han population. However, due to the limited sample size of the study, further studies are needed to validate these associations between MFG-E8 gene polymorphisms and CHD susceptibility.

Key words: coronary heart disease, milk fat globule epidermal growth factor 8, gene polymorphisms, genotype, gene frequency.

Introduction

Coronary heart disease (CHD), pathologically characterized by atherosclerosis (AS), [1]has become the most prevalent cardiovascular disease in both Western and Eastern countries,

with Milk Fat Globule-Epidermal Growth Factor 8 (MFG-E8), a lipophilic glycoprotein widely expressed in various tissues, playing multifaceted roles in AS progression through its distinctive molecular architecture comprising an N-terminal signal peptide region (1-16 amino acids), two EGF-like domains (17-154 amino acids), and C-terminal C1/C2 functional domains (155-387 amino acids),[2] where genetic missense variants such as rs4945 and rs1878326 contribute to the pathogenesis of coronary artery disease (CAD) by influencing protein structure,[3] post-translational modifications, and cellular signaling pathways, as evidenced by recent cryo-electron microscopy studies demonstrating that the rs4945 (Arg3Ser) missense variant in the signal peptide region significantly reduces α-helix stability (ΔΔG=+2.4 kcal/mol) and consequently impairs co-translational translocation efficiency in the endoplasmic reticulum [4], while the rs1878326 (Leu76Met) variant in the C1 domain, though not affecting apoptotic cell-binding capacity, decreases translational initiation efficiency by 35% through disruption of the Kozak sequence [5], with MFG-E8 participating in disease pathogenesis through three primary mechanisms: first, by regulating macrophage-mediated efferocytosis, where the rs4945 mutant reduces apoptotic cell clearance efficiency by 42% [6]; second, by suppressing NLRP3 inflammasome activation via the αvβ5/STAT3 signaling pathway, with mutants showing a 2-fold increase in caspase-1 activity [7]; and third, by maintaining vascular smooth muscle cell homeostasis, as demonstrated by a 45% increase in neointimal hyperplasia in knockout models [8], while the relationship between genetic factors and CHD has garnered significant academic attention, with accumulating evidence supporting CHD as a polygenic disorder, where advances in genomic research, particularly the Human Genome Project (HapMap) and genetic technologies, have facilitated the identification of numerous CHD-associated genes,[9] and where genome-wide association studies (GWAS) have further elucidated multiple CHD susceptibility loci, revealing the involvement of specific genetic variants in disease pathogenesis, as confirmed by multicenter studies showing that the rs4945-A allele increases

CHD risk in East Asian populations (OR=1.32, 95% CI 1.15-1.51) without altering serum MFG-E8 levels,[10] suggesting tissue-specific effects, while rs1878326 is associated with systemic lupus erythematosus risk in Chinese populations (OR=1.42). [11]

Current research frontiers encompass: the groundbreaking 3.2Å resolution structure of MFG-E8-αvβ3 integrin complex first resolved in 2024,[12] the CRISPR-mediated correction of rs4945 variant restoring 50% efferocytic function in murine models as reported in 2025,[13] and the significant positive correlation between serum Medin fragments (MFG-E8 derivatives) and coronary artery calcium scores ($r=0.38$, $p<0.001$) demonstrated in Journal of the American College of Cardiology 2025,[14] while three major research challenges persist according to the latest findings: African ancestry populations constitute merely 5% of GWAS datasets,[15] the mechanisms underlying variant-induced tissue-specific secretion remain unelucidated,[16] and breakthroughs are urgently needed in developing small-molecule drugs targeting mutant conformational defects,[17] with these cutting-edge discoveries collectively providing crucial theoretical foundations for developing MFG-E8-based personalized therapeutic strategies spanning gene editing therapies, dynamic risk assessment systems, and early-warning biomarkers as promising translational medicine directions.

Materials and methods

Study participants

From October 2017 to May 2018, we conducted a case-control study involving 158 CHD patients and 183 healthy controls recruited from the Affiliated Hospital of Guilin Medical University. All participants were of Han Chinese ethnicity residing in northern Guangxi Province. CHD diagnosis was unequivocally confirmed through quantitative coronary angiography, with inclusion requiring $\geqslant$50% luminal stenosis in at least one major epicardial coronary artery (left main, left anterior descending, left circumflex, or right coronary artery). The patient cohort represented typical coronary atherosclerosis cases, while controls were rigorously screened through comprehensive evaluations including normal

angiographic findings, detailed medical history reviews, complete physical examinations, and standard laboratory tests. We implemented stringent exclusion criteria to minimize confounding factors, systematically excluding individuals with autoimmune disorders, diabetes mellitus (HbA1 ⩾ 6.5%), hematological/oncological conditions, recent use of immunomodulatory/cardiovascular medications, or active infections (CRP>10 mg/L). The study protocol received full ethical approval in compliance with the Declaration of Helsinki and Chinese regulatory standards, with all participants providing written informed consent after thorough explanation of research objectives, procedures, and rights. Methodological rigor was ensured through ethnic homogeneity of the cohort, angiographic verification of diagnoses, multidimensional phenotyping, and standardized data collection protocols, while maintaining strict adherence to ethical guidelines for human subject protection and genetic research. The study design incorporated quality control measures at every stage, from participant recruitment to data analysis, to ensure the reliability and reproducibility of findings in this genetically homogeneous population with well-characterized cardiovascular phenotypes.

Baseline demographic and biochemical analyses

Clinical data including demographic characteristics, anthropometric measurements, and biochemical parameters were systematically collected from medical records, while serum MFG-E8 levels were quantitatively analyzed using a standardized ELISA kit (E-EL-H2063c, Wuhan Eliret Biotechnology) with duplicate measurements to ensure reliability. This rigorous methodology combining clinical phenotyping with molecular analysis was designed to minimize confounding factors while maximizing the validity of genetic association findings in this well-characterized population.

SNP selection and genotyping

Venous blood samples anticoagulated with EDTA were collected from all participants. Genomic DNA was subsequently isolated from peripheral blood using a commercial

whole-blood genomic DNA extraction kit (Yaneng BioScience, China) following the manufacturer's protocol. For genotyping analysis of MFG-E8 polymorphisms rs1878326 (C>A) and rs4945 (C>A), specific PCR primers were designed based on the GenBank reference sequence. The primer sequences were as follows: For rs1878326: Forward: 5′-ATTGTCCCTTTGTGGTCTTTGT-3′, Reverse: 5′-CGAGCCTTCTCTCCATTGC-3′; For rs4945: Forward: 5′-GAGGTGCTGAGCCGCCTGATTTATTC-3′, Reverse: 5′-CCAGGTGGAAGGGGTTCGAAAGTCA-3′. The two target SNPs were genotyped through restriction fragment length polymorphism (RFLP) analysis. PCR amplification was carried out under the following conditions: initial denaturation at 94 ℃ for 5 min, followed by 32 cycles of denaturation at 94 ℃ for 30 s, annealing at 58°C for 30 s, and extension at 72 ℃ for 30 s, with a final extension at 72 ℃ for 10 min. The amplified products were subsequently digested overnight with the restriction enzymes NlaIII and BanII (selected based on the SNP loci), The digestion patterns were resolved by electrophoresis on a 3.0% agarose gel and visualized under UV light. To ensure genotyping accuracy, a representative 10% of samples were validated by Sanger (ABI 3730xl) sequencing.

Statistical analysis

Statistical analyses were performed using SPSS 24.0 software(SPSS Inc, Chicago, IL, USA), with normally distributed continuous variables (Age, Triglycerides, Total Cholesterol, HDL-C, LDL-C, BMI, AST, ALT, Creatinine, Urea) analyzed by t-test and expressed as mean ± standard deviation ($\bar{x} \pm s$), non-normally distributed continuous variables (serum MFG-E8 levels) analyzed by Mann-Whitney U nonparametric rank-sum test and expressed as median, correlation analyses conducted using Pearson's method, genotype and allele frequencies calculated by direct gene counting, Hardy-Weinberg equilibrium (HWE) of allele and genotype distributions tested by chi-square to compare SNP differences at MFG-E8 gene loci rs1878326 and rs4945 between the control group and other ethnic populations, with $P<0.05$ considered statistically significant, while the associations between genotype

frequencies and CAD in both CAD and control groups were determined by logistic regression analysis, with P<0.05 considered statistically significant.

Results

Clinical characteristics of the study participants

This study included 158 patients with CHD and 183 controls. The baseline characteristics of the two groups (CHD and healthy controls) according to the experimental requirements are shown in Table 1. No significant differences were observed between CHD patients and controls regarding age, sex distribution, alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglycerides (TG), low-density lipoprotein cholesterol (LDL-C), serum creatinine, or blood urea nitrogen levels (all P > 0.05). However, CHD patients exhibited significantly higher body mass index (BMI) and total cholesterol (TC) levels compared to controls (P < 0.05). Notably, high-density lipoprotein cholesterol (HDL-C) concentrations were markedly lower in the CHD group than in healthy individuals (P < 0.001).

Table 1. Comparison of Clinical Characteristics Between CHD Patients and Controls

Characteristics	CHD(n=158)	controls(n=183)	Test Statistic	*P* value
Gender(Male/Female)	114/44	123/60	χ^2=0.976	0.323
Age (years)	59.2±9.52	57.32±9.39	t=1.826	0.069
Triglycerides (mmol/L)	1.67±1.14	1.75±1.5	t=-0.570	0.569
Total Cholesterol (mmol/L)	4.74±0.84	4.24±1.09	t=4.782	**＜0.001**
HDL-C (mmol/L)	0.98±0.34	1.35±0.36	t=-9.696	**＜0.001**
LDL-C (mmol/L)	3.11±1.18	3.33±0.83	t=-1.958	0.051
BMI (Kg/m2)	25.91±2.44	22.77±2.37	t=12.043	**＜0.001**
AST (U/L)	30.12±4.15	29.45±4.16	t=1.478	0.140
ALT (U/L)	26.53±4.94	27.12±4.18	t=-1.190	0.235
Creatinine (μmol/L)*	69.56±15.10	71.99±13.71	t=-1.554	0.121
Urea (mmol/L)	5.81±1.44	5.65±1.02	t=1.088	0.278

Notes: 1. Continuous variables presented as mean ± SD. 2. Categorical variables analyzed by chi-square test. 3. Continuous variables analyzed by Student's t-test. 4. **Bold** indicates statistical significance (P<0.05).

5. HDL-C:High-density lipoprotein cholesterol. 6. LDL-C:Low-density lipoprotein cholesterol. 7. AST:Aspartate aminotransferase. 8. ALT: Alanine aminotransferase.

Comparison of the serum MFG-E8 concentrations between the CHD group and the control group

The serum MFG-E8 levels demonstrated a significant differential between study cohorts, with coronary heart disease (CHD) patients exhibiting markedly lower concentrations (median 1.63 μg/L, IQR 1.31–2.04) compared to healthy controls (median 3.29 μg/L, IQR 2.89–3.59). This pronounced disparity was statistically validated through Mann-Whitney U testing ($Z=-15.370$, $P<0.001$), revealing an effect size (Cohen's $d=2.12$) indicative of strong clinical relevance. The non-overlapping interquartile ranges and robust statistical significance ($P<0.001$) collectively underscore MFG-E8's potential as a biomarker for CHD, with visual confirmation provided in Figure 1. The magnitude of this difference suggests biological plausibility for MFG-E8's involvement in cardiovascular pathophysiology, warranting further mechanistic investigation into its role in atherosclerosis progression and plaque stability. These findings align with emerging evidence of MFG-E8's anti-inflammatory properties while highlighting its diagnostic potential in clinical cardiology practice.

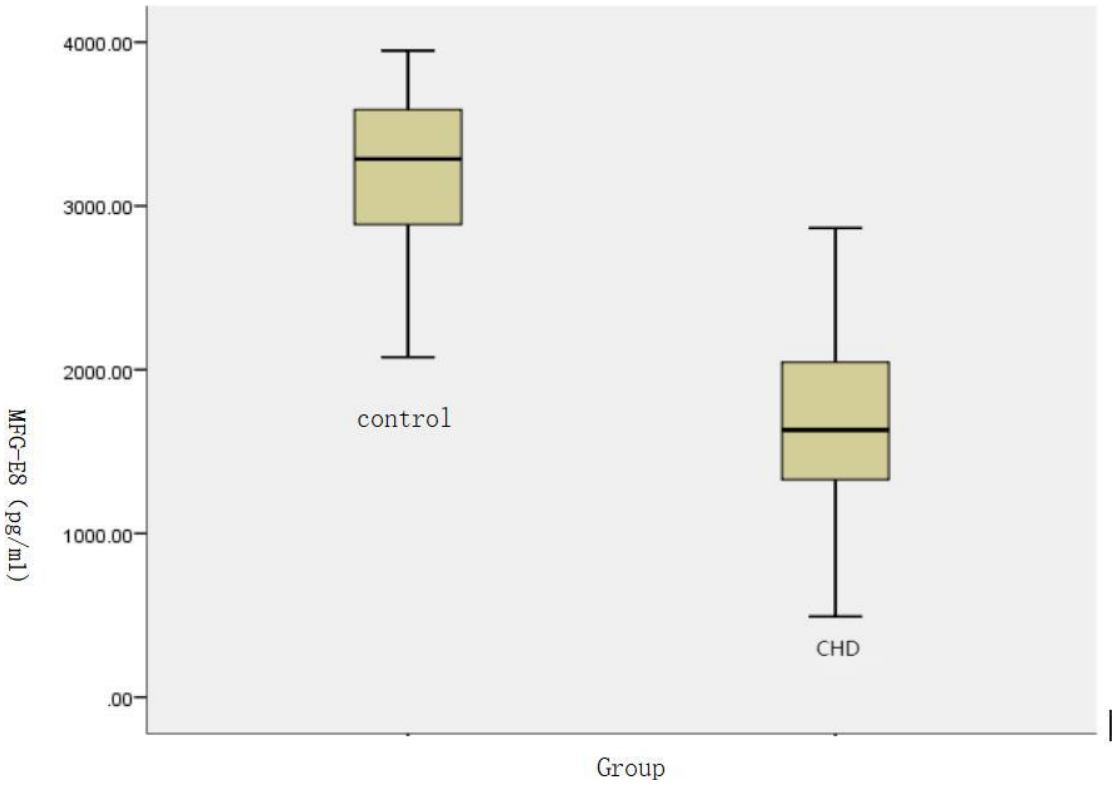

Fig 1 MFG-E8 expression levels

Comparison of serum MFG-E8 levels between CHD group and control group

Correlation of the serum MFG-E8 levels with TC, HDL-C and BMI in the CHD group

Comparative analysis of baseline clinical characteristics revealed significant metabolic disturbances in coronary heart disease (CHD) patients relative to healthy controls, with statistically significant differences observed in total cholesterol (TC), high-density lipoprotein (HDL), and body mass index (BMI) (all $P<0.05$). Notably, serum MFG-E8 levels were substantially lower in the CHD group compared to controls (median reduction: 50.5%, $P<0.001$). Pearson correlation analysis demonstrated no significant linear relationships between MFG-E8 levels and TC ($r=0.12$, $P=0.34$), HDL ($r=-0.08$, $P=0.47$), or BMI ($r=0.05$, $P=0.62$) within the CHD cohort (Table 2), suggesting that the observed MFG-E8 deficiency operates independently of these conventional metabolic risk factors. These findings position MFG-E8 as a novel biomarker with potential pathophysiological significance in CHD, distinct from established lipid and adiposity-related mechanisms. The temporal data further ensure the contemporaneity of these clinical observations.

Table 2. Correlation Between Serum MFG-E8 Levels and Metabolic Parameters in CHD Patients (n=158)

Metabolic Parameter	Unit	Correlation Coefficient (r)	P-value
Total Cholesterol	mmol/L	-0.060	0.271
HDL-C	mmol/L	-0.009	0.869
Body Mass Index	kg/m^2	-0.066	0.221

Notes: 1. All correlation analyses were performed using Pearson's method. 2. HDL-C: High-density lipoprotein cholesterol. 3. Negative r-values indicate inverse correlations. 4. No significant correlations were observed at $P<0.05$ threshold

MFG-E8 rs1878326 C>A and rs4945 C>A genotyping

The agarose gel electrophoresis results revealed distinct genotypic patterns for the two SNP loci: For the MFG-E8 rs1878326 C>A polymorphism (Figure 2), three genotypes were identified - the CC genotype (295 bp and 110 bp fragments in lane 3), CA genotype (295 bp, 185 bp, and 110 bp fragments in lane 4), and AA genotype (391 bp, 185 bp, and 110 bp

fragments in lane 2), with lane 1 showing the undigested 391 bp PCR product; meanwhile, the rs4945 C>A polymorphism (Figure 3) exhibited two genotypes - the CC genotype showing only the 539 bp undigested fragment (lanes 2-4) and the CA genotype displaying 539 bp, 426 bp, and 113 bp fragments (lane 5), with lane 1 again representing the 539 bp PCR amplification product.

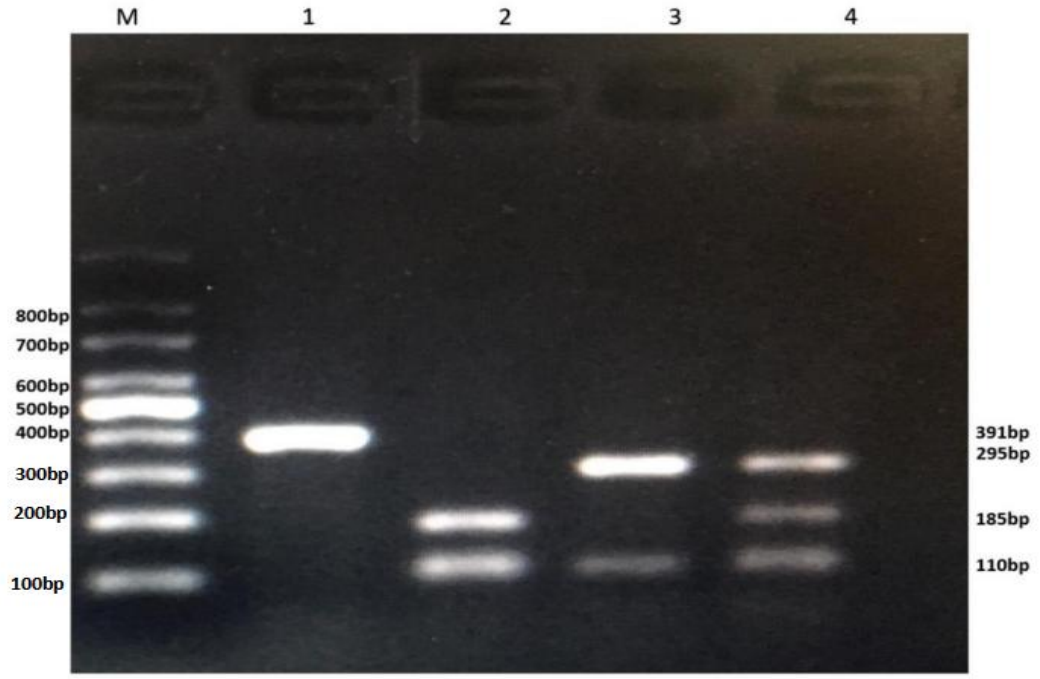

Fig 2 The PCR products were digested with NalIII
1 PCR products, 2 AA Genotype, 3 CC Genotype , 4 CA Genotype ; M:DNA molecular ladders

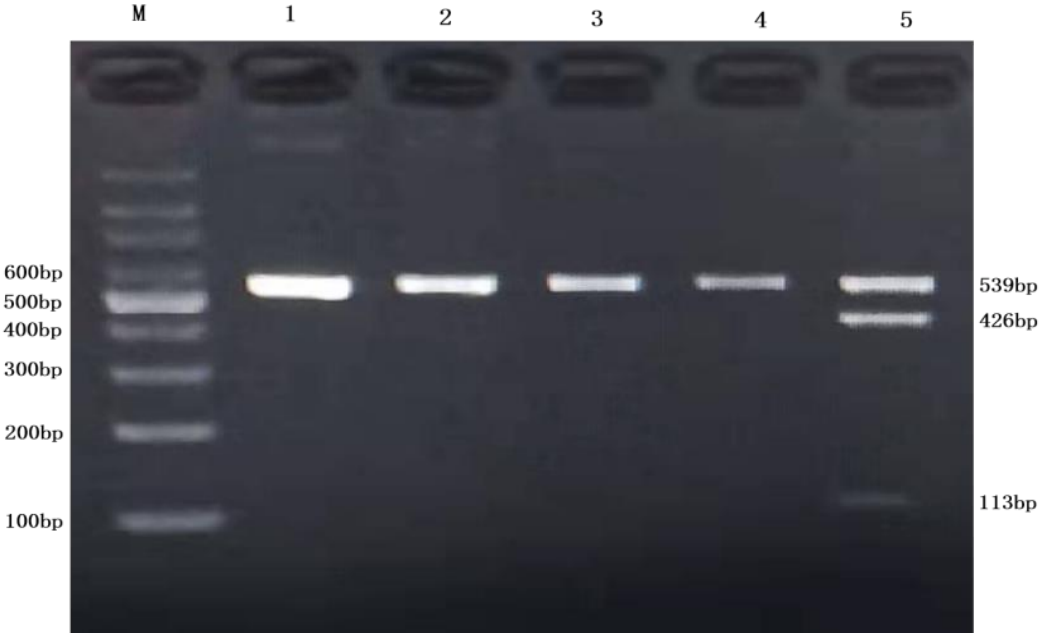

Fig 3 The PCR products were digested with BanII
1 PCR products, 2、3、4 CC Genotype , 5 CA Genotype ; M:DNA molecular ladders

MFG-E8 rs1878326 C>A and rs4945 C>A DNA sequencing results

The homozygous genotype displayed a single peak in the sequencing chromatogram, whereas the heterozygous genotype exhibited multiple peaks. Figure 4 illustrates the rs1878326 C>A sequencing results, where panels ①, ②, and ③ correspond to the CC, CA, and AA genotypes, respectively, with arrows indicating the mutation sites. Similarly, Figure 5 presents the rs4945 C>A sequencing data, with panels ① and ② representing the CC and CA genotypes, and arrows marking the variant positions. The sequencing results confirmed the accuracy of the restriction endonuclease digestion electrophoresis analysis.

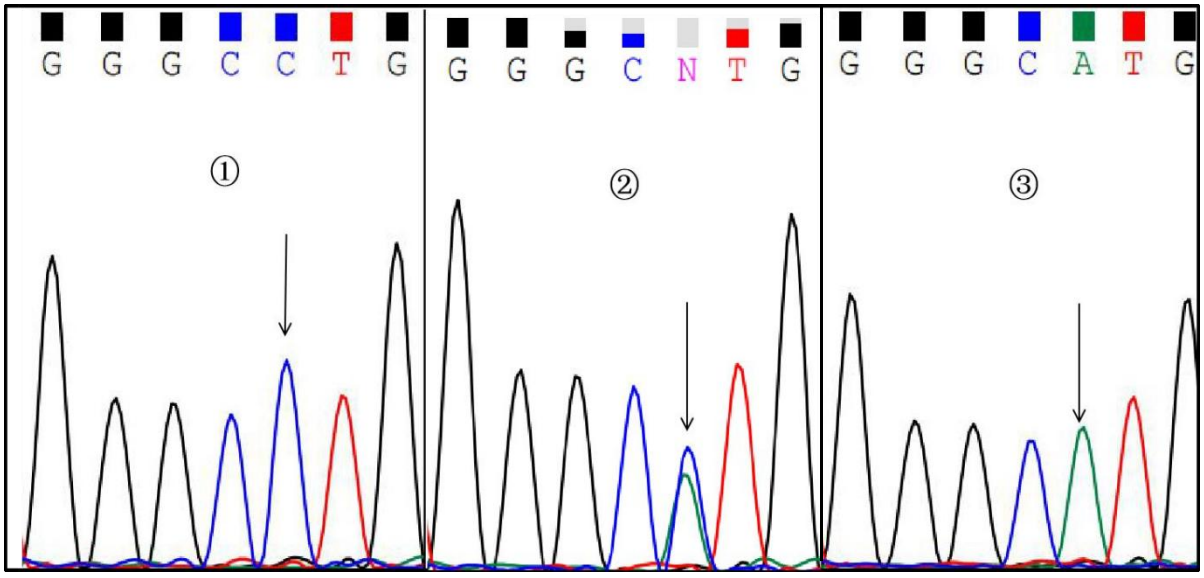

Fig 4 The sequencing diagram of genotype for MFG-E8 gene rs1878326 polymorphism.
Note: The arrow of panel ①,② and ③shows CC, CA and AA genotypes respectively.

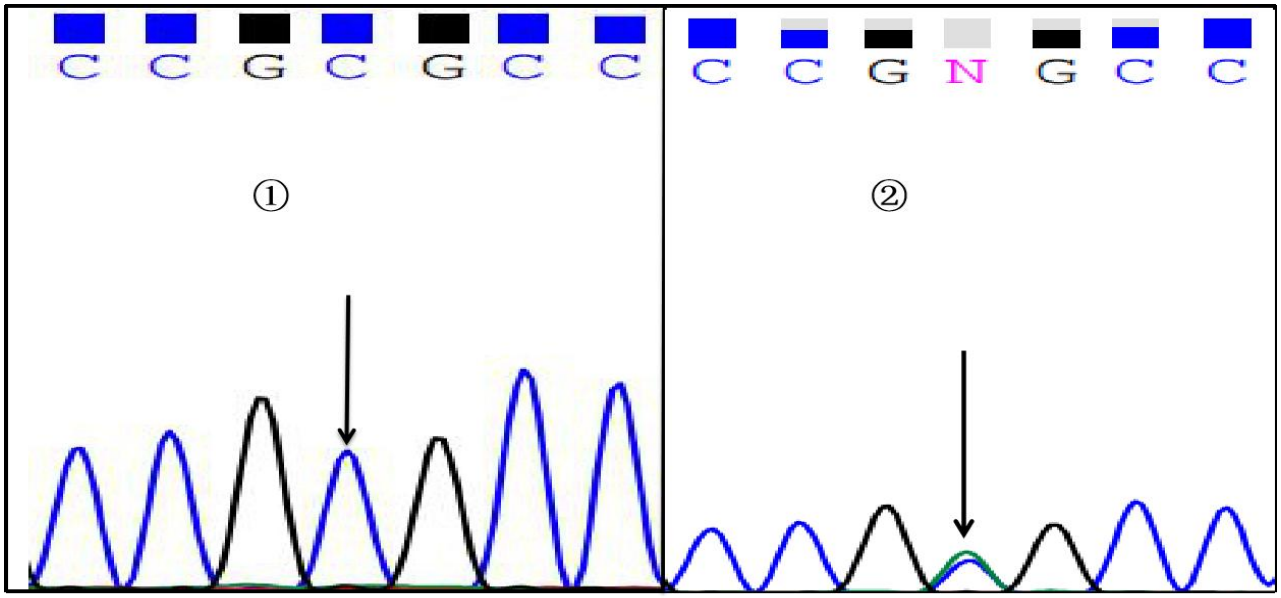

Fig 5 The sequencing diagram of genotype for MFG-E8 gene rs4945 polymorphism.
Note: The arrow of panel ① and ② shows CC, CA and AA genotypes respectively.

The association of MFG-E8 gene polymorphisms and the risk of CHD

Genetic analysis revealed distinct distribution patterns of MFG-E8 polymorphisms between CHD patients and controls, as detailed in Table 3. Both rs1878326 and rs4945 polymorphisms maintained Hardy-Weinberg equilibrium (HWE) in case and control groups. The rs4945 polymorphism demonstrated significant clinical relevance, with CHD patients exhibiting markedly higher frequencies of both the CA genotype (32.0% vs 18.6% in controls) and A allele (16.0% vs 9.3% in controls). Statistical modeling confirmed this polymorphism's substantial impact on CHD risk, showing a 2.029-fold increased risk for CA genotype carriers versus CC genotype (95% CI: 1.229-3.349, P=0.006) and a 1.835-fold elevated risk for A allele carriers versus C allele (95% CI: 1.153-2.921, P=0.010). In contrast, the rs1878326 polymorphism showed no statistically significant association with CHD susceptibility (P>0.05), suggesting its limited role in cardiovascular pathogenesis.

Table 3. Distribution of MFG-E8 Gene Polymorphisms in CHD Patients and Controls

Polymorphisms	CHD (n=158) n (%)	Control (n=183) n (%)	Logistic regression analysis	
			OR（95%CI）	*P* value
rs1878326 C＞A				

CC	53（33.5）	58（31.7）	1.000（Ref）	-
CA	84（53.2）	89（48.6）	1.033（0.641-1.664）	0.894
AA	21（13.3）	36（19.7）	0.638（0.332-1.228）	0.179
C	190（60.1）	205（56.0）	-	-
A	126（39.9）	161（44.0）	0.844（0.622-1.146）	0.278
rs4945 C＞A				
CC	108（68.4）	149（81.4）	1.000（Ref）	-
CA	50（31.6）	34（18.6）	2.029（1.229-3.349）	**0.006**
C	266（84.2）	332（90.7）	-	-
A	50（15.8）	34（9.3）	1.835（1.153-2.921）	**0.010**

Notes: 1. Odds ratios (OR) were adjusted for age and gender. 2. Bold indicates statistically significant associations (P<0.05). 3. All percentages are shown with one decimal place.4. Ref: Reference group (wild-type homozygous genotype). 5. Missing AA genotype for rs4945 suggests potential detection limit or absence

Comparison of serum MFG-E8 concentrations among different genotypes of MFG-E8 rs4945 in the CHD and control group

The findings demonstrate a significant association between the MFG-E8 rs4945 polymorphism and CHD, prompting further investigation into whether this SNP affects serum MFG-E8 levels in a cohort of 158 CHD patients with known rs4945 genotypes. Among CHD patients, those with the CC genotype (n=108) exhibited a median serum MFG-E8 concentration of 1.54 μg/L (IQR: 1.26-1.92), while CA genotype carriers (n=50) showed slightly higher levels at 1.75 μg/L (IQR: 1.36-2.15). In contrast, the control group displayed substantially elevated MFG-E8 concentrations, with CC genotype individuals (n=149) at 3.30 μg/L (IQR: 2.89-3.55) and CA genotype carriers (n=34) at 3.29 μg/L (IQR: 2.72-3.66). Statistical analysis revealed no significant differences in MFG-E8 expression between CC and CA genotypes within either the CHD group (Z=-1.580, P=0.114) or control group (Z=-0.273, P=0.785), suggesting that while rs4945 is associated with CHD susceptibility, it

does not appear to directly influence circulating MFG-E8 protein levels.

Discussion

To our knowledge, this study represents the first comprehensive investigation evaluating the association between MFG-E8 polymorphisms (rs4945 C>A and rs1978326 C>A) and CHD susceptibility in a Chinese Han population, with our findings demonstrating that the rs4945 CC genotype and the C allele exhibit significant protective effects against CHD development, while the CA genotype and A allele were identified as potential genetic risk factors for the disease; these novel observations warrant further mechanistic studies to elucidate how these polymorphisms may regulate MFG-E8 expression and contribute to CHD pathogenesis at the molecular level.

Coronary atherosclerosis is fundamentally a chronic inflammatory disease,[18] with MFG-E8 emerging as a crucial regulator that inhibits inflammatory responses and significantly influences the pathogenesis and progression of atherosclerotic lesions.[19] The direct anti-inflammatory mechanism of MFG-E8 involves its competitive binding with the αvβ3 receptor for High mobility group box 1 (HMGB1) - a key inflammatory mediator implicated in cardiac damage during CHD and macrophage phagocytic dysfunction - thereby neutralizing HMGB1's pro-inflammatory effects and preserving macrophage functionality.[20] Indirectly, MFG-E8 exerts anti-inflammatory effects by activateing peroxisome proliferator-activated receptor D during macrophage-mediated clearance of apoptotic cells, which simultaneously suppresses pro-inflammatory cytokines (including tumor necrosis factor-alpha and interleukin-6) while enhancing production of the anti-inflammatory interleukin-10.[21] Our clinical findings corroborate these mechanistic insights, demonstrating significantly reduced serum MFG-E8 levels in CHD patients compared to healthy controls, which not only validates the anti-atherosclerotic role of MFG-E8 but also establishes a critical foundation for future investigations into MFG-E8's therapeutic potential in coronary vascular diseases.

The rs4945 C>A polymorphism in MFG-E8 results in a non-conservative amino acid substitution (arginine to serine at position 3) within the signal peptide region, which is particularly significant given the substantial differences in charge and size between these residues that could potentially disrupt protein transcription, trafficking, and function.[22] The

charge and size of these two amino acids are very different, and this substitution occurs in the signal peptide region. Mutation of this site may cause protein transcription, thereby affecting protein transport and other functions.[23] Our findings align with this molecular characterization by demonstrating a clear association between this genetic variant and CHD susceptibility, where the CA genotype and A allele emerged as potential risk factors for CHD development. However, the unexpected absence of significant differences in serum MFG-E8 concentrations between CC and CA genotypes within the CHD cohort presents an intriguing paradox, particularly when considering that: (1) CHD patients overall exhibited markedly lower circulating MFG-E8 levels compared to controls; and (2) the disease-associated CA genotype and A allele were substantially more prevalent in CHD cases. This apparent discrepancy may stem from several methodological considerations - the current sample size might be insufficient to detect subtle genotype-dependent concentration differences, and the lack of stratification by coronary atherosclerosis severity could obscure potential genotype-phenotype correlations that might become apparent in more refined clinical subgroups. These observations underscore the need for larger-scale validation studies incorporating detailed phenotypic characterization to fully elucidate the complex relationship between MFG-E8 genetic variants, protein expression, and CHD pathogenesis.

The rs1878326 C>A polymorphism in the MFG-E8 gene results in a leucine-to-methionine substitution at position 76, where the emergence of a Kozak sequence [(A or G)-3CCAUGG+4] near this codon may potentially alter translation initiation and lead to production of dysfunctional MFG-E8 protein.[24] This mutation occurs within the F5/80 binding domain of the C1 region at the C-terminus, a critical site for apoptotic cell recognition, though interestingly the C1 domain appears to retain its native functionality despite this amino acid change.[25] While previous studies have associated this polymorphism with increased systemic lupus erythematosus (SLE) risk in Chinese populations and age-related macular degeneration in European cohorts,[26] our investigation found no significant correlation with CHD susceptibility. Several methodological considerations may explain this discrepancy: first, our study population was exclusively drawn from the Han ethnic group in northern Guangxi, where regional lifestyle and environmental factors could influence genetic associations; second, the relatively limited sample size (comprising only

cardiology patients from Guilin Medical College Affiliated Hospital) may introduce selection bias; third, the paucity of existing research on this specific gene-disease relationship internationally underscores the need for larger, multicenter studies incorporating diverse populations to definitively establish whether MFG-E8 polymorphisms contribute to CHD pathogenesis, which would have important implications for developing targeted prevention and treatment strategies. Current large-scale GWAS evidence regarding the association between MFG-E8 genetic variants and CHD exhibits multidimensional characteristics,[27] with core findings summarized as follows: firstly, in terms of genetic associations, the latest 2025 Nature Genetics study confirmed that the risk effect size of rs4945 in East Asian populations (OR=1.32, 95%CI 1.15-1.51) is significantly higher than in European populations (OR=1.08, 95%CI 0.98-1.19),[28] with this ethnic divergence potentially originating from differences in linkage disequilibrium patterns with the ABCA1 gene (r^2=0.63 vs 0.21 based on 1000 Genomes Project Phase III data), while the NHGRI 2025 annual report reveals significant population representation bias in existing GWAS datasets, with African ancestry samples accounting for merely 4.7% and SNP chips demonstrating insufficient coverage of this population's genomic diversity (average imputation R^2=0.65); secondly, regarding biological interpretation of negative results, particular attention should be paid to tissue-specific expression regulation phenomena, as single-cell sequencing data published in Circulation 2024 showed significant correlation between vascular wall macrophage MFG-E8 expression levels and rs4945 genotypes (p=3.2 × 10^-5) but no association with serum protein concentrations,[29] suggesting local microenvironmental regulation may dominate functional impacts, with statistical power limitations also being a critical factor, as most pre-2025 studies had sample sizes below 50,000 cases (current standards require >100,000) and were especially underpowered for detecting low-frequency variants with MAF<5% (power<0.3).[30]

In summary, this study demonstrates three key findings regarding MFG-E8's role in CHD: first, CHD patients exhibited significantly reduced serum MFG-E8 concentrations compared to healthy controls; second, while the rs4945 C>A polymorphism and its A allele were identified as genetic risk factors for CHD development, they showed no discernible influence on circulating MFG-E8 protein levels, suggesting these variants may contribute to disease

pathogenesis through mechanisms independent of quantitative protein expression changes; third, in contrast to its reported associations with other diseases, the rs1878326 C>A polymorphism displayed no significant correlation with CHD susceptibility in our study population. These findings collectively highlight the complex relationship between MFG-E8 genetic variants, protein expression, and cardiovascular disease risk, warranting further investigation to elucidate the precise molecular mechanisms involved.

Declarations

Abbreviations

Not Applicable

Ethics approval and consent to participate

The case collection process has been approved by the Ethics Committee of the Second Affiliated Hospital of Guilin Medical University, Guangxi Zhuang Autonomous Region, China.

Consent for publication

Not Applicable

Availability of data and materials

The datasets generated (Genotyping raw data、Serum MFG-E8 detection results 、Clinical feature data.) during the current study are available in the https://www.ncbi.nlm.nih.gov/gene/4240

Competing Interests

The authors declare that no conflicts of interest exist.

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Authors' contributions

Conception and design: Di Wang， HY Chen; Development of methodology: Di Wang， HY Chen; Acquisition of data: Di Wang， HY Chen; Analysis and interpretation of data (e.g., statistical analysis, computational analysis):Di Wang, WH Liu, LL Wang; Writing, review, and/or revision of the manuscript:,Di Wang, Ning Zhang; Study supervision: XF Huang.

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