ANRIL GENE POLYMORPHISM RS1333040 AND THE ASSOCIATION WITH SEVERITY OF CORONARY ARTERY STENOSIS IN PATIENTS WITH CHRONIC CORONARY SYNDROME: A MULTICENTER STUDY

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ABSTRACT

Introduction: Several studies have indicated that the rs1333040 polymorphism of the *ANRIL* gene is associated with the severity of coronary artery stenosis; however, data remain inconsistent, particularly among patients with chronic coronary syndrome. **Objective:** To determine the characteristics of the rs1333040 polymorphism of the *ANRIL* gene and its association with the severity of coronary artery stenosis. **Materials and methods:** This cross-sectional descriptive analytical study was conducted on 79 patients diagnosed with chronic coronary syndrome treated at Can Tho University of Medicine and Pharmacy Hospital, Can Tho Central General Hospital, and Can Tho Cardiovascular Hospital from June 2024 to June 2025. **Results**: The mean age of the 79 patients included in the study was 67.35 ± 8.86 years, with the majority aged ≥ 60 years. The gender distribution was nearly equal. Regarding lifestyle, more than two-thirds of patients were sedentary, and half reported tobacco smoking. Hypertension was common among patients; approximately half were overweight or obese, had dyslipidemia, and one-third had diabetes mellitus. Genotype frequencies were 41.3% TT and 58.7% TC. The T and C allele frequencies were 72.2% and 27.8%, respectively. The TT genotype group exhibited significantly higher proportions of moderate (84.8% vs. 65.9%) and severe (14.3% vs. 0%) Gensini scores compared to the TC genotype group ($p < 0.001$). Similarly, the T allele was associated with significantly higher rates of moderate and severe Gensini scores compared to the C allele ($p = 0.012$). **Conclusion:** The T allele and TT genotype of the rs1333040 polymorphism in the *ANRIL* gene were significantly associated with higher proportions of moderate and severe coronary artery stenosis according to the Gensini score compared with the C allele and TC genotype in patients with chronic coronary syndrome.

Keywords: *coronary artery stenosis, Gensini score, gene polymorphism, rs1333040, ANRIL gene, chronic coronary syndrome (CCS).*

I. INTRODUCTION

Coronary artery disease (CAD) remains the leading cause of death worldwide, particularly chronic coronary syndrome (CCS), a term introduced at the European Society of Cardiology Congress in 2019 to replace previously used terminologies such as stable angina, stable coronary artery disease, chronic ischemic heart disease, or coronary insufficiency. CCS is characterized by slowly progressive atherosclerosis, resulting in narrowing of coronary arteries and reduced myocardial blood supply, causing chronic symptoms such as chest pain or exertional dyspnea. Modern genetic research has identified multiple gene variants associated with CAD risk, with the chromosome 9p21.3 locus recognized as one

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of the most significant genetic regions [5]. Within this locus, the *ANRIL* (Antisense Non-coding RNA in the INK4 Locus), also known as CDKN2B-AS1, encodes a long non-coding RNA (lncRNA) involved in regulating the expression of multiple genes associated with cell cycle progression, aging, and atherosclerosis [11]. The single nucleotide polymorphism (SNP) rs1333040 in the *ANRIL* gene has received considerable research attention due to its reported association with CAD risk. A recent meta-analysis demonstrated a statistically significant association between the rs1333040 and the risks of myocardial infarction and CAD. However, findings from studies in Asian populations have not been entirely consistent [8].

The Gensini score is a widely utilized quantitative assessment system for evaluating CAD severity, based on the degree of arterial stenosis, the specific locations, and the functional importance of the affected coronary arteries. Recent studies have begun investigating associations between genetic polymorphisms and Gensini scores, aiming to elucidate the genetic basis underlying CAD severity. Although substantial evidence supports the role of *ANRIL* gene polymorphisms in the pathogenesis of CAD, data regarding the specific association between rs1333040 polymorphism and the severity of coronary artery stenosis, particularly among patients with CCS, remains limited and inconclusive. Understanding this relationship is particularly important in the context of precision medicine, where identifying genetic prognostic factors may help improve risk stratification and personalized treatment strategies for patients with CAD.

II. MATERIALS AND METHODS

2.1. Study population

Participants included patients diagnosed with chronic coronary syndrome (CCS) at Can Tho University of Medicine and Pharmacy Hospital, Can Tho Central General Hospital, and Can Tho Cardiovascular Hospital between June 2024 and June 2025.

Inclusion criteria:

Patients diagnosed with CCS according to the European Society of Cardiology (ESC) 2019 criteria, defined as follows [10]:

- Clinical characteristics:

+ Patients suspected of having coronary artery disease with typical stable angina and/or dyspnea.

+ Patients presenting with newly-onset heart failure symptoms or reduced left ventricular function, with suspected coronary artery disease as the underlying cause.

- Laboratory tests:

+ Electrocardiogram (ECG): Resting ECG showing ST-segment depression ≥ 0.5 mm or symmetric T-wave inversion in at least two contiguous leads, or typical necrotic Q waves (≥ 40 milliseconds wide).

+ Troponin: Negative or non-dynamic changes in high-sensitivity Troponin T levels. Dynamic change is defined as an increase above the 99th percentile of the upper reference limit measured at 3 or 6 hours compared to baseline.

+ Echocardiography: Presence of regional wall-motion abnormalities.

All patients underwent coronary angiography.

Patients aged ≥ 18 years who provided informed consent were included in the study.

Exclusion criteria:

Patients with contraindications for percutaneous coronary angiography

according to the guidelines of the Ministry of Health [1]:

- Severe active infection.
- History of anaphylaxis to contrast agents.
- Severe renal failure (stage IV or V, eGFR <30 ml/min/1.73 m²).

2.2. Research methods

Study design: Analytical cross-sectional descriptive study.

Sample size: Convenience sampling method was employed, including all patients meeting the inclusion criteria and not meeting any exclusion criteria at Can Tho University of Medicine and Pharmacy Hospital, Can Tho Central General Hospital, and Can Tho Cardiovascular Hospital during the study period. Actually, 79 eligible subjects were enrolled.

Research variables:

General characteristics: age (mean ± SD, categorized as <60 years, ≥60 years), gender (male/female), smoking history (yes/no), sedentary lifestyle (yes/no), hypertension (yes/no), diabetes mellitus (yes/no), dyslipidemia (yes/no), overweight or obesity (defined as BMI ≥23 kg/m², yes/no).

Characteristics of rs1333040 polymorphism in the *ANRIL* gene: frequencies (%) of alleles (A, C) and genotypes (AA, AC, CC).

Gensini score: Calculated as the sum of all coronary lesions multiplied by their corresponding weighting factors and categorized into mild (<16 points), moderate (16-38 points), and severe (>38 points) [6].

Distribution of Gensini scores according to genotype and allele type of the rs1333040 *ANRIL* polymorphism.

Associations between genotype and allele type of rs1333040 *ANRIL* polymorphism and categorized Gensini scores (mild, moderate, severe).

Data analysis:

Data were initially entered using Microsoft Excel 365 and subsequently processed with R software version 4.5.0, using the libraries ggplot2, stats, and contingencytables. Quantitative variables were summarized as medians and interquartile ranges (IQR) due to non-normal distributions. Differences in Gensini scores between allele and genotype groups were assessed using the Mann–Whitney U test. The Fisher–Freeman–Halton exact test was applied to evaluate associations between genotypes/alleles and the three Gensini severity categories. A p-value < 0.05 was considered statistically significant.

2.3. Ethical consideration:

The study was approved by the Biomedical Research Ethics Committee of Can Tho University of Medicine and Pharmacy (Approval No. 25.016.HV/PCT-HĐĐĐ).

III. RESULTS

During the period from June 2024 to June 2025, there were 79 patients participated in the study.

Table 1. ***General characteristics of study participants (n = 79)***

Characteristics (n = 79)		Frequency (n)	Percentage (%)
Age, mean ± SD (year)		67.35 ± 8.86	
Age group	≥ 60	66	83.5
	< 60	13	16.5

Characteristics (n = 79)		Frequency (n)	Percentage (%)
Sex	Male	41	51.9
	Female	38	48.1
Overweight-obesity	Yes	40	50.6
	No	39	49.4
Smoking	Yes	37	48.6
	No	42	53.2
Sedentary lifestyle	Yes	54	68.4
	No	25	31.6
Dyslipidemia	Yes	54	68.4
	No	25	31.6
Diabetes	Yes	23	29.1
	No	56	70.9
Hypertension	Yes	74	93.7
	No	5	6.3

The mean age of participants was 67.35 ± 8.86 years, with most patients aged ≥ 60 years. The male-to-female ratio was approximately equal. Regarding lifestyle, over two-thirds of patients reported sedentary habits, and half were smokers. Hypertension was prevalent among patients, while about half had overweight or obesity, dyslipidemia, and approximately one-third had type 2 diabetes mellitus.

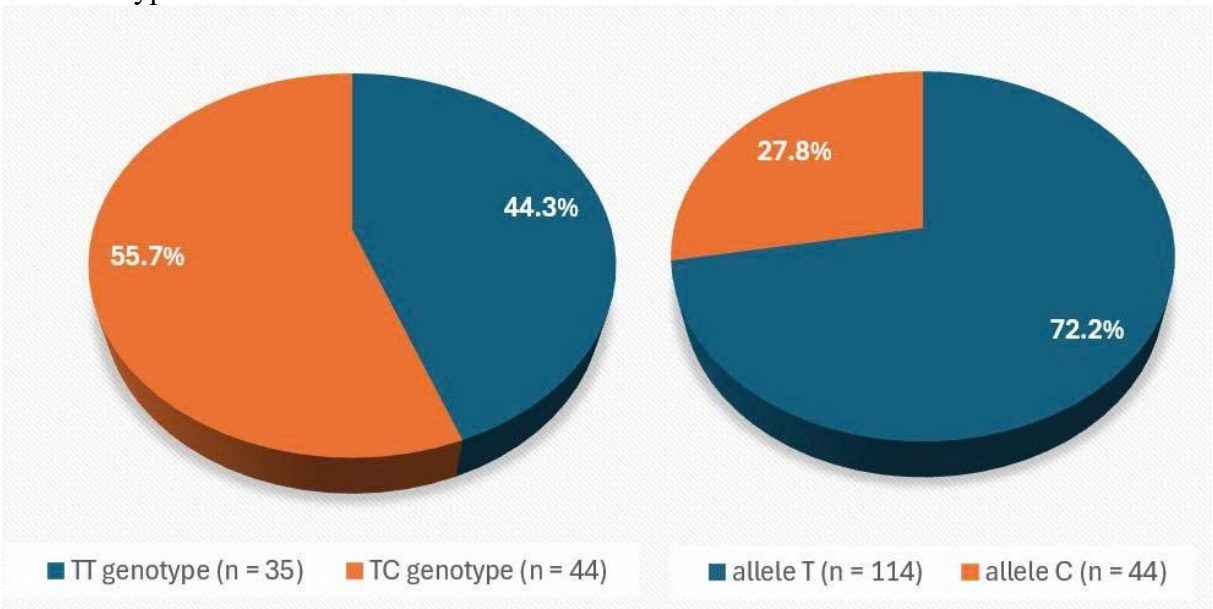

Figure 1. ***Genotype and allele frequencies of the rs1333040 polymorphism in the* ANRIL *gene***

Analysis of genotype distribution revealed that among 79 CCS patients, the frequencies of TT, TC, and CC genotypes were 44.3%, 55.7%, and 0%, respectively. The T allele was predominant, accounting for nearly three-quarters (72.2%) of total alleles..

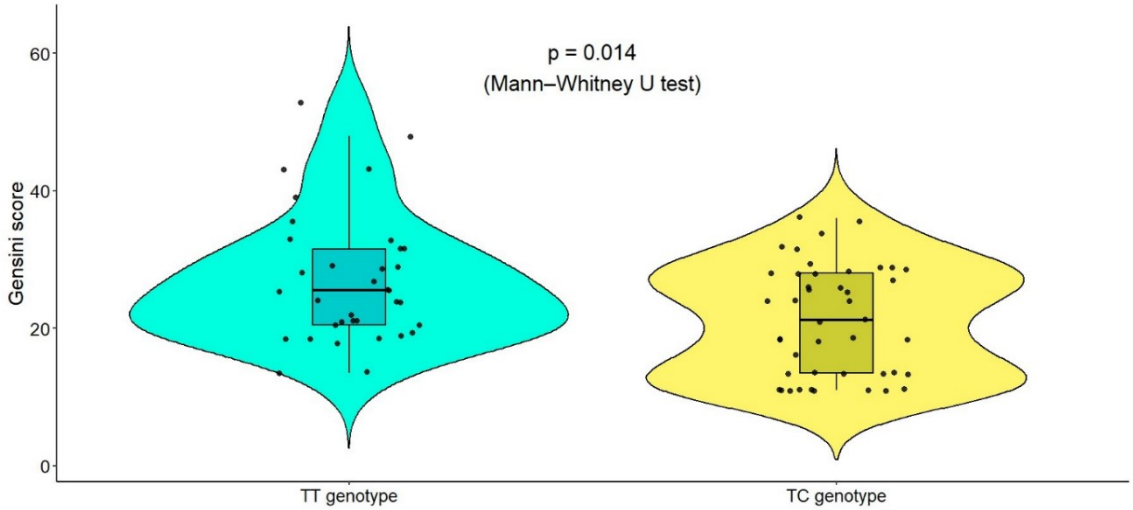

Figure 2. ***Distribution of Gensini scores according to TT and TC genotypes***

Analysis of Gensini score distribution by genotype (TT and TC) revealed a statistically significant difference between the two groups (p = 0.014). The TC genotype group tended to exhibit higher Gensini scores compared to the TT genotype, as demonstrated by a higher median value and a concentration of observations at higher scores.

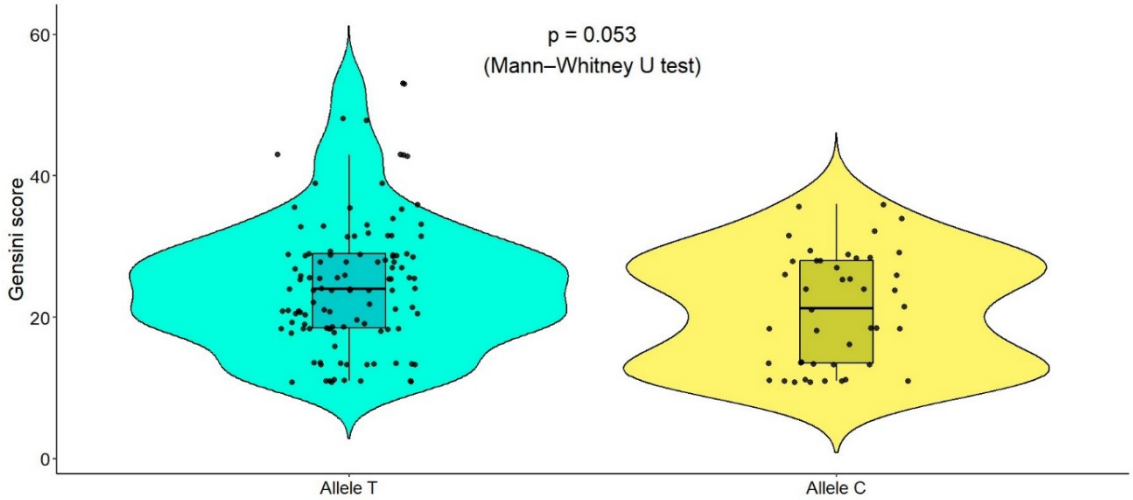

Figure 3. ***Distribution of Gensini scores according to T and C alleles***

The comparison of Gensini scores by individual alleles (T and C) did not show a statistically significant difference between the two allele groups (p = 0.053). However, there was a slight trend toward higher Gensini scores for allele T compared to allele C.

Table 2. ***The association of genotype and allele groups with the severity of coronary artery stenosis according to the Gensini score***

Characteristics		Mild	Moderate	Severe	p
Genotype	TT (n = 35)	2 (5.7)	28 (84.8)	5 (14.3)	**<0.001**
	TC (n = 44)	15 (34.1)	29 (65.9)	0 (0.0)	
Allele	T (n = 114)	19 (16.7)	85 (74.6)	10 (8.8)	**0.012**
	C (n = 44)	15 (34.1)	29 (65.9)	0 (0.0)	
Fisher Freeman Halton Exact Test					

Our findings showed that patients carrying the TT genotype had significantly higher proportions of moderate (84.8% vs. 65.9%) and severe (14.3% vs. 0%) Gensini scores compared to those with the TC genotype ($p < 0.001$). Similarly, the T allele was associated with significantly higher proportions of moderate and severe Gensini scores compared to the C allele ($p = 0.012$).

IV. DISCUSSION

This study included 79 patients recruited from three major cardiovascular centers in Can Tho city - Can Tho University of Medicine and Pharmacy Hospital, Can Tho Central General Hospital, and Can Tho Cardiovascular Hospital from June 2024 to June 2025. The genotype distribution of the *ANRIL* rs1333040 polymorphism among these patients was TT (44.3%), TC (55.7%), and CC (0%), with allele T predominating, accounting for approximately three-quarters (72.2%) of the total alleles. The T allele and the TT genotype were significantly associated with moderate to severe coronary artery stenosis as assessed by the Gensini score compared with the C allele and TC genotype.

Our results revealed that the average age of the 79 CCS patients was 67.35 ± 8.86 years, with most patients aged ≥ 60 years, a typical epidemiological characteristic consistent with other studies [4]. Regarding cardiovascular risk factors, more than two-thirds of patients had a sedentary lifestyle, and half were smokers, consistent with established risk factors previously reported in CAD patients. Additionally, the prevalence of hypertension, overweight/obesity, dyslipidemia, and diabetes mellitus was comparable to that observed in other Asian populations where metabolic syndrome is rapidly increasing. For instance, a study by Bui Minh Nghia on CCS patients in Vietnam reported a similar prevalence of overweight/obesity (62.6%), hypertension (58.7%), and diabetes mellitus (41.3%), aligning closely with our findings [4].

The genotype distribution in our study (TT 41.3% and TC 58.7%; T allele 72.2% and C allele 27.8%) differed from a previous study conducted in 80 Vietnamese patients with acute coronary syndrome (ACS), which reported TT genotype (58.8%), TC genotype (33.7%), and CC genotype (7.5%) [2]. These discrepancies might be attributable to phenotypic differences between chronic and acute coronary syndromes or natural genetic variations within subpopulations. Furthermore, the relatively small sample sizes in both studies could explain the observed variation in the CC genotype frequency. However, our observed allele frequencies were consistent with data from other Asian populations. For instance, a 2019 meta-analysis by Lina Hu et al. involving five studies of *ANRIL* rs1333040 polymorphisms reported that the T allele was more prevalent, particularly among Asian populations. Similarly, a Vietnamese study by Tran Thi Thu Lan on ACS patients reported a T allele frequency of 75.6% [2]. Another study by Tran Nguyen Thao Lien reported a 70% T allele frequency among acute myocardial infarction patients with hypertension [3].

Our results demonstrate a statistically significant association between the *ANRIL* rs1333040 polymorphism and the severity of coronary artery stenosis assessed by the Gensini score. Specifically, the TT genotype group had significantly higher rates of moderate (84.8% vs. 65.9%) and severe

(14.3% vs. 0%) Gensini scores compared with the TC genotype ($p<0.001$). Similarly, the T allele was associated with significantly higher moderate and severe Gensini scores compared to the C allele ($p=0.012$). Currently, direct evidence assessing the association between the rs1333040 polymorphism and coronary stenosis severity is scarce. However, clinical studies have demonstrated that the T allele and TT genotype are significant risk factors for CAD. Indeed, a meta-analysis by Lina Hu et al. reported an association between the T allele and CAD risk (OR = 1.25, 95% CI: 1.16–1.34, $p < 0.0001$). Dong-Ling Huang et al. also showed a significant correlation between the TT genotype and acute myocardial infarction ($p < 0.001$) [9].

In terms of mechanism, these findings align with experimental evidence highlighting the *ANRIL* gene's involvement in atherosclerosis. *ANRIL* acts as a regulatory RNA influencing the expression of CDKN2A/B genes, thus modulating cell cycle progression and vascular cell senescence. *ANRIL* also regulates vascular endothelial growth factor (VEGF) expression and activates the NF-κB pathway, promoting angiogenesis and inflammation [11]. At the molecular level, the rs1333040 polymorphism affects not only *ANRIL* expression but also RNA splicing, shifting the balance between linear and circular *ANRIL* forms. Genotype-dependent *ANRIL* expression impacts pathways critical to atherosclerosis progression [7]. Specifically, the TT genotype and T allele may promote linear *ANRIL* expression, enhancing vascular smooth muscle cell proliferation and migration, reducing apoptosis, and increasing inflammatory responses, contributing to more complex and stenotic atherosclerotic lesions. Furthermore, *ANRIL* affects ribosome biogenesis, altering protein synthesis in vascular cells, driving a phenotypic shift from contractile to synthetic cells - a hallmark of advanced, stenotic atherosclerotic lesions [7]. Thus, these molecular mechanisms may explain the association observed between the rs1333040 polymorphism and the severity of coronary artery stenosis measured by the Gensini score in our study.

Despite these significant findings, our study has several limitations. First, although this was a multicenter study, all participating hospitals were in Can Tho City, with a relatively small sample size limiting generalizability. Additionally, the cross-sectional design precludes direct causality inference. Several potential confounders influencing coronary stenosis severity were not fully collected or analyzed. Nevertheless, this study is among the first to explore the relationship between rs1333040 polymorphism and coronary stenosis severity in CCS patients. These preliminary findings serve as a foundation for future larger, well-designed studies to clarify the precise role of the rs1333040 polymorphism in CCS and other atherosclerotic cardiovascular diseases.

V. CONCLUSION

Our preliminary findings indicate that the T allele and TT genotype of the rs1333040 polymorphism in the *ANRIL* gene are associated with higher proportions of moderate and severe coronary artery stenosis, as assessed by the Gensini score, compared to the C allele and TC genotype in patients with chronic coronary syndrome.

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