Медицинская генетика 2023. Том 22. Номер 4
Medical genetics 2023. Vol. 22. Issue 4

https://doi.org/10.25557/2073-7998.2023.04.32-37

Полиморфизм гена ANRIL и предрасположенность к атеросклерозу

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Проведен метаанализ для оценки связи между полиморфизмом *ANRIL* rs2383207 и предрасположенностью к атеросклерозу. Для выявления подходящих исследований был проведен систематический поиск литературы в базах данных Google Scholar и PubMed. Всего для мета анализа было отобрано 12 исследований. Ассоциацию оценивали по статистическому отношению шансов (ОШ) с 95% доверительным интервалом (ДИ). Программное обеспечение RevMan (Cochrane Collaboration, 5.3. Копенгаген) использовали для мета анализа. При объединении исследуемых популяций в одну выборку показано, что ген *ANRIL* был связан с риском атеросклероза. Дальнейший стратифицированный анализ по этническому признаку показал, что ген *ANRIL* был связан с предрасположенностью к атеросклерозу как у азиатов, так и у европеоидов. Наши результаты показывают, что *ANRIL* может служить генетическим биомаркером атеросклероза. Для подтверждения наблюдаемой связи потребуются дальнейшие исследования.

Ключевые слова: *ANRIL*, днРНК, атеросклероз, метаанализ.

Для цитирования: Тимофеева С.В., Шерчкова Т.А., Шкурат Т.П. Полиморфизм гена *ANRIL* и предрасположенность к атеросклерозу. *Медицинская генетика* 2023; 22(4): 32-37.

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Финансирование. Исследование не имело внешнего финансирования.
Конфликт интересов: Авторы декларируют об отсутствии конфликта интересов.
Поступила 14.04.2023

ANRIL gene polymorphism and susceptibility to atherosclerosis

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We conducted this meta-analysis to estimate associations between polymorphisms of ANRIL rs2383207 and susceptibility to atherosclerosis. A systematic literature research of Google Scholar and PubMed was performed to identify eligible studies. Overall, 12 studies were included for meta-analyses. The association was assessed by statistical odds ratio (OR) with 95% confidence interval (CI). RevMan software (Cochrane Collaboration, 5.3. Copenhagen) was used for the meta-analysis. Pooled overall analyses showed that ANRIL was associated with risk of atherosclerosis in the whole population. Further analyses by ethnicity revealed that ANRIL was associated with susceptibility to atherosclerosis in Asians and Caucasions. Our results suggest that ANRIL, might serve as genetic biomarkers of atherosclerosis. Further studies will be required to confirm the observed association.

Keywords: ANRIL, lncRNA, atherosclerosis, metaanalysis

For citation: Timofeeva S.V., Sherchkova T.A., Shkurat T.P.*ANRIL* gene polymorphism and susceptibility to atherosclerosis. *Medical genetics [Medicinskaya genetika]* 2023; 22(4): 32-37.

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Funding. This research received no external funding.
Conflict of interest: The authors declare no conflict of interest
Accepted 14.04.2023

1. Introduction

Cardiovascular disease (CVD), including atherosclerosis, remain the leading causes of death worldwide [1]. A number of candidate genes are known to be involved in the etiopathogenesis of atherosclerosis, but at the same time, of great importance in the emergence and development atherosclerosis is influenced by epigenetic factors that regulate gene expression, including non-coding RNA (ncRNAs). NoncodingRNAs are transcribed but are not translated into proteins, performing their biological functions at the RNA level [2]. One of the

most intensively studied biomarkers in recent years are long non-coding RNAs (lncRNAs), a class of non-coding RNAs over 200 nucleotides in length. LncRNAs play a regulatory role in various biological processes, such as apoptosis, cell cycle, proliferation, cell differentiation, etc [3]. The development and progression of various diseases including atherosclerosis, may be associated with both activation and decrease in the expression of lncRNA in cells. Therefore, approaches to gene therapy aimed at activating or suppressing the expression of specific lncRNAs for atherosclerosis are currently being actively developed [4]. ANRIL is located at the human CDKN2A/B locus at 9p21.3. There is an assumption that 9p21.3 region of the genome affects the cell proliferation and can lead to CVD. The question of the influence of the 9p21.3 loci is also discussed on the integrity of the atherosclerotic plaque [5]. Recently, studies have already investigated potential associations between several variants CDKN2B-AS polymorphisms and the susceptibility to atherosclerosis. In contrast to our previous study for polymorphism rs2383207 in this loci [6], we expanded the sample by searching for slightly different keywords and found 4 more studies that were included in the meta-analysis.

2. Materials and methods

2.1. Literature Search and Inclusion Criteria

This meta-analysis conformed with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guideline [7]. Potentially related literature published prior to October 2022 were extracted from Google Scholar and PubMed using the following key phrases: (ANRIL OR CDKN2B-AS long non-coding RNA OR CDKN2B antisense RNA) and (allele OR mutation OR genotype OR variant OR polymorphism) and (atherosclerosis OR coronary heart disease) and (rs2383207).

The inclusion criteria for publications in this meta-analysis were: (1) Studies evaluating the association between the rs2383207 polymorphisms and the risk of atherosclerosis using the case-control method; (2) Diagnosis of atherosclerosis confirmed by ultrasound evaluation of the carotid arteries; (3) Studies with or without Hardy-Weinberg bias. Equlibrium (HWE) for the control group; (4) DNA copy number was measured using previously described methods based on PCR technology; (5) the sample only included adults over the age of 18; (6) all data were presented as OR and 95% CI.

The main exclusion criteria were: (1) reviews, short communications, conference abstracts, comments; (2) studies without case-control statistics; (3) studies without providing sample data; (4) research in the field of cerebrovascular diseases; (5) duplicate data.

2.2. Data Extraction and Quality Assessment

In accordance with the selection criteria, all publications were independently reviewed by two reviewers. The extracted data included basic information: (a) first author name, (b) year of publication, (c) country of origin, (d) ethnicity, (e) genotyping method, (f) sample size, (g) allele and genotype frequencies rs2383207. In addition, we also assessed the methodological quality of the publications included in our meta-analysis using the Newcastle–Ottawa scale (NOS) [8]. The NOS contains 8 items in 3 areas, and the total maximum score is 9 [9].

2.3. Statistical Analyses

The data were analyzed using Review Manager 5.3 software (RevMan 5.3) to calculate odds ratio (OR) with 95% confidence interval (CI). Heterogeneity was assessed in all studies using the Cochran and I squared tests [10, 11]. The total OR was calculated for the dominant (GG+CG vs.CC), recessive (GG vs. CC + CG), and allelic (C vs. G) models, as well as for the homozygous (GG vs. CC) and heterozygous (CG vs. CC) models. Z-scores were used to assess the statistical significance of pooled ORs, and a p-value <0.05 was considered statistically significant. I squared statistics were used to assess interstudy heterogeneity. If I squared was higher than 50%, random effects models were used to pool the data. Otherwise, fixed effect models were chosen. Subgroup analyses by ethnicity of participants and type of disease were also performed. Stabilities of synthetic results were evaluated with sensitivity analyses, and publication bias was evaluated with funnel plots.

3. Results

We found 138 potential relevant papers. Among these articles, 12 eligible studies were included for pooled analyses (Figure 1). The NOS score of eligible articles ranged from 7 to 8, which indicated that they were of high quality. Baseline characteristics of included studies are shown in **Table 1**.

3.1. Characteristics of Included Studies

In this meta-analysis, 13842 participants were included: 6383 in the atherosclerosis group and 7459 cases in the control group. Twelve articles were selected, which four investigated rs2383207 in Caucasian population [12-15], five investigated rs2383207 in Asian population [16-23].

The allele frequencies at each generation are obtained by pooling together the alleles from each genotype of the

same generation according to the expected contribution from the homozygote and heterozygote genotypes. All investigated polymorphisms contain a major allele (M) and a minor allele (m), the genotypic distributions of investigated polymorphisms were summarized by MM/Mm/mm.

3.2. Overall and Subgroup Analyses

To investigate potential associations of *ANRIL* rs2383207 polymorphism with susceptibility to atherosclerosis 12 studies were included for analyses. Significant association with atherosclerosis in total group was observed for rs2383207 only in dominant model (P =<0.00001, OR 0.84, 95%CI: 0.79 - 0.90) **Figure 2**.

No significant associations were observed in overall analyses for other genetic models (allele model: P= 0.96, OR 1.00, 95%CI: 0.87, 1.15; recessive model: P=0.60, OR 1.06, 95%CI: 0.86-1.29; heterozygous model: P =0.13, OR 0.94, 95%CI: 0.86-1.02; homozygous model: P =0.80, OR 1.04, 95%CI: 0.75-1.46).

Further subgroup analyses by ethnicity revealed that rs2383207 investigated polymorphism were significantly associated with susceptibility to atherosclerosis in Asians in several models (allele model: P =0.05, OR 0.89, 95%CI: 0.80-1.00; heterozygous model: P =0.04, OR 0.92, 95%CI: 0.85-1.00; dominant model: P = <0.00001, OR 0.84, 95%CI: 0.78-0.91). Moreover, rs2383207 polymorphism

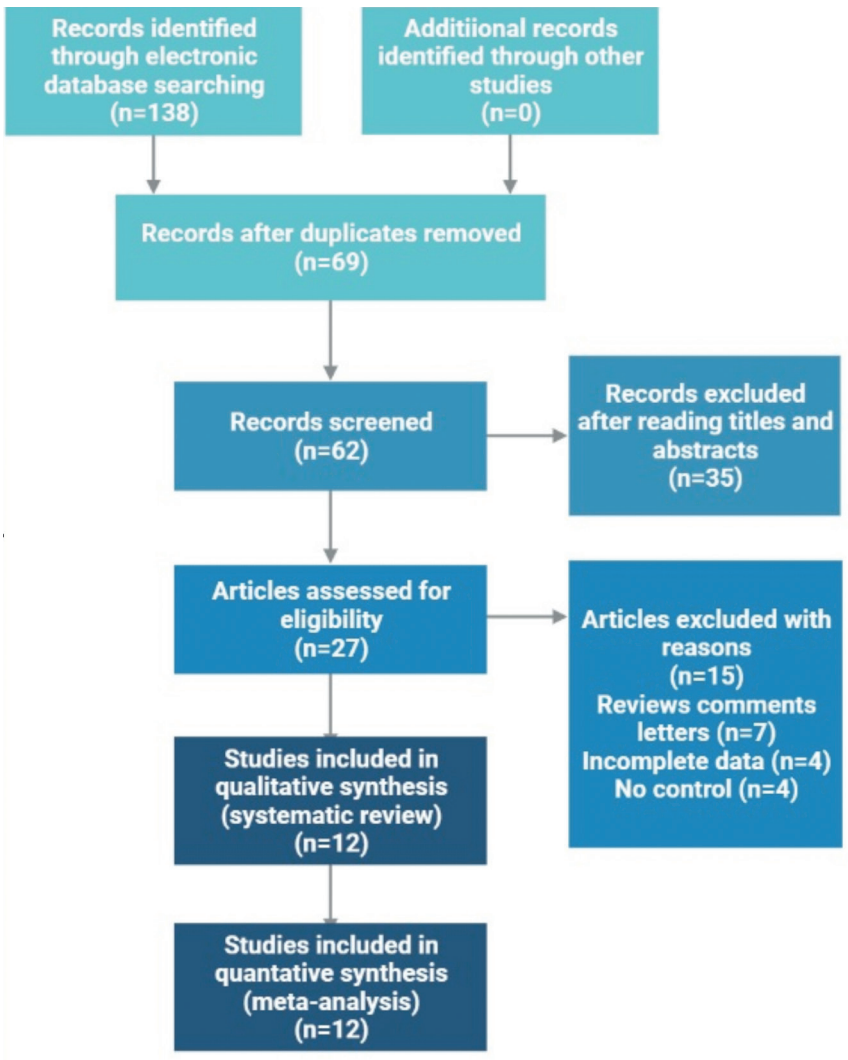

Рисунок 1. Блок-схема выбора исследований для настоящего анализа.

Figure 1. Flowchart of study selection for the present study.

https://doi.org/10.25557/2073-7998.2023.04.32-37 ***Medical genetics* 2023. Vol. 22. Issue 4**

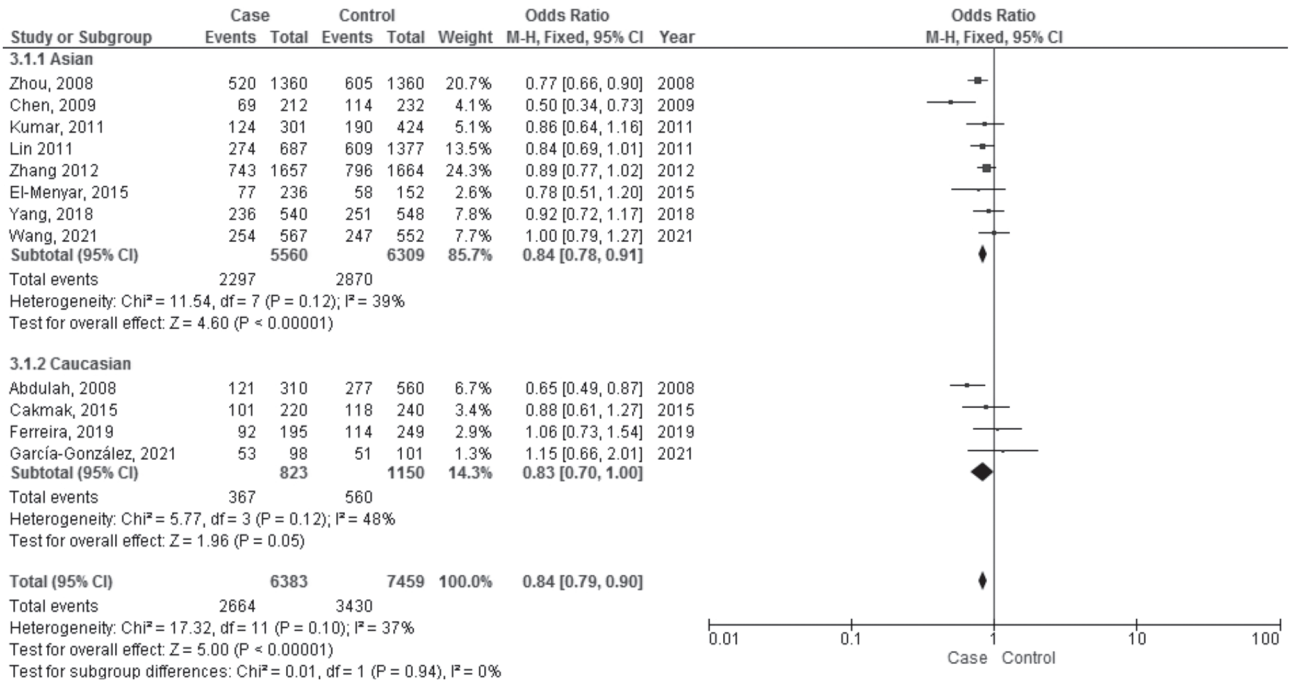

Рисунок 2. Результаты анализа доминантной генетической модели ассоциации ANRIL rs2383207 с атеросклерозом для европеоидного и азиатского населения с использованием модели с фиксированными эффектами.

Figure 2. Forest plots for dominant genetic models of ANRIL rs2383207 for Caucasian and Asian population, using a fixed-effects model.

Таблица 1. Характеристики включенных в анализ исследований

Table 1. The Characteristics of Included Studies

First author, year	Country	Ethnicity	Sample size	Genotype distribution (MM/Mm/mm)		P value for HWE	NOS score
				Cases control			
Abdulah, 2008	United States	Caucasian	310/560	139/121/50	147/277/136	0.88	8
Zhou, 2008	China	Asia	1360/1360	702/520/138	592/605/163	0.75	8
Chen, 2009	China	Asia	212/232	107/69/36	71/114/47	0.96	8
Kumar, 2011	India	Asia	301/424	137/124/40	174/190/60	0.63	7
Lin, 2011	China	Asia	687/1377	65/274/288	172/609/568	0.75	8
Zhang, 2012	China	Asia	1657/1664	214/743/700	216/796/652	0.42	8
Cakmak, 2015	Turkey	Caucasian	220/240	83/101/36	102/118/20	0.21	7
El-Menyar, 2015	Egypt	Asia	236/152	146/77/12	84/58/10	1	7
Yang, 2018	China	Asia	540/548	247/236/57	244/251/53	0.49	8
Ferreira, 2019	Brasilian	Caucasian	195/249	36/92/67	100/114/35	0.85	7
García-González, 2021	Yucatan	Caucasian	98/101	23/53/22	26/51/24	0.94	8
Wang, 2021	China	Asia	567/552	253/254/60	243/247/62	0.97	8

Abbreviations: HWE, Hardy-Weinberg equilibrium; NOS, Newcastle-Ottawa scale.

was associated with susceptibility to atherosclerosis in Caucasians (dominant model: P= 0.05, OR 0.83, 95%CI: 0.70 – 1.00), **Table 2**.

3.3 Sensitivity Analyses

We accomplished sensitivity analyses to test stabilities of pooled results by excluding studies that violated the HWE. Altered results were not noticed in any comparisons, which suggested that our findings were statistically stable.

3.4. Publication Biases

We used funnel plots to assess publication bias. We did not find obvious asymmetry of funnel plots in any comparisons, which suggested that our findings were unlikely to be severely impacted by publication bias.

4. Discussion

Recent studies have reported an association of the rs2383207 (A) risk allele with cardiovascular disease (CHD) [24], CAD manifestation [25], and CAD severity [22]. Akiniemi et al. reported that rs2383207 increased the incidence of ischemic stroke (IS) in native West African men [26]. In addition, GWAS has also been used to demonstrate that the CDKN2B-AS rs2383207 variant increases the risk of IS and coronary heart disease in the Caucasoid population [27, 28]. It is noteworthy that, studying variants of chromosome 9p21 in Chinese populations, they came to the conclusion that mutations in rs2383207 can reduce the risk of IS [24]. In the present study, we focused on the relationship between atherosclerosis and rs2383207.

This is not our first meta-analysis study on rs2383207 (9p21.3) polymorphism and the risk of atherosclerosis [6]. Previously we also conductected meta-analysis in Caucasian and Asian populations, but in this study we expanded count of samples. We changed keywords during the search of suitable publications. Thus, we included 13842 samples from participants in this meta-analysis. The atherosclerosis and control groups contained 6383 and 7459 individuals, respectively. Statistical significance existed only in heterozygous model comparisons of rs2383207. With the significant outcome in allele comparison, we can come to the conclusion that carrying the mutated allele G on rs2383207 associated with atherosclerosis. Subgroup analysis represented relation between rs2383207 and atherosclerosis for recessive model in Caucasian subgroup and for homozygous model in Asian population. However, studies include no environment factor such as smoking and alcohol.

Thus, rs2383207 is a variant of atherosclerosis susceptibility in Caucasian and Asian populations. Interestingly, even by expanding the sample, the overall results of our previous and this study do not differ, which only confirms the significance of the studied locus as a biomarker for the diagnosis of atherosclerosis. In our opinion, it is necessary

Таблица 2. Результаты общего анализа и анализа подгрупп.
Table 2. Results of Overall and Subgroup Analyses.

Genetic model	Population	Number of studies	Test of association		Test of heterogeneity		
			OR (95%CI)	P	P_h	I^2 ,%	M
C vs. G allele	Total	12	1.00 [0.87, 1.15]	0.96	<0.00001	85	R
	Asian	8	**0.89 [0.80, 1.00]**	**0.05**	**0.0008**	72	R
	Caucasian	4	1.35 [0.91, 2.00]	0.13	<0.0001	88	R
CG vs. CC hetero	Total	12	0.94 [0.86, 1.02]	0.13	<0.00001	84	R
	Asian	8	**0.92 [0.85, 1.00]**	**0.04**	**<0.00001**	80	R
	Caucasian	4	1.01 [0.75, 1.35]	0.96	<0.00001	91	R
GG vs. CC homo	Total	12	1.04 [0.75, 1.46]	0.80	<0.00001	87	R
	Asian	8	0.93 [0.72, 1.19]	0.54	0.001	71	R
	Caucasian	4	1.47 [0.40, 5.34]	0.56	<0.00001	95	R
GG+CGvs.CC dominant	Total	12	0.84 [0.79, 0.90]	**<0.00001**	0.10	37	F
	Asian	8	0.84 [0.78, 0.91]	**<0.00001**	0.12	39	F
	Caucasian	4	0.83 [0.70, 1.00]	**0.05**	0.12	48	F
GGvs.CC+CG recessive	Total	12	1.06 [0.86, 1.29]	0.60	<0.00001	76	R
	Asian	8	1.02 [0.93, 1.12]	0.64	0.42	1	F
	Caucasian	4	1.39 [0.59, 3.30]	0.45	<0.00001	92	R

Abbreviations: CI, confidence interval; OR, odds ratio; M, model; F, fixed model; R, random model.

to continue studying this polymorphic variant of ANRIL in conjunction with environmental factors.

5. Conclusion

Thus, based on the obtained results, it can be assumed that the ANRIL rs2383207 polymorphism may be associated with the risk of atherosclerosis among the Asian and Caucasian population. More studies with larger samples from other ethnic groups are needed to refute or confirm the current findings.

Author Contributions: Conceptualization, S.V.T. and T.P.S.; methodology, T.A.S.; formal analysis, S.V.T. and T.A.S.; data curation, T.A.S.; writing—original draft preparation, S.V.T.; writing—review and editing, T.P.S.; visualization, S.V.T.; supervision, T.P.S. All authors have read and agreed to the published version of the manuscript.

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