UDC 575.
https://doi.org/10.2298/GENSR2202933M
Original scientific article

MIR-146A GENE VARIANT RS2910164 MIGHT BE ASSOCIATED WITH CORONARY IN-STENT RESTENOSIS RISK: RESULTS FROM A PILOT STUDY AND META-ANALYSIS

Milica T. MIHAJLOVIC[1], Marija SAVIC VESELINOVIC[1], Mihajlo FARKIC[2], Katarina ZELJIC[1*]

[1]University of Belgrade, Faculty of Biology, Belgrade, Serbia
[2] "Dedinje" Cardiovascular Institute, Belgrade, Serbia

Mihajlovic M., M. Savic Veselinovic, M. Farkic, K. Zeljic (2022). *MIR-146A gene variant RS2910164 might be associated with coronary in-stent restenosis risk: results from a pilot study and meta-analysis*. - Genetika, Vol 54, No.2, 933-946.

Coronary in-stent restenosis (ISR) is an adverse effect that occurs in 20-35% of patients who have undergone percutaneous coronary intervention (PCI) with stent implantation. The fact that not all patients will develop ISR indicates that genetic factors contribute to ISR susceptibility. Previous studies have reported that various micro RNA (miRNA) molecules regulate biological processes underlying ISR development, including miR-146a which is involved in regulation of vascular smooth muscle cells proliferation and neointima formation. Nucleotide variants in miRNA genes can affect the function of mature miRNAs. *mir-146a* rs2910164 gene variant is located in the seed region of mature miR-146a, key region for the regulation of target mRNAs.

The current study aimed to examine the association between rs2910164 variant in *mir-146a* gene and coronary ISR risk in a group of Serbian patients and to enhance the study by performing a meta-analysis. Samples of peripheral blood were obtained from 61 patients who previously underwent PCI with stent implantation, 25 (41%) of which had angiographically confirmed ISR.

There were no significant differences in allele and genotype distribution of rs2910164 variant between patients with and without ISR. In a Serbian group of patients, the analyzed variant was not associated with the ISR risk. Results of the meta-analysis

Corresponding author: Katarina Zeljic, University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia, phone: +381638452350, E-mail: katarina.zeljic@bio.bg.ac.rs

showed that heterozygous GC genotype is associated with decreased risk to ISR (OR=0.475, P=0.006), indicating its protective role in ISR formation.

Key words: coronary in-stent restenosis, miR-146a, percutaneous coronary intervention, rs2910164

INTRODUCTION

Coronary heart disease (CHD), a type of ischemic cardiovascular disease, is one of the leading causes of mortality worldwide (https://www.who.int/health-topics/cardiovascular-diseases/#tab=tab_1). It occurs when coronary arteries are narrowed, usually as a consequence of atherosclerotic plaque forming. Percutaneous coronary intervention (PCI) with stent implantation is the most frequently performed intervention for restoring normal blood flow and blood vessel diameter. One of the important drawbacks of this procedure is the occurrence of coronary in-stent restenosis (ISR). ISR represents a narrowing of the blood vessel of more or equal to 50% of diameter stenosis in a previous stent implantation segment (CHENG *et al.,* 2019). This condition affects 20-35% of patients following the implantation of bare metal stents (BMS), while upon drug eluting stent (DES) implantation ISR incidence was decreased to 5-10% (PLEVA *et al*., 2018). ISR represents a non-specific immune response to an injury inflicted by stent implantation (BUCCHERI *et al.,* 2016). Forming of ISR is a complex process, consisting of several mechanisms: elastic recoil, thrombus formation, formation of neointima and remodeling of the blood vessel (BENNETT and O'SULLIVAN, 2001). Restenosis is shown to be an independent predictor of 4-year mortality, along with age, diabetes mellitus, smoking, left ventricular ejection fraction reduction, and gender (CASSESE *et al.,* 2015). Conditions that may occur together with ISR are hypertension, diabetes, elevated hsCRP and LDL-c, as well as longer stent and longer target lesion, when compared with CHD patients without ISR (ZHANG *et al.,* 2020). ISR is dependent on many factors: epidemiological, mechanical (procedural), and, as proposed in recent years, genetic and epigenetic factors. However, genetic background of ISR is not fully elucidated. Its comprehensive understanding is of importance in the search for potential biomarkers that might be applied in the identification of patients with the risk for ISR development.

Various non-coding RNAs have been identified to regulate processes related to ISR, among which microRNAs (miRNAs) are also extensively studied (LIU *et al.,* 2018). MicroRNAs are molecules 18-22 nucleotides long that regulate gene expression on post-transcriptional level by complementary binding of a miRNAs 5'-end (seed region) to a 3'-UTR of a target mRNA (GEBERT and MACRAE, 2019). This process initiates mRNA degradation or disables translation (KIM *et al.,* 2009; GEBERT and MACRAE, 2019). Any nucleotide change in the seed region could endanger the functional integrity of the miRNA, its processing, and affect the range of target RNAs by changed complementarity (JAZDZEWSKI *et al.,* 2008; JAZDZEWSKI *et al.,* 2009). Many biological processes, including cell differentiation, proliferation and apoptosis, are regulated by miRNAs (KIM *et al.,* 2009).

miR-146a has been shown to increase proliferation of vascular smooth muscle cells and neointimal hyperplasia, thus contributing to complex biological mechanisms of ISR formation (SUN *et al.,* 2011; DONG *et al.,* 2013; GARERI *et al.,* 2016). Also, increased miR-146a expression was reported in CHD patients with ISR after drug eluting stent implantation (ZHANG *et al.,*

2020). Gene variant rs2910164, a G to C substitution, has been identified in the pre-miR-146a, within the seed region of the 3p strand of miR-146a (JAZDZEWSKI *et al.*, 2008; RYAN *et al.*, 2010). Discordant results were obtained regarding the effects of this variant on miR-146a expression. There are reports that the C allele and CC genotype are associated with higher mature miR-146a expression (RAMKARAN *et al.*, 2014; XIONG *et al.*, 2014), while according to the others C allele negatively affects the levels of pre-miR-146a and mature miR-146a (JAZDZEWSKI *et al.*, 2008).

Despite therapeutic advances in the treatment of patients with coronary heart disease by PCI with stent implantation, the rate of ISR occurrence remains high. In order to manage patients with ISR risk, it is desirable to identify novel molecular biomarkers. Considering the base substitution site of rs2910164 variant, implications for its function, and role in cardiovascular diseases, it might be assumed that variant rs2910164 is associated with the ISR susceptibility. Therefore, the aim of the current study was to examine the association of *miR-146a* gene variant rs2910164 with the risk of coronary in-stent restenosis occurrence in the Serbian group of patients and to enhance the study by conducting a meta-analysis.

MATERIAL AND METHODS

Ethical approval

The ethics committee of the "Dedinje" Cardiovascular Institute approved the conduction of the current study according to the Helsinki declaration (number of ethical approval 3337, July 16th 2019). Informed consent was obtained from all patients included in the study.

Study population

The study group consisted of 61 patients who underwent repeat coronary angiography after PCI with stent implantation in the "Dedinje" Cardiovascular Institute, Belgrade, Serbia. All members of the study group were from the Serbian population, Caucasians of the same ethnicity. In 25 patients (41%) ISR was confirmed by coronary angiography, while 36 (59%) did not develop ISR at least nine months or maximum 12 months after PCI. Repeat coronary angiography was indicated by referring cardiologist due to reoccurrence of chest pain and/or non-invasive testing suggesting myocardial ischemia. Patients with confirmed ISR in place of bifurcation lesion, chronic total occlusion and primary PCI in acute myocardial infarction were not considered as candidates for the current study due to known high risk for development of ISR. From the included patients, peripheral blood samples were collected and stored with EDTA anticoagulant at -20℃ until DNA isolation. Following data and parameters were retrieved from the medical records: gender, age, risk factors for coronary heart disease (presence of diabetes, smoking status, hypercholesterolemia, arterial hypertension, statin use) and procedural indicator of intervention (type of stent, number of stented blood vessels, stent diameter, length of stented fragment).

DNA isolation

DNA was isolated from the collected peripheral blood by using a commercial GeneJet DNA isolation kit (Thermofisher Scientific, USA) according to the manufacturer's instructions, or by salting out method following in-house's developed protocol. Integrity of isolated DNA was analyzed by agarose gel-electrophoresis, while DNA concentration was measured by spectrophotometer. DNA was stored at -20℃until further analysis.

Genotyping

Genotyping of *mir-146a* gene variant rs2910164 was done by using commercially available TaqMan® SNP genotyping assay (assay ID: C_15946974_10) and 2xUniversal TaqMan Master Mix (Applied Biosystems, USA). The allelic discrimination was performed on a QuantStudio™ 3 Real Time PCR system (Applied Biosystems, USA). Obtained genotypes were automatically analyzed by software and inspected manually.

Statistical and in silico analysis

The obtained results in our study were analyzed in SPSS software, version 20.00 (IBM Inc., USA). Descriptive and non-parametric statistical tests were used. Contingency tables were analyzed by χ^2 test or Fisher's exact test when appropriate. Mann-Whitney U test was used for comparison of mean ranks of variables with continuous distribution. Odds ratio (OR) with 95% confidence interval (CI) was calculated by binary logistic regression. For calculation of adjusted OR, an adjustment was done by diabetes mellitus, as the most important confounding factor of ISR. Post-hoc power of the study was calculated by using on line calculator OpenEpi, version 3.01 (http://www.openepi.com/). P values were two-tailed and considered significant when p values were less than 0.05.

Prediction of the functional effect of analyzed gene variant rs2910164 was performed by *in silico* software RNAfold (http://rna.tbi.univie.ac.at/).

Meta-analysis

Search through the PubMed database was carried out by a different combination of keywords: miRNA-146a, miR-146a, micro RNA 146a, rs2910164, coronary in-stent restenosis, coronary in stent restenosis, coronary ISR, ISR. The last search was done on 27th November 2020. In order to be included in the meta-analysis, publications were selected if the following criteria were fulfilled: (a) original articles published in English; (b) studies that performed genotyping of *mir-146a* gene variant rs2910164 in patients with ISR and patients who did not develop ISR after PCI; (c) studies with clearly reported allele and/or genotype frequency in the publication. Exclusion criteria were as follows: studies published in other languages than English, those with no reported frequency of rs2910164 alleles and/or genotypes, and studies in which control group consisted of healthy controls that did not undergo PCI. Frequency of alleles and genotypes were collected from included studies and used for meta-analysis.

Meta-analysis was performed by using free software: MetaGenyo (MARTORELL-MARUGAN *et al.*, 2017) and OpenMeta Analyst (http://www.cebm.brown.edu/openmeta/index.html). The heterogeneity between the studies included in the meta-analysis was estimated by the inconsistency index (I^2) and the statistical

significance of the heterogeneity was determined by the Cohran's Q test. If heterogeneity was significant ($I^2 \geq 0.5$, $p<0.1$), data synthesis was calculated by random effects statistical model, otherwise, if there was no heterogeneity, fixed statistical model was used. Weighting values, which depend on the effect sizes of the included studies, were also taken into account. Association analysis was performed for multiple genetic models: allelic (C vs. G), recessive (CC vs. GC+GG), dominant (CC+GC vs. GG), overdominant (GC vs. CC+GG) and pairwise (CC vs. GG, CC vs. GC, GC vs. GG). For each model OR with 95% CI was calculated. The results of individual studies included in the meta-analysis and quantitative synthesis of the results are presented graphically by the Forest plot, generated in the OpenMeta Analyst software.

RESULTS

The demographic, clinical and procedural characteristics of patients involved in the study, and genotype and allele distribution of *miR-146a* rs2910164 variant are presented in Table 1. There were no significant differences in gender, age, presence of diabetes mellitus, smoking status, hyperlipoproteinemia, left ventricular ejection fraction, arterial hypertension, stent type, number of stented blood vessels, stent diameter, length of stented fragment, genotypes and alleles distribution between the group of patients with and without ISR. There were no departures from Hardy-Weinberg equilibrium for rs2910164 variant in the group of patients with (P=0.692) and without ISR (P=0.812).

Table 2 presents the association of analyzed gene variant rs2910164 with the occurrence of coronary in-stent restenosis risk. According to the OR results, joint heterozygous CG and mutated CC genotypes compared with *wild type* GG, were not associated with the risk of coronary ISR. The absence of association was also noticed after adjustment by diabetes mellitus status in patients, as an important etiological factor for coronary ISR (Table 2).

There was no significant difference in the distribution of *mir-146a* rs2910164 gene variant genotypes in a group of coronary ISR patients according to demographic, clinical and procedural characteristics (Table 3).

The absence of associations in our pilot study might be a result of the small sample size and low statistical power (post-hoc power=18.6%). In order to overcome problems of the small study group, conduction of a meta-analysis was justified. For performing a meta-analysis, the minimal number of studies is two (VALENTINE *et al.*, 2010). Only one study fulfilled all inclusion criteria (HAMANN *et al.*, 2014) and was analyzed in a meta-analysis together with the data obtained in our pilot study. The pooled data of the meta-analysis included 126 patients with ISR, and 141 without ISR. The overall results for all tested genetic models in the meta-analysis are summarized in Table 4. rs2910164 variant was associated with the overall decreased susceptibility to ISR ($OR_{CG\ vs.\ GG}$=0.475, 95% CI=0.280-0.806, P=0.006; $OR_{overdom}$=0.430, 95% CI=0.255-0.725, P=0.002). The results of the meta-analysis showed that the heterozygous GC genotype significantly reduced the risk for the occurrence of coronary ISR compared with the GG genotype (P=0.006) (Table 4, Figure 1). All other tested genetic models (allelic, recessive, dominant, pairwise CC vs. GG and CC vs. CG) were not associated with susceptibility to coronary in-stent restenosis.

Table 1. Demographic, clinical and procedural characteristics of the patients with and without coronary in-stent restenosis and rs2910164 genotype and allele distribution.

		With ISR		Without ISR		P
		N	%	N	%	
Gender	male	21	84	27	75	0.530
	female	4	16	9	25	
Age	mean ± SD	61.88 ± 6.84		61.44 ± 9.45		0.849‡
Diabetes mellitus	yes	13	52	10	27.8	0.066
	no	12	48	26	72.2	
Smoking status	active smoker	2	8	5	13.9	
	non smoker	11	44	15	41.7	0.775
	ex smoker	12	48	16	44.4	
HLP	yes	18	72	31	86.1	0.203
	no	7	28	5	13.9	
HTA	yes	22	88	32	88.9	1.000
	no	3	12	4	11.1	
LVEF	mean ± SD	45.80 ± 10.38		48.03 ± 9.64		0.457‡
Statin dose*	20mg	8	32	8	22.2	0.555
	40mg	17	68	28	77.8	
Stent type	BMS	14	56	21	58.3	0.862
	DES	11	44	15	41.7	
Number of stented blood vessels	mean ± SD	1.04 ± 0.200		1.06 ± 0.232		0.784‡
Stent diameter	mean ± SD	3.14 ± 0.402		3.229 ± 0.497		0.549‡
Length of stented segment	mean ± SD	23.92 ± 5.972		20.58 ± 6.674		0.062‡
Time from PCI to repeat angiography†	mean ± SD	10.24 ± 1.052		10.50±1.231		0.385‡
mir-146a rs2910164	GG	18	72	22	61.1	
	GC	7	28	13	36.1	0.533
	CC	0	0	1	2.8	
	G	43	86	57	79.2	0.334
	C	7	14	15	20.8	

*Equivalent of atorvastatin dose.

† Time in months from PCI to repeat angiography and in-stent restenosis diagnosis.

‡Mann-Whitney U test

ISR – in-stent restenosis; N – total number of patients; SD – standard deviation;

HLP – hyperlipoproteinemia; HTA – arterial hypertension; LVEF – left ventricular ejection fraction; BMS - bare metal stent; DES - drug eluting stent; PCI - percutaneous coronary intervention.

Table 2. mir-146a gene variant rs2910164 association with coronary in-stent restenosis risk.

mir-146a rs2910164	OR (95%CI)	P	Adjusted OR* (95%CI)	P
GG	1.000	Ref.	1.000	Ref.
GC	0.658 (0.217-1.997)	0.460	0.596 (0.186-1.907)	0.384
CC	NC	NC	NC	NC
Dominant (GC+CC vs. GG)	0.611 (0.203-1.837)	0.380	0.538 (0.170-1.701)	0.291

OR - odds ratio; 95% CI - 95% confidence interval; Ref – referent; NC – not calculated; vs. - versus

*Adjustment by diabetes mellitus

Table 3. mir-146a gene variant rs2910164 association with demographic, clinical and procedural characteristics of patients with coronary in-stent restenosis.

Variable		*mir-146a* rs2910164			P
		GG	GC	CC	
Gender	male	15	6	0	1.000
	female	3	1	0	
Diabetes mellitus	yes	10	3	0	0.673
	no	8	4	0	
Smoking status	active smoker	2	0	0	0.201
	non smoker	6	5	0	
	ex smoker	10	2	0	
HLP	yes	14	4	0	0.355
	no	4	3	0	
HTA	yes	1	2	0	0.180
	no	17	5	0	
Stent type	BMS	8	6	0	0.090
	DES	10	1	0	

HLP – hyperlipoproteinemia; HTA – arterial hypertension; BMS - bare metal stent; DES - drug eluting stent

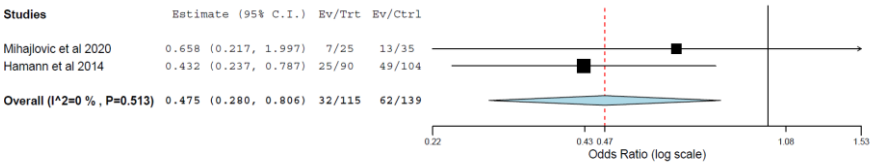
Table 4. Meta-analysis of association between variant rs2910164 in miR-146a gene and risk for coronary in-stent restenosis.

Genetic models	Overall OR (95% CI)	P	Test of heterogeneity	
			I^2 (%)	P
Allelic (C vs. G)	0.877 (0.581- 1.324)	0.533	0	0.442
Recessive (CC vs. GC+GG)	3.103 (0.125 - 76.756)	0.489	65	0.091
Dominant (GC + CC vs. GG)	0.609 (0.370 - 1.003)	0.052	0	0.996
Overdominant (GC vs. CC + GG)	0.430 (0.255 - 0.725)	**0.002**	0	0.345
CC vs. GG	3.762 (0.652 - 21.699)	0.138	60.4	0.112
CC vs. GC	4.574 (0.141 - 148.216)	0.392	68.6	0.074
GC vs. GG	0.475 (0.280 - 0.805)	**0.006**	0	0.513

OR - odds ratio; 95% CI - 95% confidence interval; I^2 – Inconsistency index; vs. – versus

P values less than 0.05 are indicated in bold.

(a)



(b)

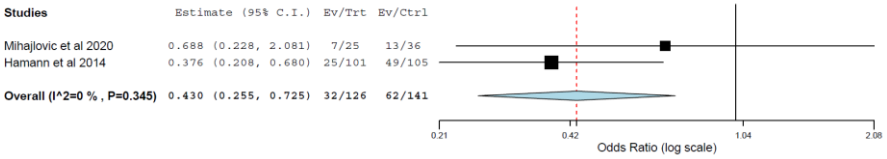


Figure 1. Meta-analysis of the association between rs2910164 variant in *mir-146a* gene and risk to coronary in-stent restenosis – forest plots of significant genetic models (a) pair-wise GC vs. GG (b) overdominant (GC vs. CC + GG)

P values are from heterogeneity test; I^2 – I^2 inconsistency index; OR - odds ratio; 95% CI - 95% confidence interval

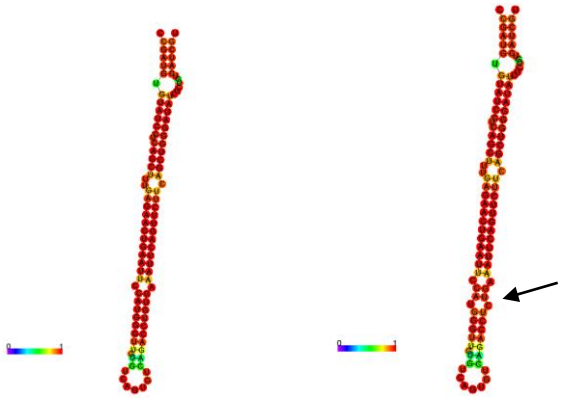


Figure 2. Secondary structure of pre-miR-146a depending on allele rs2910164 variant

(a) G allele (G=-43.33 kcal/mol) (b) C allele (G=-40.49 kcal/mol) in pre-miR-146a

G – Gibs free energy. Arrow indicates the position of the C allele and consequent changes in the secondary structure of pre-miR-146a. The figure was generated in the RNA fold software.

Performed *in silico* analysis by using RNAfold, indicated that depending on the present allele of the rs2910164 variant, there were differences in the secondary structure and thermodynamic stability in the pre-miR-146a (Figure 2.). However, this result should be taken with caution, since it might not necessarily imply real biological effects.

DISCUSSION

Since ISR presents the major drawback of PCI with stent implantation and still occurs in up to 30% of patients, it is of high importance to search for potential molecular biomarkers that can be used in the identification of patients at risk for ISR development. Due to their role in regulation of key biological processes, miRNAs, including miR-146a, have also been recognized as important novel players in ISR development. As a potential genetic component, this study has investigated the association of the rs2910164 variant in *mir-146a* gene with the risk for ISR. There was no significant difference in the distribution of the alleles and genotypes of the analyzed variant between patients with and without ISR. According to the OR analysis, heterozygous genotype was not associated with ISR risk, even after including diabetes mellitus as a confounding factor. Our results are in line with findings in Mexican patients (FRAGOSO *et al.,* 2019). The absence of association in our study might be attributed to the small sample size and low statistical power. A small cohort size is not representative of the entire population and diminishes the chances of identifying a variant associated with the disease. It has been suggested that studies related to ISR should contain more than twenty thousand examinees to have sufficient statistical power (HAMANN *et al.,* 2014). Apart from the size of the study group, the success of an association also depends on population stratification. It implies different frequencies of alleles and genotypes in different subpopulations, making a variant associated with the disease in one subpopulation, but not in the other. Even though the *miR-146a* rs2910164 gene variant was not associated with the risk for ISR in a Serbian group of patients, conducted meta-analysis confirmed that enlarging the study group could increase the power of the study in the detection of the significant association. That justifies the continuation of studies of rs2910164 association with ISR risk in a larger group of patients of different ethnicities.

In order to overcome the limitations of a small study group, a meta-analysis was performed. In the meta-analysis, which included results from the current pilot study and HAMANN *et al.* (2014), heterozygous GC genotype was associated with decreased ISR risk. That indicates a protective effect of heterozygous compared with GG genotype. The protective effect of the GC genotype might be explained by the simultaneous presence of three functional miRNAs: miR-146a, from the leading strand and miR-146a*G and miR-146a*C isoforms, from the passenger strand (JAZDZEWSKI *et al.,* 2009). In this way, every miR-146a isoform regulates a different set of target mRNAs. Heterozygosity in this context contributes to epistasis (JAZDZEWSKI *et al.,* 2009), as gene variants of miRNA can have different type of interactions with other coding and non-coding genes (HUFFAKER *et al.,* 2012; LI *et al.,* 2020). It has been reported that processes altered with GC genotype are apoptosis, localization, cell differentiation and blood vessel development (JAZDZEWSKI *et al.,* 2009). Our *in silico* analysis showed a difference in the secondary structure and thermodynamic stability in the pre-miR-146a, depending on which allele of the rs2910164 variant was present. This could affect mature miRNA through the choice of the lead strand, which could be influenced by thermodynamic

stability and the processing of the pre-miR-146a. Guide strand usually originates from the strand with lower 5'-end stability, although it is not always the rule: functional miRNAs could be produced from both strands (RYAN *et al.,* 2010; O'BRIEN *et al.,* 2018). Results of *in silico* analyses may be used as guidance for appropriate functional studies, as these results may not reflect *in vitro* or *in vivo* conditions. In order to fully elucidate the biological mechanism of decreasing the risk of ISR related to the heterozygous genotype of rs2910164 variant requires further in-depth functional studies. Noticed association might also be a result of linkage disequilibrium of analyzed rs2910164 variant with other variants with functional effects in the same or surrounding genes.

Our results showed that the overdominant genetic model (GC vs. CC+GG) was associated with decreased ISR risk. This might be explained by low frequency of CC genotype in our analysis (none detected in ISR patients and 2.8% in a group without ISR in our study; in the study by HAMANN *et al.* (2014) 10.9% in ISR and 1% in a group without ISR). The low frequency of CC genotype in our and the study by HAMANN *et al.* (2014) might be explained by the fact that genetic variants in the stem and loop regions of pre-miRNAs, such as rs2910164, are more frequent compared to variants that could influence its function (SAUNDERS *et al.,* 2007). The low frequency of functionally significant variants might be a result of its elimination by purifying selection (SAUNDERS *et al.,* 2007).

It is interesting to notice that CC genotype was associated with an increased risk to ISR in the study by HAMANN *et al.* (2014). However, the results of the meta-analysis did not confirm association from a single study, probably as there were no ISR patients with CC genotype in our study.

Complex conditions, such as ISR, arise as a result of the interaction of genetic background and environmental factors. Apart from gene variants that might contribute to ISR susceptibility, clinical and procedural characteristics should be taken into consideration. For instance, it is known that the type of implanted stent, particularly BMS, is associated with a greater risk to ISR development. DESs almost completely replaced BMSs in clinical practice, reducing the rate of restenosis to <10% (MOSES *et al.,* 2003; STONE *et al.,* 2004).

Results obtained in association studies considering only one gene variant have its limitations. Complex conditions, such as ISR can rarely have one major cause because there are many variants with different frequencies in different genes that could contribute to ISR development. Detailed insight into the complex molecular etiology of ISR might be provided by high throughput methods, such as NGS and genome wide association studies (LEOPOLD and LOSCALZO, 2018). The origin of complex phenotypes can be dependent on multiple genes, where *mir-146a* is just one of the many causal genes contributing to ISR. In this context interactions between various miRNA genes as well as between miRNA and their target genes are important. Also, the pleiotropic effect of miR-146a can be inferred from the fact that it is associated with immune response in different organs, but further functional annotation associated with miR-146a is needed (RUSCA and MONTICELLI, 2011; PRICE *et al.,* 2015; MARSCHNER *et al.,* 2020). Expression levels of miR-146a are different in various diseases depending on the alleles and genotypes present (JAZDZEWSKI *et al.,* 2008; RAMKARAN *et al.,* 2014; XIONG *et al.,* 2014). This fact emphasizes the need to elucidate the relation between gene variant and its function. Further studies on a larger group of patients that will include gene-gene and gene-environment

interaction, as well as functional analysis, would provide more information on the role and functional relevance of rs2910164 in ISR occurrence.

To sum up, although no association of rs2910164 variant in *mir-146a* gene and ISR risk was detected in a Serbian group of patients, the employed meta-analysis revealed that heterozygous GC genotype has a potential protective effect by decreasing risk to ISR development. These findings justify a further examination of rs2910164 gene variant, as a potential molecular biomarker for the identification of patients with the risk of developing ISR after PCI. In order to fully confirm the noticed association, a larger group of patients of different ethnicities is needed. Elucidation of protective effects of heterozygous genotype should be studied in further mechanistic studies.

ACKNOWLEDGEMENTS

This study was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia, number: 451-03-9/2021-14/200178.

Disclosure form: The study was derived from the MSc thesis of Milica T. Mihajlovic. Part of the results from this study was presented at the VI Congress of the Genetic society of Serbia in the form of an abstract and poster presentation.

Received, December 24th, 2021
Accepted June 28th, 2022

REFERENCES

BENNETT, M.R., M., O'SULLIVAN (2001): Mechanisms of angioplasty and stent restenosis: implications for design of rational therapy. Pharmacol Ther., *91*(2): 149-166.

BUCCHERI, D., D., PIRAINO, G., ANDOLINA, B., CORTESE (2016): Understanding and managing in-stent restenosis: a review of clinical data, from pathogenesis to treatment. J. Thorac. Dis., *8*(10): E1150-E1162.

CASSESE, S., R.A., BYRNE, S., SCHULZ, HOPPMAN, J., KREUTZER, A., FEUCHTENBERGER, T., IBRAHIM, I., OTT, M., FUSARO, H., SCHUNKERT, K.L., LAUGWITZ, A., KASTRATI (2015): Prognostic role of restenosis in 10 004 patients undergoing routine control angiography after coronary stenting. Eur. Heart J., *36*(2): 94-9.

CHENG, G., F.J., CHANG, Y., WANG, P.H., YOU, H.C., CHEN, W., HAN, J., WANG, N., ZHONG, Z., MIN (2019): Factors Influencing Stent Restenosis After Percutaneous Coronary Intervention in Patients with Coronary Heart Disease: A Clinical Trial Based on 1-Year Follow-Up. Med. Sci. Monit., *25*: 240-247.

DONG, S., W., XIONG, J., YUAN, J., LI, J., LIU, X., XU (2013): MiRNA-146a regulates the maturation and differentiation of vascular smooth muscle cells by targeting NF-κB expression. Mol. Med. Rep., *8*(2): 407-412.

FRAGOSO, J.M., J., RAMÍREZ-BELLO, M.A., MARTÍNEZ-RÍOS, M.A., PEÑA-DUQUE, R., POSADAS-SÁNCHEZ, H., DELGADILLO-RODRÍGUEZ, M., JIMÉNEZ-MORALES, C., POSADAS-ROMERO, G., VARGAS-ALARCÓN (2019): miR-196a2 (rs11614913) polymorphism is associated with coronary artery disease, but not with in-stent coronary restenosis. Inflammation Res., *68*(3): 215-221.

GARERI, C., S., DE ROSSA, C., INDOLFI (2016): MicroRNAs for Restenosis and Thrombosis After Vascular Injury. Circ Res, *118*(7):1170-1184.

GEBERT, L.F., I.J., MACRAE (2019): Regulation of microRNA function in animals. Nature reviews Mol Cell Biol, *20*(1): 21-37.

HAMANN, L., C., GLAESER, S., SCHULZ, M., GROSS, A., FRANKE, U., NÖTHLINGS, R.R., SCHUMANN (2014): A micro RNA-146a polymorphism is associated with coronary restenosis. Int. J. Immunogenet, *41*(5):393-396.

HUFFAKER, T., HU, R., RUNTSCH, M., BAKE, E., CHEN, X., ZHAO, J., ROUND, J., BALTIMORE, R., O'CONNELL (2012): Epistasis between MicroRNAs 155 and 146a during T Cell-Mediated Antitumor Immunity. Cell Reports, *2*: 1697–1709.

JAZDZEWSKI, K., E.L., MURRAY, K., FRANSILLA, B., JARZAB, D.R., SCHOENBERG, A., DE LA CHAPELLE (2008): Common SNP in pre-miR-146a decreases mature miR expression and predisposes to papillary thyroid carcinoma. Proc. Nac. Acad. Sci. USA, *105*(20): 7269-7274.

JAZDZEWSKI, K., S., LIYANARACHCHI, M., SWIERNIAK, J., PACHUCKI, M.D., RINGEL, B., JARZAB, A., DE LA CHAPELLE (2009): Polymorphic mature microRNAs from passenger strand of pre-miR-146a contribute to thyroid cancer. Proc. Nac. Acad. Sci. USA, *106*(5)*:*1502-1505.

KIM, V.N., J., HAN, M.C., SIOMI (2009): Biogenesis of small RNAs in animals. Nat. Rev. Mol. Cell. Biol., *10*(2):126–139.

LEOPOLD, J.A., J., LOSCALZO (2018): Emerging Role of Precision Medicine in Cardiovascular Disease. Circ. Res., *122*(9): 1302-1315.

LI, Y., H., CHO, F., WANG, O., CANELA-XANDRI, C., LUO, K., RAWLIK, S., ARCHACKI C., XU, A., TENESA, Q., CHEN, Q., WANG (2020): Epistasis of Cardiovascular Risk Genomic Variants From Genome-Wide Association Studies. J. Am. Heart Assoc., *9*:e014146.

LIU, S., Y., YANG, S., JIANG, N., TANG, J., TIAN, M., PONNUSAMY, M.A., TARIQ, Z., LIAN, H., XIN, T., YU (2018): Understanding the role of non-coding RNA (ncRNA) in stent restenosis. Atherosclerosis, *272*: 153-161.

MARSCHNER, D., M., FALK, N.R., JAVORNICZKY, K., HANKE-MÜLLER, J., RAWLUK, A., SCHMITT-GRAEFF, F., SIMONETTA, E., HARING, S., DICKS, M., KU, S., DUQUESNE, K., AUMANN, D., RAFEI-SHAMSABADI, F., MEISS, P., MARSCHNER, M., BOERRIES, R.S., NEGRIN, J., DUYSTER, R., ZEISER, N., KÖHLER (2020): MicroRNA-146a regulates immune-related adverse events caused by immune checkpoint inhibitors. JCI Insight, *5*(6): e132334.

MARTORELL-MARUGAN, J., D., TORO-DOMINGUEZ, M.E., ALARCON-RIQUELME, P., CARMONA-SAEZ (2017). MetaGenyo: a web tool for meta-analysis of genetic association studies. BMC bioinformatics, *18*(1): 563.

MOSES, J.W., M.B., LEON, J.J., POPMA, P.J., FITZGERALD, D.R., HOLMES, C., O'SHAUGHNESSY, R.P., CAPUTO, D.J., KEREIAKES, D.O., WILLIAMS, P.S., TEIRSTEIN, J.L., JAEGER, R.E., KUNTZ, SIRIUS INVESTIGATORS (2003): Sirolimus eluting stents versus standard stents in patients with stenosis in a native coronary artery. N. Engl. J. Med., *349*(14): 1315-1323.

O'BRIEN, J., H., HAYDER, Y., ZAYED, C., PENG (2018): Overview of MicroRNA Biogenesis, Mechanisms of Actions, and Circulation. Front Endocrinol (Lausanne), *9*:402.

PLEVA, L., P., KUKLA, O., HLINOMAZ (2018): Treatment of coronary in-stent restenosis: a systematic review. J. Geriatr. Cardiol., *15*(2):173-184.

PRICE, A.L., C.C.A., SPENCER, P., DONNELLY (2015): Progress and promise in understanding the genetic basis of common diseases. Proc. Biol. Sci., *282*(1821):20151684.

RAMKARAN, P., S., KHAN, A., PHULUKDAREE, D., MOODLEY, A.A., CHUTURGOON (2014): miR-146a Polymorphism Influences Levels of miR-146a, IRAK-1, and TRAF-6 in Young Patients with Coronary Artery Disease. Cell Biochem. Biophys., *68*(2): 259-266.

RUSCA, N., S., MONTICELLI (2011): MiR-146a in immunity and disease. Mol. Biol. Int., *2011*(2011): 437301.

RYAN, B.M., A.I., ROBLES, C.C., HARRIS (2010): Genetic variation in microRNA networks: the implication for cancer research. Nat. Rev. Cancer, *10*(6):389-402.

SAUNDERS, M.A., H., LIANG, W.H., LI (2007): Human polymorphism at microRNAs and microRNA target sites. Proc. Nac. Ac. Sci. USA, *104*(9):3300-3305.

STONE, G.W., S.G., ELLIS, D.A., COX, J., HERMILLER, C., O'SHAUGHNESSY, J.T., MANN, M., TURCO, R., CAPUTO, P., BERGIN, J., GREENBERG, J.J., POPMA, M.E., RUSSELL, TAXUS-IV INVESTIGATORS (2004): A polymer-based, paclitaxel-eluting stent in patients with coronary artery disease. N. Engl. J. Med., *350*(3):221-231.

SUN, S.G., B., ZHENG, M., HAN, X.M., FANG, H.X., LI, S.B., MIAO, M., SU, Y., HAN, H.J., SHI, J.K., WEN (2010): miR-146a and Kruppel-like factor 4 form a feedback loop to participate in vascular smooth muscle cell proliferation. EMBO Rep., *12*(1): 56-62.

VALENTINE, J. C., T. D, PIGOTT, H. R., ROTHSTEIN (2010): How many studies do you need? A primer on statistical power for meta-analysis. *JEBS*, *35*(2): 215-247.

XIONG, X.D., M., CHO, X.P., CAI, J., CHENG, X., JING, J.M., CEN, X., LIU, X.L., YANG, Y., SUH (2014): A common variant in pre-miR-146 is associated with coronary artery disease risk and its mature miRNA expression. Mutat. Res., *761*:15-20.

ZHANG, H., Q., ZHANG, Y., LIU, T., XUE (2020): miR-146a and miR-146b predict increased restenosis and rapid angiographic stenotic progression risk in coronary heart disease patients who underwent percuataneous coronary intervention. Ir. J. Med. Sci., *189*(2): 467-474.

MIR-146A GENSKA VARIJANTA RS2910164 MOŽE BITI POVEZANA SA RIZIKOM ZA KORONARNU IN-STENT RESTENOZU: REZULTATI PILOT STUDIJE I META-ANALIZE

Milica T. MIHAJLOVIĆ[1], Marija SAVIĆ VESELINOVIĆ[1], Mihajlo FARKIĆ[2], Katarina ZELJIĆ[1]

[1]Univerzitet u Beogradu, Biološki fakultet, Beograd, Srbija
[2]Institut za kardiovaskularne bolesti "Dedinje", Beograd, Srbija

Izvod

Koronarna restenoza u stentu (ISR) je neželjeni efekat koji se javlja kod 20-35% pacijenata koji su podvrgnuti perkutanoj koronarnoj intervenciji (PCI) sa implantacijom stenta. S obzirom da neće svi pacijenti razviti ISR ukazuje da genetički faktori mogu da doprinosu razvoju ISR-a. Prethodne studije ukazuju da različiti molekuli mikro RNK (miRNK) regulišu biološke procese u osnovi razvoja ISR, uključujući miR-146a koji je uključen u regulaciju proliferacije vaskularnih glatkih mišićnih ćelija i stvaranje neointime. Nukleotidne varijante u miRNK genima mogu uticati na funkciju zrele forme miRNK. Varijanta rs2910164 gena *mir-146a* se nalazi u regionu tzv. semena zrele miR-146a, ključnog regiona za regulaciju ciljnih iRNK. Cilj ove studije je bilo ispitivanje postojanja asocijacije varijante rs2910164 u genu *mir-146a* sa rizikom za razvoj ISR-a u grupi srpskih pacijenata i pojačati istraživanje sprovođenjem meta-analize. Uzorci periferne krvi dobijeni su od 61 pacijenta podvrgnutih PCI sa implantacijom stenta, od kojih je 25 (41%) imalo angiografski potvrđenu ISR. Nije bilo značajnih razlika u distribuciji alela i genotipova varijante rs2910164 između pacijenata sa i bez ISR. U srpskoj grupi pacijenata, analizirana varijanta nije bila povezana sa rizikom za razvoj ISR-a. Rezultati meta-analize pokazali su da je heterozigotni GC genotip povezan sa smanjenim rizikom za ISR (OR=0.475, P=0.006), što ukazuje na njegovu potencijalnu protektivnu ulogu u formiranju ISR-a.

Primljeno 24.XII.2021.
Odobreno 28.VI.2022.