ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

Association of CDKN2B-AS1 Gene polymorphism with Acute Myocardial Infarction.

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Abstract

Background and objectives: Several genetic studies have demonstrated an association between genetic variants in a region on chromosome 9p21 and acute myocardial infarction. We aimed to investigate the association between the cyclin-dependent kinase inhibitor 2B antisense RNA rs1333049 single nucleotide polymorphisms on chromosome 9p21 and acute myocardial infarction, in addition to determine the association between risk genotypes and 1- year outcome in such patients.

Methods: A total of 150 subjects (100 patients with acute myocardial infarction and 50 participants as a controls) were enrolled in the study. All subjects underwent genotyping of rs1333049. Patients were followed up for 1 year for development of reoccurrence of myocardial infarction, re-coronary intervention and cardiovascular death.

Results: The frequency of the GC and CC genotypes of rs1333049 is higher in patients in comparison with controls (54% vs 36% and 36% vs 18% respectively, p<0.001), while controls demonstrated increased frequency of GG genotypes (46% vs 10%, p<0.001). In a multivariate logistic regression analysis. GC and CC genotypes were independent risk factors for myocardial infarction (Odd`s ratio was 174.67, p=0.005). New myocardial ischemic event was significantly higher among the carriers of the CC and CG genotypes compared with patients with homozygous GG genotype (43.3% vs 10%, p value= 0.041).

Conclusion: Cyclin-dependent kinase inhibitor 2B antisense RNA rs1333049 single nucleotide polymorphisms is associated with acute myocardial infarction. The carriers of the CC and CG genotypes are at risk for new coronary events rather than homozygous GG genotype in 1 year follow up.

Key words: cardiogenetics, chromosome 9p21, myocardial infarction.

Introduction

Acue myocardial infarction (AMI) is a leading cause of death and morbidity worldwide.[1] AMI is diagnosed by clinical evaluation (chest pain), the electrocardiogram (ST segment shift), elevated cardiac markers, noninvasive imaging and coronary angiography. AMI is classified according to ST segment shift into ST-segment elevation-MI (STEMI) and Non-ST segment elevation MI (NSTEMI).[2] The main pathological event of MI is rupture of a coronary plaque, which activates blood platelets and clotting factors thus causing artery occlusion by thrombus. The instability of coronary plaque doesn't relate to its size, but to its contents and cap (vulnerable plaque has high lipid core and thin cap). The commonest risk factors of MI are dyslipidemia, diabetes mellitus, hypertension, family history of CAD, lack of physical activity, smoking, alcohol consumption, increased body mass index, chronic life stress and age.[3]

In the last decade, a genetic role in initiation of MI was investigated, but remains principally unidentified. Recent genetic studies have reported that cyclin-dependent kinase inhibitor 2B antisense RNA (CDKN2B-AS) is a risk gene for CAD susceptibility.[4,5] These studies have demonstrated an association between genetic variants in a region on chromosome 9p21 and MI.[6,7] Several genetic-polymorphisms have been identified in this region, and these are clustered around the gene loci for CDKN2B, CDKN2A and the 3′ end of CDKN2B-AS, which has been termed antisense noncoding RNA in the INK4 locus.[8-10]

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Aim of the work

This study aimed to investigate the association between the CDKN2B-AS1 RNA rs 1333049 single nucleotide polymorphism on chromosome 9p21 and AMI, in addition to determine the association between risk genotypes and 1- year outcome in patients with MI.

Subjects & methods

Study populations:

It was observational study that included a total of 150 subjects and carried out by co-operation between both Molecular Biology and Cardiology Departments, faculty of Medicine, university of Menoufia, Egypt in the period between December 2019 and March 2021. Subjects were divided into 2 groups: the first group included 100 patients who were admitted by AMI. Patients with significant valvular heart disease, heart failure, chronic kidney disease and chronic liver disease were excluded from the study. The 2nd group (control group) included 50 subjects without history or signs of ischemic heart diseases. All participants have signed a written informed consent. The study was reviewed and approved by the institutional review board of Menoufia university (IRB approval number: 12/2019 CARD23).

Methods: All patients were subjected to the followings:

1. Electrocardiogram:

STEMI was diagnosed according to the fourth universal definition of AMI by the magnitude of ST segment elevation in ECG leads, sex category and patient's age. Non-STEMI was defined by elevated cardiac enzymes without persistent ST elevation or with ST depression.[11]

2.Transthoracic Echocardiography:

The modified Simpson's method has been used to measure the left ventricular ejection fraction after estimating the end-diastolic and end-systolic left ventricular volumes in the apical 4-chamber and 2-chamber views.[12]

3. Sample collection and assay:

Samples were collected after 12 hours of fasting and within the first 24 hours after MI, **7** ml of venous blood were taken from all subjects and divided as follow: 2.5 ml were put in a tube containing EDTA for DNA extraction and 4.5 ml of blood were transferred slowly into a plain tube and then allowed to clot for 30 minutes before centrifugation for 15 minutes at approximately 4000 rpm. Serum was separated and kept frozen in aliquots at - 80°C for colorimetric measurement of serum creatinine and lipid profile. Enzyme-linked immunosorbent assay (ELISA) was done for the measurement of serum troponin-I.

4. Assessment of CDKN2B-AS1 gene (rs1333049):

DNA Extraction from the whole blood using the QIAGEN extraction kit (Hilden, Germany). CDKN2B-AS1 gene rs1333049 polymorphism was genotyped using allelic discrimination assay by real time PCR technique using TaqMan probe, Applied Biosystems, USA. The maxima probe qPCR Master Mix (40X), primers and probes were supplied from Thermo Fisher Scientific. The probe sequence labeled with VIC and FAM fluorescent dyes was as follows: forward was 5´CATACTAACCATATGATCAACAGTT 3´and the reverse primer was 5´AAAAGCAGCCACTCGCAGAGGTAAG 3´. Ten µl of master mix was added to 1.25 µl of the genotyping assay of primer/ probe mix and 3.75 µl of DNAase-free water. Five µl of genomic DNA extract for every sample and 5 µl of DNAase-free water for the negative control reaction were applied. The following cycling conditions were used: initial denaturation was done at 95°C for 10 minutes, followed by 40 cycles of denaturation at 94°C for 15 seconds, primer annealing at 50°C for I minute then extension at 72°C for 2 minutes and the last extension at 72°C for 1 minute. Analysis of data was completed using 7500 Real-Time PCR instrument, version 2.0.1, Applied Bio systems. Figure 1 and 2.

Statistical analysis

Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov- Smirnov was used to verify the normality of distribution of variables; Comparisons between groups for categorical variables were assessed using Chi-square test (Fisher's Exact correction). Comparisons between the different two periods for categorical variables were assessed using McNemar-Bowker Test. Student t-test was used to compare two groups for normally distributed quantitative variables while Mann Whitney test was used to compare two groups for not normally distributed quantitative variables. The population of the studied sample was explored to find its equilibrium with Hardy-Weinberg

ISSN: 0975-3583, 0976-2833 VOL 12, ISSUE 03, 2021

equation, while ANOVA was used for comparing the four studied groups and followed. Kruskal Wallis test was used to compare different groups for abnormally distributed quantitative variables. Regression To detect the most independent factors that are associated with acute myocardial infarction. Significance of the obtained results was judged at the 5% level.

Results

Table 1 demonstrated characteristics of the study population. Age and sex were matched between patients and control groups (P>0.05). From a total of 100 AMI patients, 50% were hypertensive, 43% were diabetic, 54% were smoker, 45% were dyslipidemic and 22% had a positive family history. Patients with AMI had higher frequency of cardiovascular risk factors except for family history of coronary artery disease as only 22 (22%) of patients had a family history while 6 (12%) of the control group had a family history (p=0.138). The percentage of STEMI in patient group was 57% while NSTEMI was 43%. Cardiac tropnini-1 was significantly higher in patients than in controls (p<0.001), LVEF% was significantly lower in patients than in controls (p<0.001).

Table 2 shows the distribution of genotype frequencies of CDKN2B-AS1 rs1333049 with increased frequency of the GC and CC genotypes in AMI patients in comparison with controls (54% vs 36% and 36% vs 18% respectively, p<0.001), while controls demonstrated increased frequency of GG genotypes (46% vs 10%, p<0.001) and G allele (64% vs 37%, p<0.001). Patients had increased frequency of C allele rather than controls (63% vs 36%, p<0.001).

The GC and CC genotype of CDKN2B-AS1 rs1333049 increase the risk of AMI (Odds ratio for GC genotype was 6.9 [95% CI: 2.76 – 17.2] and for CC genotype was 9.2 [95% CI: 3.2 – 26]). The C allele increases the risk of AMI (Odds ratio was 3.02 [95% CI: 1.83 – 4.98]).

Table 3 demonstrated the differences in patient's characteristics, clinical, biochemical and echocardiographic data of the AMI patients in different genotypes of CDKN2B-AS1. There were no significant differences between CDKN2B-AS1 genotypes in regard to different parameters except for LDL-C. Patients with GC genotype had higher LDL-C than other genotypes (p=0.012).

Factors that are associated with occurrence of myocardial infarction (diabetes mellitus, hypertension, smoking dyslipidemia, LV ejection fraction and CDKN2B-AS1 genotypes were evaluated in a multivariate logistic regression analysis. GC and CC genotypes were independent risk factors for AMI (Odd`s ratio was 174.67, p=0.005). Table 4.

The association between CDKN2B-AS1 rs1333049 genotypes and 1 year follow up in patients with AMI was demonstrated in table 5. Within the follow-up period after hospital discharge, 40% of patients with AMI were admitted with ACS, 29% of patients underwent a new PCI and mortality was 2.2%. Those with genetic polymorphism (CC and CG) have demonstrated new coronary events in comparison with GG genotype (43.3% vs 10%, p value= 0.041). Although re- coronary intervention was higher among patients with CC and GC genotypes compared with patients with GG genotypes, however, such difference did not reach statistically significant level (31.1% vs 10%, p value= 0.163).

Discussion

Cardiovascular disease is a world-wide cause of morbidity and mortality in humans and its incidence is on the rise.[13] The high frequency of coronary artery disease and acute coronary syndrome reported in genetic studies attracts attention of the researchers to study the relation between genetic polymorphism and myocardial infarction in order to support and strengthen the myocardial infarction prevention programs.[14] One of the important finding in these studies is the association between the 9p21.3 locus and myocardial infarction.[15-19] However, the underlying mechanism remains unclear. a non -coding RNA of locus 9p21.3 increased cardiac expression of cyclin dependent kinase inhibitor 2A and 2B antisense (CDKN2A-AS1, CDKN2B-AS1) and may lead to progression of coronary atherosclerosis and plaque rupture.[20] The 9p21.3 region has been also associated with premature coronary atherosclerosis and progression of carotid atherosclerosis.[21, 22]

The main finding of the current study is the association between single nucleotide polymorphism rs1333049 and AMI. The GC and CC genotypes of CDKN2B-AS1 increase the risk of AMI. Patients with GC genotype had higher LDL-C than other genotypes which might suggest a certain role of rs1333049 polymorphism and the metabolic disorder of LDL-C with subsequent higher frequency of AMI. In the logistic regression analysis. GC and CC genotypes were independent risk factors for the acute coronary syndrome. Furthermore, re-acute coronary syndrome was significantly higher among the carriers of the CC

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and CG genotypes compared with patients with homozygous GG genotype. Despite the influence of the genotype on reoccurrence of myocardial infarction, no statistical evidence was found to support its impact on the risk of the need for subsequent coronary artery revascularization or cardiovascular death. These results are in agreement with previous studies suggesting an association between genetic variations in the region of 9p21.3 and both CAD and ACS but involved different ribosomes.[9,23] Multiple single nucleotide polymorphisms have been identified in this chromosomal region of thousands bases, but they might represent the same genetic signal.[24,25] Ardissino D et al reported association between 9p21.3 variant rs 1333040 and risk of AMI.[26] Nikulina S et al reported association between 9p21.3 variant rs 10757278 and risk of AMI.[27] Shesternya PA et al reported an association between chromosome 9p21.3 polymorphisms and the family history of CAD in patients with AMI.[28] Chan K and co-authors reported an association between the same chromosome polymorphism locus and the CAD severity assessed by coronary angiography.[29] In contrast, Horne et al who informed that the 9p21.3 genetic variant rs2383206 was not associated with coronary thrombosis, but with development of coronary artery disease.[30] The difference in results of these studies suggests that transcription factor 21 gene polymorphism may have variant disease elements in the development of coronary atherosclerosis, and the association between 9p21.3 and CAD predisposition may have an impact on patients' risk levels.

Conclusion

CDKN2B-AS1 rs 1333049 single nucleotide polymorphisms on chromosome 9p21 were associated with myocardial infarction. The carriers of the CC and CG genotypes are at risk for new coronary events rather than homozygous GG genotype in 1 year follow up.

Study limitations

The drug therapy for the selected patients was not uniform which may affect the clinical and laboratory data. microRNAs, which has a role in post-transcriptional regulation of gene expression, did not assessed in the current study. The study included a small population, the results should be confirmed by further studies.

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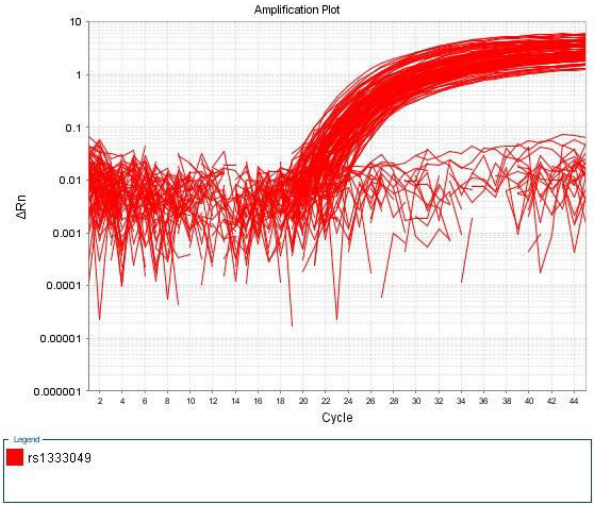

Figure 1: Amplification plot rs1333049 gene polymorphism

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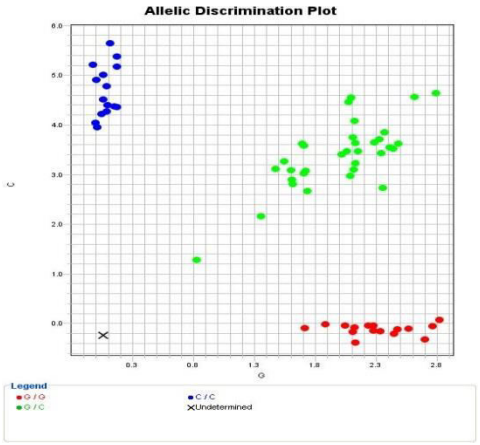

Figure 2: Allelic discrimination plot showing CC, GC and GG genotypes.

Table 1: Patient's characteristics, laboratory and echocardiographic data in the two studied groups

	Patients (n = 100)	Control (n = 50)	Test of significance	p
Age (years)	56.9 ± 10.9	55 ± 7.6	t=1.094	0.276
Sex, n., %				
Male	76 (76%)	34(68%)	$\chi 2$=1.091	0.296
Female	24 (24%)	16(32%)		
Smoking, n., %	54 (54%)	10 (20%)	$\chi 2$=15.752	<0.001
Hypertension, n., %	50 (50%)	16 (32%)	$\chi 2$=4.383	0.036
Diabetes mellitus, n., %	43 (43%)	9 (18%)	$\chi 2$=9.198	0.002
Family history of CAD, n., %	22 (22%)	6 (12%)	$\chi 2$=2.196	0.138
Hyperlipidemia, n., %	45 (45%)	14 (28%)	$\chi 2$=4.037	0.045
Heart rate, beat/ min.	77.4 ± 13.6	69.3 ± 4.9	t=5.305	<0.001
Systolic blood pressure, mmHg	112.9 ± 15	113.2 ± 7.1	t=0.166	0.869
Diastolic blood pressure, mmHg	73.8 ± 9.2	75.9 ± 4.6	t=1.851	0.066
Troponin I, ng/ml	30.7 ± 104.7	0.2 ± 0.2	U=1297	<0.001
Total cholesterol, mg/dl	209.4 ± 53.8	193.6 ± 31.2	t=2.267	0.025
LDL-C, mg/dl	129.8 ± 47.3	128.4 ± 30.1	U=2426	0.768
HDL-C, mg/dl	40.9 ± 11.9	38.2 ± 6.9	t=1.741	0.084
Triglycerides, mg/dl	165 ± 136.3	137.2 ± 39.9	U=1949	0.028
Creatinine, mg/dl	1.1 ± 0.5	1 ± 0.2	U=2497	0.990
LVEF (%)	49.9 ± 8.1	61.7 ± 5.5	t=9.293	<0.001

HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol. LVEF: left ventricular ejection fraction

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Table 2: CDKN2B-AS1 genotypes of the two studied groups

	Patients (n = 100)	Control (n = 50)	$\chi 2$	P	OR (95% C.I)
CDKN2B-AS1					
SNP					
GG	**10 (10%)**	**23 (46%)**			
GC	**54 (54%)**	**18 (36%)**	**25.486**	**<0.001**	**6.9 (2.766 – 17.214)**
CC	**36 (36%)**	**9 (18%)**		**<0.001**	**9.2 (3.247 – 26.068)**
Allele					
G	**74 (37%)**	**64 (64%)**			
C	**126 (63%)**	**36 (36%)**	**19.565**	**<0.001**	**3.027(1.838 – 4.986)**

$\chi 2$: Chi square test, CI: Confidence interval, OR: Odds rat

Table 3: Patient's characteristics of the studied patients in different genotypes of CDKN2B-AS1

	GG (n= 10)	GC (n= 54)	CC (n= 36)	Test of Sig	P
Age (years)	**57.1 ± 8.6**	**56.8 ± 11**	**57.1 ± 11.6**	**F=0.010**	**0.990**
Sex					
Male	**9 (90%)**	**39 (72.2%)**	**28 (77.8%)**	**χ^2=1.559**	**0.459**
Female	**1 (10%)**	**15 (27.8%)**	**8 (22.2%)**		
Smoking, n., %	**6 (60%)**	**28 (51.9%)**	**20 (55.6%)**	**χ^2=0.280**	**0.869**
Hypertension, n., %	**5(50%)**	**30(55.6%)**	**15(41.7%)**	**χ^2=1.667**	**0.435**
Diabetes mellitus, n., %	**5 (50)**	**24 (44.4)**	**14 (38.9)**	**χ^2=0.494**	**0.781**
Family history of CAD, n., %	**2(20%)**	**13(24.1%)**	**7(19.4%)**	**χ^2=0.296**	**0.863**
Hyperlipidemia, n., %	**5(50%)**	**26(48.1%)**	**14(38.9%)**	**χ^2=0.860**	**0.650**
Heart rate, beat/ min.	**85.5 ± 13.1**	**77.2 ± 12.5**	**75.3 ± 14.7**	**F=2.295**	**0.106**
Systolic blood pressure, mmHg	**115 ± 15.1**	**111.9 ± 15.4**	**113.9 ± 14.8**	**F=0.302**	**0.740**
Diastolic blood pressure, mmHg	**73 ± 8.2**	**73.6 ± 8.9**	**74.3 ± 9.9**	**F=0.098**	**0.907**
Troponin I, ng/ml	**55 ± 149.8**	**33.9 ± 119**	**19.1 ± 59.1**	**H=0.456**	**0.796**
Total cholesterol, mg/dl	**213.4 ± 56.5**	**216.7 ± 54.5**	**197.2 ± 51.3**	**F=1.460**	**0.237**
LDL-C, mg/dl	**113.1 ± 45.5**	**140.7 ± 53.9**	**118 ± 31.5**	**H=8.830**	**0.012**
HDL-C, mg/dl	**37.6 ± 11.3**	**39.8 ± 9.9**	**43.4 ± 14.4**	**F=1.442**	**0.242**
Triglycerides, mg/dl	**239.6 ± 366.9**	**156.9 ± 97.3**	**156.4 ± 43**	**H=3.899**	**0.142**
Creatinine, mg/dl	**1 ± 0.5**	**1.1 ± 0.6**	**1 ± 0.3**	**H=1.894**	**0.388**
LVEF (%)	**48 ± 5.1**	**49.1 ± 7.9**	**51.5 ± 8.9**	**F=1.185**	**0.310**

HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol. LVEF: left ventricular ejection fraction

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Table 4: Multivariate Logistic regression analysis of predicting factors associated with AMI

	p	OR (95% C.I)
Hypertension	0.962	1.045 (0.172–6.326)
Diabetes mellitus	0.002	54.749 (4.184–716.433)
Hyperlipidemia	0.708	0.703 (0.111–4.450)
Smoking	0.019	9.854 (1.466–66.255)
Troponin I	0.070	22.533 (0.777–653.112)
LVEF (%)	<0.001	0.673 (0.550–0.824)
CDKN2B-AS1 SNP		
GC+CC	0.005	174.671 (4.682–6516.333)

OR: Odd`s ratio, C.I: Confidence interval, LL: Lower limit, UL: Upper Limit

Table 5: Association between CDKN2B-AS1 rs1333049 genotypes and 1 year follow up

	GG (n=10)	GC+CC (n=90)	p
ACS	1(10%)	39 (43.3%)	0.041
Re-PCI	1(10%)	28 (31.1%)	0.163
Mortality	0(0%)	2(2.2%)	0.633

ACS: new acute coronary syndrome, PCI: percutaneous coronary intervention