Prognostic value of platelet glycoprotein Ibα (Kozak) gene polymorphism in patients with coronary heart disease

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Background Coronary heart disease (CHD) is one of the leading causes of death worldwide for both men and women. It is caused by the disproportion between myocardial oxygen demand and its supply. Platelets play an important role in thrombosis and hemostasis by forming a plug at the exposed subendothelium preventing blood loss; if this process is uncontrolled it results in thrombotic events causing life-threatening disease such as myocardial infarction (MI) or ischemic stroke. Glycoprotein (GP) Ib-IX-V is a platelet membrane receptor complex containing four polypeptides, GPIbα, GPIbB, GPIX, and GPV, which plays a key role in mediating platelet activity and thrombosis. This study aimed to evaluate the role of platelet GPIbα (Kozak) gene polymorphism as a risk factor of CHD.

Patients and methods This study was conducted on 120 newly diagnosed patients of CHD admitted to the Internal Medicine Hospital [46 patients with unstable angina (UA) and 74 patients with MI]. Thirty apparently healthy individuals served as a control group. The EDTA blood samples collected from patients and the control group were subjected to DNA extraction, followed by PCR amplification for the Kozak gene.

Results This study showed that by taking rs2243093 TT as the reference genotype and T as the reference allele. TC, CC, TC+CC genotypes, and C allele showed a lower frequency in cases when compared with the control group, with protective effect against CHD susceptibility ($P<0.05$). On the other hand, no association was found in GP1bα genotypes and alleles between UA and MI subgroups ($P>0.05$).

Conclusion Individuals carrying the C allele of (rs2243093) had protective effect against CHD including UA, MI. The rs2243093 had no association with the risk of MI in those having UA. Wild genotype (TT) was associated with higher creatine kinase-MB, while genotypes containing the risk allele (C) had lower creatine kinase-MB in CHD patients.

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Introduction

Cardiovascular diseases account for more than 17 million deaths globally each year (30% of all deaths), 80% of which occur in low-income and middle-income countries, and this figure is expected to grow to 23.6 million by 2030. Cardiovascular disease is a category of disorders that include coronary heart disease (CHD), coronary artery disease, and acute coronary syndrome (ACS) named unstable angina (UA) [1]. CHD is an inflammatory disease affecting coronary arteries and it includes angina pectoris, myocardial infarction (MI), and silent myocardial ischemia [2].

The incidence and the prevalence of CHD risk factors vary greatly according to the geographical region, sex, and ethnic background. CHD is caused by increased myocardial oxygen demand at the time of the provision of coronary artery spasm or intravascular blood clotting at the site of the ruptured atherosclerotic plaque. This results in limiting the coronary flow. It is possible to combine all of those mechanisms at one time [3].

Platelets have an essential role in thrombosis and hemostasis that hold the integrity of the vascular system. At sites of vascular injury, platelets adhere to the exposed subendothelial components as collagens or laminins, forming a plug to prevent blood loss. However, if this process is uncontrolled it may lead to thrombotic events causing life-threatening disease such as MI or ischemic stroke [4]. Platelet adhesion and activation is a multistep process which involves multiple platelet receptor–ligand interactions. During vessel wall injury, circulating platelets are decelerated by transient interactions between the glycoprotein (GP) Ib-IX-V complex and von Willebrand factor bound to the collagen [5].

GPIb-IX-V is a platelet membrane receptor complex, which plays a key role in mediating platelet activity and thrombosis [6]. The GPIb-IX-V complex contains four polypeptides: GPIbα, GPIbB, GPIX, and GPV. The largest subunit is GPIbα which contains all of the known extracellular ligand-binding sites. It also contains the binding site for von Willebrand factor and for several other proteins important in the genesis of vascular disease [7].

There are four polymorphisms associated with GPIbα. The first one is the human platelet antigen-2 system characterized by amino acid dimorphism (Thr/Met) at residue 145. The second polymorphism is the variable number of tandem repeats in the macroglycopeptide region [8]. The third one is the Kozak sequence which contains a single-nucleotide substitution. The fourth one is Taq polymorphism in the 3' untranslated region [9].

The GPIbα gene (Kozak) sequence polymorphism is the result of either a T (thymine) or a C (cytosine) at position −5 relative to the ATG start codon [10]. It was observed that the close proximity of C allele to the Kozak consensus sequence leads to increased expression of GPIB/IX/V complex on the platelet surface [11].

The association between Kozak sequence polymorphism and CHD is controversial. Croft *et al.* [12] found no association with carrier ship of the C allele of the Kozak sequence and cardiovascular risk factors. Also, Corral *et al.* [13] observed no association between the Kozak genotype and CHD. On the other hand, many studies on the Kozak genotype frequency and haplotype analysis confirmed that there were significant differences of the CC genotype distribution between the control group and the CHD group. As the association between Kozak polymorphism and CHD is controversy, this study aimed to evaluate the role of platelet GPIbα (Kozak) polymorphism as a risk factor of CHD.

Patients and methods

After approval of the Local Ethics Committee of Mansoura University, Faculty of Medicine and obtaining written informed consent from all patients, this study was conducted on 120 newly diagnosed patients of CHD admitted to the Internal Medicine Hospital (74 patients with MI and 46 patients with UA) in the period from December 2017 to June 2019. In addition, 30 apparently healthy individuals served as a control group. The following parameters were carried out to all patients: full history taking, complete clinical examination, laboratory investigations, ECG, and ECHO.

EDTA blood samples were taken from the patients and the control group for DNA extraction followed by PCR amplification for the Kozak gene using the primers F. 5′: GGG AGT AGG GAG GAC AGG AG. 3′ and R 5′: AGT GTA AGG CAT CAG GGT TG. 3′. The annealing temperature was 60°C. The PCR products (348 bp) were separated by electrophoresis on 2% agarose gel and then digested by Eco471 restriction enzyme (#FD0314) (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) using the PCR-RFLP technique. The restriction products were 129 and 219 bp for the homozygous TT genotype, 348 bp for the homozygous cc genotype, and 219, 219, and 348 bp for the heterozygous TC genotype. Statistical analysis of collected data was done using the Statistical Package for the Social Sciences (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0.; IBM Corp., Armonk, New York, USA). Data were presented and suitable analysis was done according to the type of data obtained for each parameter.

Results

This study was conducted on 120 newly diagnosed patients of CHD (46 patients with UA and 74 patients with MI).Their mean age was 55.9 years; there were 86 (71.7%) men and 34 (28.3%) women in addition to 30 apparently healthy individuals as a control group of matched age and sex. No significant differences were found in age and sex between UA, MI versus the control group. Moreover, no significant differences were found in age and sex between UA and MI. It was noticed that UA was associated with hypertension when compared with MI. Otherwise, no significant differences were found in risk factors between UA and MI subgroups. Also, no significant differences were found in the laboratory data as regards complete blood count, international normalized ratio (INR), creatine kinase-MB (CK-MB), and cardiac troponins ($P \geq 0.005$) between UA and MI subgroups. The allelic distribution of Kozak gene in patients and control groups is illustrated in Table 1.

Taking rs2243093 TT as the reference genotype and T as the reference allele, TC, CC, TC+CC genotypes, and C allele showed lower frequency in cases when compared with the control group, with protective effect against CHD susceptibility ($P<0.05$). On the other hand, no association was found in GP1BA genotypes and alleles between UA and MI

Table 1 Comparison of glycoprotein 1bα genotypes and alleles between total coronary heart disease, unstable angina, myocardial infarction cases, and control groups

GP1bα genotypes	Control (*N*=30) [*n* (%)]	CHD (*N*=120) [*n* (%)]	UA (*N*=46) [*n* (%)]	MI (*N*=74) [*n* (%)]	*P*1	*P*2	*P*3	*P*4
TT	14 (46.7)	88 (73.3)	36 (78.3)	52 (70.3)	–	–	–	–
TC	12 (40)	28 (23.3)	8 (17.4)	20 (27)	**0.029**	**0.014**	0.092	0.244
CC	4 (13.3)	4 (3.4)	2(4.3)	2 (2.7)	**0.020**	0.071	**0.027**	0.719
TC+CC	16 (53.3)	32 (26.7)	10 (21.7)	22 (29.7)	**0.006**	**0.005**	**0.025**	0.338
Alleles								
T	40 (66.7)	204 (85)	80 (87)	124 (83.8)	–	–	–	–
C	20 (33.3)	36 (15)	12 (13)	24 (16.2)	**0.002**	**0.004**	**0.007**	0.504

CHD, coronary heart disease; GP, glycoprotein; MI, myocardial infarction; UA, unstable angina. *P*1: control versus CHD. *P*2: control versus UA. *P*3: control versus MI. *P*4: UA versus MI. The bold values for the significant one.

Table 2 Comparison of laboratory data between glycoprotein 1bα genotypes in all studied coronary heart disease cases

Lab data	TT (*N*=88)	TC (*N*=28)	CC (*N*=4)	TC+CC (*N*=32)	*P*1	*P*2
Hemoglobin (g/dl)						
Mean±SD	11.7±2.2	11.2±2.3	11.3±1.5	11.2±2.2	0.582^{A}	0.299^{T}
TLC ($\times10^9$/L)						
Mean ±SD	6.7±1.4	7.2±1.8	12.9±1.3	7.9±2.1	0.073^{A}	0.113^{T}
Platelet ($\times10^9$/l)						
Mean±SD	244.2±86.8	262.8±79.8	280.0±23.1	264.9±73.6	0.551^{A}	0.298^{T}
Prothrombin time (s)						
Mean±SD	12.2±1.3	12.0±1.5	12.3±0.3	12.0±1.4	0.867^{A}	0.681^{T}
INR						
Mean±SD	1.1±0.1	1.0±0.1	1.1±0.1	1.0±0.2	0.822^{A}	0.581^{T}
CK-MB (IU/l)						
Median	30.5	27	12.5	26.5	**0.031K**	**0.033M**
Minimum–maximum	5–70	5–40	5–20	5–40		
Cardiac troponins (pg/ml) [*n* (%)]						
Negative	36 (40.9)	12 (42.9)	2 (50.0)	14 (43.8)	9.927^{F}	0.780^{C}
Positive	52 (59.1)	16 (57.1)	2 (50.0)	18 (56.3)		

CK-MB, creatine kinase-MB; INR, international normalized ratio; TLC, total leukocyte count. *P*1, comparison between: A, analysis of variance; C, χ^2 test; F, Fisher's exact test; T, Student's *t* test; TT, TC, CC; *P*2, comparison between TC+CC versus TT. The bold values for the significant one.

subgroups ($P>0.05$). As regards the laboratory data, CK-MB decreased gradually in patients with TT, TC, and CC, respectively, with significant differences between them ($P=0.031$), as well as patients carrying C allele had significantly lower CK-MB when compared with those having wild allele (26.5 vs. 30.5, $P=0.033$). Otherwise, no significant differences were found in laboratory data between rs2243093 genotypes in all studied CHD cases as shown in Table 2.

Logistic regression analysis was conducted for the prediction of CHD susceptibility using age, sex, history of similar condition, hypertension, diabetes mellitus (DM), obesity, smoking, INR, CK-MB, cardiac troponin, and rs2243093 genotypes as covariates. History of similar condition, hypertension, obesity, smoking, CK-MB, and positive cardiac troponin were associated with the risk of CHD, while rs2243093 (TC+CC) had protective effect against CHD development in univariable analysis. However, taking significant covariate in univariable analysis into multivariable analysis showed that obesity, smoking, CK-MB, and positive cardiac troponin were considered risk predictors for CHD susceptibility. In addition, rs2243093 (TC+CC) was considered a protective predictor against CHD development as described in Table 3.

Discussion

The pathogenesis of ischemic heart disease was modified over many decades. Marvelous efforts were invested to identify the genes and specific DNA sequence changes, responsible for this heritability as genetic polymorphisms, may be the risk factors, predisposing to ischemic heart disease. Several recent genetic risk factors have been identified [1,14]. The current study aimed to evaluate the role of platelet GPIbα Kozak polymorphism as a risk factor of CHD. The present study included 120 patients of CHD in

Table 3 Regression analysis for the prediction of coronary heart disease susceptibility

	Univariable				Multivariable			
	P	OR	95% CI		*P*	OR	95% CI	
Age	0.275	1.023	0.982	1.066				
Sex	0.728	1.167	0.490	2.780				
History of similar condition	**0.028**	3.500	1.145	10.700	0.322	2.115	0.480	9.313
Hypertension	**0.009**	3.000	1.317	6.833	0.103	2.467	0.833	7.312
DM	0.104	2.042	0.865	4.821				
Obesity	**0.011**	3.286	1.311	8.233	**0.007**	2.889	1.633	12.235
Smoking	**0.025**	2.667	1.129	6.297	<**0.001**	2.458	1.697	13.167
INR	0.243	2.620	0.277	8.149				
CK-MB	<**0.001**	1.064	1.028	1.102	**0.021**	1.067	1.010	1.127
Positive cardiac troponins	<**0.001**	3.875	2.145	7.001	**0.003**	2.823	1.313	6.434
rs2243093 (TC+CC)	**0.006**	0.516	0.320	0.831	**0.003**	0.166	0.051	0.535

Logistic regression test was used. CI, confidence interval; CK-MB, creatine kinase-MB; DM, diabetes mellitus; INR, international normalized ratio; OR, odds ratio. The bold values for the significant one.

addition to 30 apparently healthy individuals as a control group.

In this study, taking rs2243093 TT as the reference genotype and T as the reference allele, TC, CC, TC +CC genotypes and C allele showed lower frequency in cases when compared with the control group, with protective effect against CHD susceptibility (i.e. TT was risky for CHD) [odds ratio (OR)=0.566, 0.335, 0.516, 0.353; 95% confidence interval (95% CI)= 0.34–0.943, 0.134–0.842, 0.320–0.831, 0.186–0.671; *P*=0.029, 0.020, 0.006, 0.002, respectively]. TC, TC +CC genotypes and C allele showed lower frequency in UA cases when compared with the control group, with protective effect against UA susceptibility (i.e. TT was risky for UA) (OR=0.433, 0.416, and0.300; 95% CI=0.222–0.845, 0.225–0.769, and 0.133-0.674; *P*=0.014, 0.005, and 0.004, respectively). CC, TC +CC genotypes and C allele showed lower frequency in MI cases when compared with the control group (OR=0.292, 0.549, and 0.387; 95% CI=0.098–0.871, 0.324–0.929, and 0.194–0.773; *P*=0.027, 0.025, and 0.007), with protective effect against MI susceptibility (i.e. TT was risky for MI).

Frank *et al*. [9] carried out genotype evaluation of the Kozak sequence polymorphism of GPIbα in a resident-based research of 18–44 year old women with nonfatal MI (*n*=78), nonfatal stroke (*n*=106), and 384 demographically comparable woman control individuals. Analyses of T/C genotypes showed that one or more copies of the C allele were present in 14.1% of MI cases, 23.6% of stroke cases, and 23.7% of controls. The age-adjusted OR for MI in women carrying one or more copies of the C allele was 0.53 (95%). The age-modified OR for stroke in women carrying one or more copies of the C allele was 0.99 (95%). Analyses stratified by stroke type (ischemia, hemorrhage) yielded the same outcomes. They demonstrated that young women carrying the C allele of the Kozak sequence polymorphism of GPIbα were not at elevated possibility of MI or stroke. They illustrated that, paradoxically, the C allele may be accompanied with a reduced possibility of MI in this people.

Ozelo *et al*. [15] found no correlation was seen with Kozak polymorphism and possibility of MI when they compare the cases (*n*=180) presenting as survivors of MI with 180 controls matched by age, sex, and race. Likewise, Candore *et al*. [16] recorded that they could not show any association among the Kozak polymorphisms and possibility of MI.

Golcuk *et al*. [17] found that the incidence of the C allele in the initial-onset ACS group in comparison with the control group did not differ significantly (*P*=0.993), denoting that the C allele is not a risk factor for initial-onset ACS in residents in Turkey. Another research revealed that there was no association among Kozak polymorphism and acute ischemic stroke, which indicates that there was no significant differences among early-onset ACS cases and controls, in genotype (*P*=0.89) or in allele frequency [18]. On the other hand, Meisel *et al*. [19] reported that there is association between the C allele of Kozak sequence and ischemic complications of percutaneous transluminal coronary intervention in White people suffered from ACS. Douglas *et al*. [10] found TT homozygote was related to MI, while the C allele in TC heterozygote was a protective factor against. Also, Zhang *et al*. [20] studied the Kozak genotype frequency and haplotype analysis in CHD; they confirmed that there were significant differences of CC genotype distribution between the control group and the CHD group.

In this study, there were no significant differences found between rs2243093 genotypes in all studied CHD cases regarding age, sex, obesity, hypertension, smoking, and DM. Golcuk *et al.* [17] found that in the ACS group, the mean age, smoking, family history, hypertension, hyperlipidemia, and DM revealed no significant difference between the TT and CT+CC groups.

Logistic regression analysis was conducted for the prediction of CHD susceptibility using age, sex, history of similar condition, hypertension, DM, obesity, smoking, INR, CK-MB, cardiac troponin, rs2243093 genotypes as covariates. History of similar condition, hypertension, obesity, smoking, CK-MB, and positive cardiac troponin were associated with risk of CHD, while rs2243093 (TC+CC) had a protective effect against CHD development in univariable analysis. However, taking significant covariate in univariable analysis into multivariable analysis showed that obesity, smoking, CK-MB, and positive cardiac troponin were considered to be risk predictors for CHD susceptibility. In addition, rs2243093 (TC +CC) was considered to be a protective predictor against CHD development.In this study, we found that positive cardiac CK-MB were considered risk predictors for CHD susceptibility. Scientific community is utilizing CK-MB as a biomarker for acute MI. This can be explained as CK is an essential enzyme responsible for the reversible transfer of active phosphate group present in phosphocreatine for the generation of energy packs in the form of ATP.

CK-MB isoenzyme (CK-MB) is present in a comparatively high level in the myocardium and is a favored biomarker of cardiac damage from the last years. A prior study reported that in the emergency department CK-MB levels provide objective information for the prediction of ischemic complications in stable chest pain patients [21].

Cardiac troponin is a specific biomarker for the prediction of CHD outcomes. In this study, we found that positive cardiac troponin was considered risk predictors for CHD susceptibility. In contrast, novel researches of cases with confirmed CHD by Omland *et al.* [22] showed that troponin expect fatal and nonfatal MI rather than heart failure occasions.

Conclusion

From this study, we could conclude that healthy individuals carrying the C allele of rs2243093,had protective effect against CHD, UA, MI while the rs2243093 had no association with the risk of MI in those having UA. Wild genotype (TT) was associated with higher CK-MB, while genotypes containing risk allele (C) had lower CK-MB in CHD patients. The platelet GPIbα (Kozak) gene could be incorporated into the routine prognostic workup of CHD patients as it is easily determined by PCR-RFLP.

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Conflicts of interest

There are no conflicts of interest.

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