THE CO-EXSISTANCE OF β-FIBRINOGEN -455 G/A POLYMORPHISM AND PROTHROMBIN 20210 G/A MUTATION IN CORONARY HEART DISEASE IN EGYPTIAN PATIENTS

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List of Abbreviations

CVDs	Cardio vascular diseases
CHD	Coronary heart disease
FGA	Fibrinogen alpha
FGB	Fibrinogen beta
FGG	Fibrinogen gamma
PDA	Posterior descending artery
RCA	Right coronary artery
CX	Circumflex artery
LVMT	Left ventricular mural thrombus
RV	Right ventricle
VFWR	Ventricular free wall rupture
VSR	Ventricular septal rupture
MR	Mitral regurgitation
APC	Activated protein C
FVa	Activated factor five
FVIIIa	Activated factor eight
TFPI	Tissue factor pathway inhibitor
TF	Tissue factor
Serpin	Serine protease inhibitor
t-PA	Tissue plasminogen activator
PGI^2	Prostacyclin
HMWK	High-molecular-weight kininogen
ZPI	Protein Z-related protease inhibitor
PAI-1	Plasminogen activator inhibitor-1
PAI-2	Plasminogen activator inhibitor-2
PT	Prothrombin time

INR	International normalized ratio
APTT	Activated partial thromboplastin time
CT	Clotting time
GP	Glycoprotein
Arg	Arginine aminoacid
Lys	Lysine aminoacid
ASVD	Arterio-Sclerotic Vascular Disease
HDL	High density lipoproteins
LDL	Low density lipoproteins
CRP	C reactive protein
SHM	Somatic hypermutation
DNA	Deoxy-ribonucleic acid
RNA	Ribonucleic acid
HX	Hypo-xanthin
5MeC	5-methylcytosine
AP site	A purinic site
UV light	Ultra violet light
SNP	Single nucleotide polymorphism
KD	Kilo Dalton
Gla	Gamma carboxyglutamic acid
PARs	Protease activated receptors
HRT	Hormone replacement therapy
DVT	Deep venous thrombosis
OCP	Oral contraceptive pills
IL-6	Interleukin 6
CFD	Congenital fibrinogen deficiency
ICVDs	Ischaemic cardio-vascular diseases
CABG	Coronary artery bypass graft surgery

ICHD	Ischaemic coronary heart disease
ECG	Electrocardiogram
TAG	Triglycerides
AST	Aspartate transaminase
ALT	Alanine transaminase
PCR	Polymerase chain reaction
DNAT	Denaturation (solution)
NBT	Nitro blue tetrazolium
BCIP	5-bromo-4-chloro-3-indolyl phosphate
EDTA	Ethylene di-amine tetra-acetic acid
SD	Standard deviation
OR	Odds ratio
RR	Relative risk
DM	Diabetes mellitus
HTN	Hypertension
IMT	Intimal medial thickening
EA	European ancestry
AA	African ancestry
MTHFR	Methylene tetrahydrofolate reductase

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Abstract

Coronary Heart Disease (CHD) is a complex disease with both environmental and genetic determinants. The aim of this study was to assess the presence of FGB-455 G/A and prothrombin 20210 G/A polymorphisms in CHD Egyptian patients and to correlate the co-exsistance of both polymorphisms with the clinical and laboratory data of the patients. Thirty adult patients with angiographically documented CHD from National Heart Institute, scheduled for elective coronary artery bypass graft surgery (CABG) were included in this study and compared with thirty age and sex matched healthy individuals. DNA was extracted from peripheral blood and identification of FGB-455 and prothrombin 20210 genotypes was done. Fibrinogen 455 locus showed 3 polymorphisms (GG, GA & AA), while prothrombin 20210 locus showed 2 polymorphisms (GG & GA). The genotype distribution and frequency of mutated alleles in both polymorphisms showed no significant difference between patients and control groups ($P > 0.05$). Accordingly, none of the polymorphisms can be accused as a risk factor for CHD in the studied Egyptian patients. The co-exsistance of FGB-455 G/A and prothrombin 20210 G/A is not reported in the studied Egyptian subjects and can not be considered implicated in the occurrence of CHD.

Key words

Coronary Heart Disease, Thrombosis, Fibrinogen-455 G/A gene polymorphism, Prothrombin 20210 G/A gene polymorphism and Myocardial Infarction.

Cardiovascular diseases (CVDs) refer to the class of diseases that involve the heart and/or blood vessels (arteries and veins). Most countries face high and increasing rates of cardiovascular diseases. One of the most dangerous CVDs is the Coronary heart disease (CHD) that kills more Americans than cancer (1, 2).

CHD is a complex disease with both environmental and genetic determinants. Many risk factors are incorporated such as hypertension, hyper-cholesterolemia, diabetes mellitus, obesity, unhealthy diet, protein C deficiency, protein S deficiency, antithrombin III deficiency, elevated clotting factors VIII, IX, XI and elevated fibrinogen. In addition, a positive family history is a strong independent risk factor for CHD (3).

The development of CHD and myocardial infarction (MI) involves hyperplasia of arterial smooth muscles, the presence of fatty streaks, atheroma formation, plaque rupture, thrombus formation and vessel occlusion. The presence of a first- degree relative with MI was found to be associated with seven fold increased risk for MI (4). Also it was found that among the underlying causes of CHD, the blood coagulation system is thought to determine the onset of MI through its role in blood clots formation (5).

The coagulation cascade is a series of reactions, in which a zymogen (inactive enzyme precursor) and its glycoprotein co-factor are activated to become active components that then catalyze the next reaction in the cascade, ultimately resulting in cross-linked fibrin. A very important step in thrombus formation is prothrombin conversion to thrombin which converts fibrinogen to fibrin (6).

Fibrinogen is a protein that is composed of alpha, beta & gamma polypeptide chains and plays a key role in blood clotting. It is a sticky,

fibrous coagulant in the blood that appears to significantly increase the risk of experiencing one of the leading causes of death and disability, either stroke or MI (7). Patients with CHD tended to have higher fibrinogen levels than those without the disease (8).The percent mortality rate jumped by over seven-fold in those with the highest fibrinogen levels, compared to those with the lowest levels (1).

The 3 polypeptide chains of fibrinogen (α, β, and γ chains), are encoded by 3 different genes clustered on chromosome 4 in region q28. In vitro studies have suggested that β-chain synthesis limits the rate of production of mature fibrinogen. Thus, most studies focus on the association of polymorphisms in the fibrinogen β-chain (FGB) gene with MI. The FGB -455 G/A promoter polymorphism was found to be associated with increased plasma fibrinogen levels, but the role of the FGB polymorphisms as a risk factor of MI has been debated (9).

Another component of the coagulation cascade is prothrombin which is a blood clotting protein needed to form fibrin. It is also called factor II. A common point mutation in the 3'-untranslated region of the prothrombin gene (20210 G/A) has been reported to be associated with elevated plasma prothrombin levels, making the blood more likely to clot and increasing the risk for venous thrombosis by 3 to 5 folds. Thus, attention has been paid to investigate whether there is a relationship between that mutation and CHD and MI (10).

- **Aim of work**

1. To assess the frequency of FGB -455 G/A polymorphism and its association with CHD in the Egyptian patients.

2. To assess the frequency of prothrombin 20210 G/A polymorphism and its association with CHD in the Egyptian patients.

3. To correlate the co-exsistance of both polymorphisms with the clinical and biochemical data of the CHD patients.

Coronary circulation is the circulation of blood in the blood vessels of the heart muscle. Although blood fills the chambers of the heart, the muscle tissue of the heart (the myocardium) is so thick that it requires coronary blood vessels to deliver blood deep into it. The vessels that deliver oxygenated blood to the myocardium are known as coronary arteries. The vessels that remove the deoxygenated blood from the heart muscle are known as coronary veins (11, 12).

The coronary arteries that run on the surface of the heart are called epicardial coronary arteries. These arteries, when healthy, are capable of autoregulation to maintain coronary blood flow at levels appropriate to the needs of the heart muscle (12, 13). These relatively narrow vessels are commonly affected by atherosclerosis and can become blocked, causing angina or a heart attack (14). The arteries that run deep within the myocardium are referred to as subendocardial coronary arteries (13) (figure 1).

- **Anatomy and Classification of coronary arteries**

The coronary arteries are classified as "end circulation", since they represent the only source of blood supply to the myocardium i.e. there is very little redundant blood supply, which is why blockage of these vessels can be so critical (13, 14). The exact anatomy of the myocardial blood supply system varies considerably from person to person. A full evaluation of the coronary arteries requires cardiac catheterization or CT coronary angiography (15).

In general there are two main coronary arteries, left and right (figure 1), both of these arteries originate from the beginning (root) of the aorta, immediately above the aortic valve. The left coronary artery originates

from the left aortic sinus, while the right coronary artery originates from the right aortic sinus (13).

Four percent (4%) of people have a third, the posterior coronary artery. In rare cases, a person will have one coronary artery that runs around the root of the aorta (13, 15).

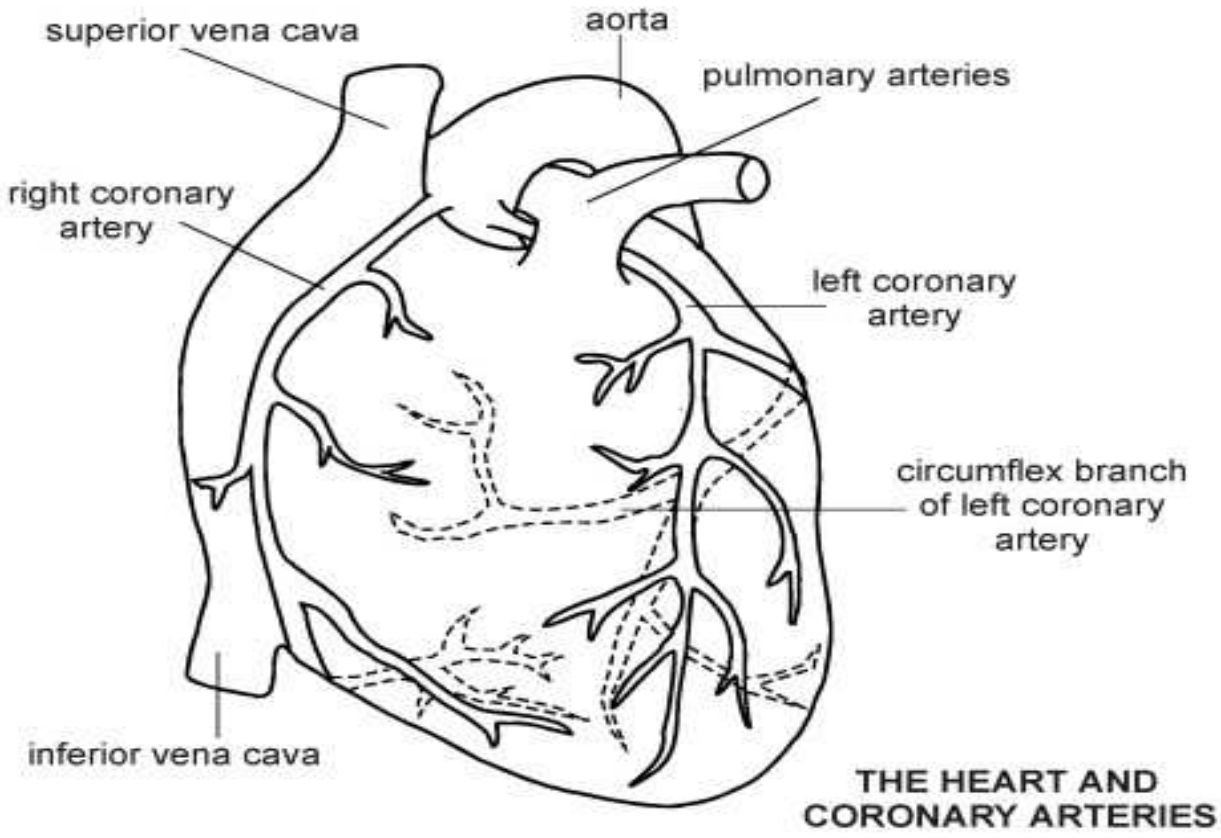

Figure [1] The heart and coronary arteries (13).

Occasionally, a coronary artery may exist as a double structure (i.e. there are two arteries, parallel to each other, where ordinarily there would be one). The artery that gives origin for the posterior descending artery (PDA) determines the coronary dominance (11).

- **Coronary dominance**

❖ If the posterior descending artery (PDA) originates from the right coronary artery (RCA), the coronary circulation can be classified as "right-dominant".

❖ If the posterior descending artery (PDA) originates from the circumflex artery (CX), a branch of the left coronary artery, the coronary circulation can be classified as "left-dominant".

❖ If the posterior descending artery (PDA) originates from both the right coronary artery and the circumflex artery, the coronary circulation can be classified as "co-dominant".

Approximately 70% of the general populations are right-dominant, 20% are co-dominant, and 10% are left-dominant (11). Blood supply to different cardiac regions is shown in (table 1).

Table [1] Blood supply of important cardiac regions (14).

Anatomic Region of the Heart	Coronary Artery most likely associated
Inferior	**Right coronary**
Anteroseptal	**Left anterior descending**
Anteroapical	**Left anterior descending (distal)**
Anterolateral	**Circumflex**
Posterior	**Right coronary artery**

- **Physiology of coronary flow**

During contraction of the ventricular myocardium (systole), the subendocardial coronary vessels (the vessels that enter the myocardium) are compressed due to the high intraventricular pressure. However, the epicardial coronary vessels (the vessels that run along the outer surface of