The Correlation between Genetic Polymorphism of P2Y12, PON1, CYP2C19, CES-1 and PEAR-1 and Clinical Response to Dual Antiplatelet Treatment in Egyptian Patients with Acute Coronary Syndrome

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Shaimaa Fathy Khalil

List of Abbreviations

Abbreviation	Stands for
ABCB1	Adenosine Triphosphate (ATP)-Binding Cassette, Sub-Family B
AC	Adenylate Cyclase
ACC	American College of Cardiology
ACE	Angiotensin Converting Enzyme
ACS	Acute Coronary Syndrome
ACTIVE	Atrial Fibrillation Clopidogrel Trial with Irbesartan for Prevention of Vascular Events
ADP	Adenosine Diphosphate
AHA	American Heart Association
AMI	Acute Myocardial Infarction
APCs	Antigen-Presenting Cells
APS	Adenosine 5' Phosphosulfate
ARBs	Angiotensin Receptor Blockers
AST	Aspartate Aminotransferase
ATP	Adenosine Triphosphate
BAFF	B-cell Activating Factor
BCHE	Butyrylcholinesterase
BMI	Body Mass Index
BNP	B-Natriuretic Peptide
bp	Base Pair
CABG	Coronary Artery Bypass Grafting
CAD	Coronary Artery Disease
cAMP	Cyclic Adenosine Monophosphate
CCBs	Calcium Channel Blockers
CD4	Cluster of Differentiation 4
CESI	Carboxylesterase
CI	Confidence Interval
CK	Creatine Kinase
CK-MB	Creatine Kinase (Cardiac isoenzymes)
$CK\text{-}MB_{1 \text{ and } 2}$	Creatine Kinase isoforms
COX-1	Cyclo-Oxygenase 1
CPIC	Clinical Pharmacogenetics Implementation Consortium
CRP	C-Reactive Protein
CT	Computed tomography
cTn	Cardiac Troponin
cTnI	Cardiac Troponin I
cTnT	Cardiac Troponin T
CVD	Cardiovascular Diseases

CURE	Clopidogrel in Unstable Angina to Prevent Recurrent Events
CYP2C19	Cytochrome P450 2C19
CYP450	Cytochrome P450
DAPT	Dual Antiplatelet Therapy
DC	Dendritic Cells
DM	Diabetes mellitus
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
ECG	Electro-Cardiogram
ED	Emergency Department
EM	Extensive Metabolizers
ER	Endoplasmic Reticulum
ESC	European Society of Cardiology
FAST-MI	French Registry of Acute ST-Elevation and Non-ST-Elevation Myocardial Infarction
FDA	Food and Drug Administration
GDF-15	Growth Differentiation Factor-15
GoF	Gain of function
GP	Glycoprotein
GRACE score	Global Registry of Acute Coronary Events
GPBB	Glycogen Phosphorylase Isoenzyme in Brain and Heart
GWAS	Genome-Wide Association Studies
H- FABP	Heart Fatty Acid Binding Protein
HF	Heart failure
hs-CRP	High Sensitive C-Reactive Protein
hs-Tn	High Sensitivity Tn
IL	Interleukin
IM	Intermediate Metabolizers
IFN-γ	Interferon-Gamma
IHD	Ischemic Heart Disease
ITGB	Integrin Subunit Beta 3
IV	Intravenous
LD	Loading Dose
LDH	Lactate Dehydrogenase
LDL	Low Density lipoprotien
LMWHs	Low Molecular weight heparin
LOF	Loss-of-Function Allele
LV	Left ventricular
LVEF	Left Ventricular Ejection Fraction
MACE	Major Adverse Cardiac Events
MAF	Minor Allele Frequencies

MDR1	Multi-Drug Resistance 1 Protein
MDRS	MPO-Derived Reactive Species
MI	Myocardial Infarction
MMPs	Matrix Degrading Metalloproteinases
MPI	Myocardial perfusion imaging
MPO	Myeloperoxidase
MS	Metabolic syndrome
NETs	Neutrophil Extracellular Traps
NGS	Next Generation DNA Sequencing
NLRP	Nucleotide-binding domain and Leucine-Rich repeat containing Proteins
NSTEMI	Non-ST Elevation Myocardial Infarction
OPR	On-treatment Platelet Reactivity
OR	Odd Ratio
PAI-1	Plasminogen Activator Inhibitor-1
PAD	Peripheral Artery Disease
PAR	Platelet Protease-Activated Receptor
PCI	Percutaneous Coronary Interventions
PCR	Polymerase Chain Reaction
PD	Pharmacodynamics
PDE-3	Platelet Phosphodiesterase-3
P-GP	Permeability G-protein
PK	Pharmacokinetics
P2Y12	ADP platelets receptor
PLATO	Platelet Inhibition And Patient Outcomes
PM	Poor Metabolizers
PMN	Polymorphonuclear Leukocytes
PO	Per oral
PPIs	Proton Pump Inhibitors
PPi	Pyrophosphate
PTGSI	Prostaglandin G/H synthase I
PTT	Partial Thromboplastin ime
RBG	Random Blood Glucose
SaO_2	Oxygen Saturation
SC	Subcutaneous
SHH	Sonic hedgehog gene
SNPs	Single Nucleotide Polymorphisms
STEMI	ST Elevation Myocardial Infarction
TAE	Tris/Acetate/EDTA buffer
T2D	Type 2 Diabetes
TGF-β	Transforming Growth Factor-βeta
Th	T helper Lymphocytes

TIMI score	Thrombolysis in Myocardial Infarction
TLR	Toll-Like Receptor
Tn	Troponin
Tn (I, C, T)	Troponin isoforms
TNF	Tumor Necrosis Factor
TPβ	Thromboxane Receptor
tPA	Tissue-type Plasminogen Activators
Treg	Regulatory T cells
TRITON-TIMI 38	Trial to Assess Improvement in Therapeutic Outcomes by Optimizing Platelet Inhibition with Prasugrel–Thrombolysis In Myocardial Infarction38
TXA2	Thromboxane A2
UA	Unstable Angina
UFH	Unfractionated Heparin
UM	ultrarapid Metabolizers
uPA	Urokinase-type Plasminogen Activators
VASP	Vasodilator-Stimulated Phosphoprotein
VASP-P	Phosporylated Vasodilator-Stimulated Phosphoprotein
vWF	von Willebrand Factor
WHO	World Health Organization

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ABSTRACT

Dual antiplatelet therapy (DAPT) with aspirin and Clopidogrel reduces the risk for recurrent cardiovascular events after acute coronary syndrome (ACS) and percutaneous coronary intervention. However, there is significant variation in response to DAPT that may be influenced by both genetic and non-genetic factors. The aim of the present study was to assess the effect of genetic polymorphisms in *PON1*, *PEAR-1*, *P2Y12*, *CES1* and *CYP2C19* along with clinical, demographic, and social factors, on variation in response to Clopidogrel plus aspirin therapy in Egyptians. This study included 230 Egyptian patients treated with Clopidogrel 75 mg/day and aspirin 81 mg/day for at least 12 months following their first ACS. It was found that *CYP2C19**2 polymorphism was the only genetic predictor of major adverse cardiovascular events (MACE), defined as the occurrence of recurrent ACS, ischemic stroke, stent-related revascularization or death (OR 2.23, 95% CI: 1.15 – 4.33, p=0.01). Moreover, the use of proton pump inhibitor (PPI) (OR 4.77, 95% CI: 1.47 – 15.54, p=0.009) and diabetes (OR 1.83, 95% CI: 1.03 – 3.26, p=0.03) were associated with higher cardiovascular risk, while the use of statins was associated with lower risk (OR 0.43, 95% CI: 0.25 – 0.76, p=0.003). The contribution of these five genetic and non-genetic factors explained 19% of the variability in risk for MACE in Egyptians treated with Clopidogrel and aspirin. The previously mentioned results showed that *CYP2C19*2* polymorphism, along with diabetes, and use of PPI and statins are important factors highly associated with the variability in clinical response to Clopidogrel plus aspirin following ACS in Egyptians.

Keywords: Clopidogrel, aspirin, dual antiplatelet, Egyptians, P2RY12, CES1, CYP2C19.

INTRODUCTION

Acute coronary syndrome (ACS), a form of coronary heart disease, has a substantial economic and societal impact in various countries. Epidemiological data showed that re-infarction and death are the two major clinical outcomes following ACS. It was established that people 40 years old or more (*18% of males and 23% of females*) may suffer from death after 1 year of myocardial infarction MI ***(Lloyd et al., 2011).*** Information concerning the demographics in Egyptian patient with ACS is incompletely clear.

Platelet attachment, activation, and aggregation after plaque rupture or abrasion are significant determinants of blood vessel thrombosis prompting ACS. Antiplatelet treatment, which targets pathways of platelet activation and aggregation, is essential in the treatment of ischemic occasions in ACS patients. Pharmacotherapy for ACS includes anticoagulants, fibrinolytics, and antiplatelet drugs to diminish the rate of cardiovascular events ***(Kushner et al., 2009).***

Dual antiplatelet therapy (DAPT) as Clopidogrel, prasugrel or ticagrelor in conjunction with aspirin, is the treatment of choice in ACS patients. Drug choice should be based on the individual patient's characteristics and contraindications; Clopidogrel will be used if, prasugrel and ticagrelor are contraindicated or not obtainable. There is a strong evidence that DAPT with aspirin and thienopyridines (e.g. Clopidogrel), can significantly reduce the cardiovascular events in patients with all types of *ACS* ***(Anderson et al., 2007)***, with or without percutaneous coronary intervention. On the basis of this evidence, antiplatelet therapies are generally accepted as the standard of care for the management of ACS (***Kushner et al., 2009).***

It is recognized that atherothrombosis is the most widely recognized cause behind ACS, and has a pivotal role in developing

complications. Vascular endothelial damage and plaque burst are events prompted for platelet adhesion, and aggregation causing thrombosis, ischemia, and infarction that is the main pathology of ACS. Inhibition of platelet aggregation (IPA) by pharmacological treatment prevents formation and progression of thrombotic processes ***(Jennings, 2009).***

Aspirin or DAPT with aspirin and Clopidogrel, a thienopyridine, has been considered as the cornerstone of antiplatelet therapy in an attempt to prevent complications among patients with ACS ***(Scott et al., 2013, Levine et al., 2014).*** Around 85% of Clopidogrel is hydrolyzed in the liver during the first-pass metabolism by esterase, essentially carboxylesterase 1 (CES1), to inactive metabolites. the remaining 15% of Clopidogrel prodrug will be converted to an active metabolite that happens in the liver by two consecutive oxidation steps that include several cytochrome P450 (CYP450) enzymes (CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4/5) (***Scott et al., 2013).***

An association between *CYP2C19* gene polymorphisms and the enzyme activity has been demonstrated. The most common genetic variation, designated as *CYP2C19*2*, leads to a splicing defect that functionally affects the enzyme resulting in loss of function. The *CYP2C19*2* allelic variant has been associated with higher levels of ADP-induced platelet aggregation values in Clopidogrel-treated patients and, consequently, a higher risk of adverse cardiovascular events, as stent thrombosis ***(Collet et al., 2009).*** Moreover, an allelic variant recently reported, *CYP2C19*17* has been associated with increased enzyme function ***(Simon et al., 2009)***. Thus, individuals harboring this genetic variant could have an enhanced response to antiplatelet treatment with Clopidogrel, improving the prevention of thrombotic events, but, on the other hand, having a putative increased risk of bleeding ***(Sibbing et al., 2011).***

In humans, carboxylesterase **CES1** is the most predominant hydrolytic enzyme, catalyzing the hydrolysis of numerous ester- and