The association between rs636832 & rs2740348 single nucleotide polymorphisms and the primary immune thrombocytopenic purpura in the Egyptian population

Thesis

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By

Wafaa Mohamed Abd Elghany Hassan

(M.B., B.Ch., M.Sc, MD.)

Supervised By

Prof. Dr. Mohamed Osman Azzazi

Professor of Internal Medicine & Clinical Haematology

Faculty of Medicine, Ain Shams University

Dr. Haydi Sayed Mohamed

Lecturer of Internal Medicine & Clinical Haematology

Faculty of Medicine, Ain Shams University

Dr. Inas Abdel Moaty Mohamed Eid

Lecturer of Internal Medicine & Clinical Haematology

Faculty of Medicine, Ain Shams University

Faculty of Medicine

Ain Shams University

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بسم الله الرحمن الرحيم

"قالوا سبحانك لا علم لنا إلا ما علمتنا إنك

أنت العليم الحكيم"

صدق الله العظيم

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Abstract

BACKGROUND: Primary ITP is an autoimmune disorder characterized by isolated thrombocytopenia. The pathogenesis of primary ITP remains incompletely understood, yet it appears to be highly multifactorial. The primary role of microRNA (miRNAs) is being to regulate the translation of many genes, that are involved in a variety of diseases and immune processes. **OBJECTIVE**: we aimed to investigate the relations between rs636832 & rs2740348 SNPs of AGO1 gene and Gemin4 gene respectively of miRNA biogenesis genes and the risk to primary ITP as well as response to therapy. **PATIENTS and METHODS:** This study involved 100 patients with primary ITP and 100 age and sex matched healthy controls. Real time polymerase chain reaction (PCR) was used for detection of rs636832 & rs2740348. **RESULTS:** *No statistically significance* was found between cases and controls regarding the genotype & alleles frequencies of both variants. *Regarding rs636832*, older age at onset of disease was observed with GG genotype (p-value=0.022). The non-cutaneous bleeding manifestations were more frequent in (AA+AG) genotypes with (*p value*= 0.066). Linkage disequilibrium (LD) was observed among studied groups with D'=0.41, r^2=0.04 & p=0.007 in ITP cases. **CONCLUSION:** our data suggest no association between rs636832 & rs2740348 and risk of ITP but rs636832 GG genotype appears to be associated with less aggressive clinical course of the disease and to be linked with rs2740348 inheritance.

Keywords: Primary, ITP, miRNAs, Linkage disequilibrium

List of Abbreviations

Ab	Antibody
ADAR1/B1	Adenosine deaminase, RNA specific/B1
Ag	Antigen
AGO	Argonaute
Anti-D	Anti-D immunoglobulin
APC	Antigen presenting cell
AREs	AU-rich elements
BM	bone marrow
BRCA1/2	breast cancergenes1/2
CBC	Complete blood count
CCL	Chemokine (C-C motif) ligand
CD40L	CD40 ligand
CMV	Cytomegalovirus
CNS	Central nervous system
CR	Complete response
CRC	Colorectal carcinoma
CXCL	C-X-C Motif Chemokine Ligand
DCs	Dendritic cells
DFS	Disease-free survival
DGCR8	Drosha and DiGeorge Syndrome Critical Region 8
DM	Dermatomyositis
DNA	Deoxy-nucleotide acid
DNMT1	DNA Methyltransferase 1
dsDNA	Double stranded DNA
DXM	Dexamethasone
EDTA	Ethylene diamine tetra-acetic acid
EIF	Eukaryotic initiation factors
FcγR	Fcγ receptors
FLSs	Fibroblast-like synoviocytes
FXR1	Fragile-x-mental retardation related protein 1
GD	Graves' disease
GP	Glycoprotein
H	Hours
H. pylori	Helicobacter pylori
HCV	Hepatitis C virus
HD-DXM	High dose DXM
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen
ICAM-1	Intercellular Adhesion Molecule 1

IDO1	Indoleamine 2,3-dioxygenase 1
IFN-γ	Interferon-gamma
IIM	Idiopathic inflammatory myopathy
IL	*Interleukins*
ITP	Immune thrombocytopenic purpura
IVIg	Intravenous immunoglobulin
IWG	International Working Group
LD	*Linkage disequilibrium*
LIN28A/B	Lin 28 homolog A/B
lncRNAs	Long non-coding RNAs
LPS	Lipopolysaccharide
m7G	Mirtrons and 7-methylguanine capped
MAPK	Microtubule associated protein kinase
MGB	Minor groove binder
MHC	Major histocompatibility complex
microRNPs	miRNA-protein complex
MiRISC	miRNA-induced silencing complex
MiRNA	MicroRNA
MKs	Macrophages
MREs	miRNA response elements
NcRNAs	Non-coding RNAs
NF-Kb	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural Killer
OS	Overall survival
PAIgGs	Platelet-associated immunoglobin Gs
PARC	Pediatric and Adult Intercontinental Registry
PBMCs	Peripheral blood mononuclear cells
PCR	Polymerase chain reaction
PFS	Progression free survival
PI3K-Akt	Phosphatidylinositol 3-kinase/Protein Kinase B
PM	Polymyositis
pre-miRNA	precursor-miRNA
pri-miRNAs	primary miRNAs
Pss	Sjogren’s syndrome
R	Response
RA	Rheumatoid arthritis
RfVIIa	Recombinant factor VIIa
RNA	Ribo-nucleotide acid
RNase	Ribonuclease
RTX	Rituximab
SiRNA	Small interfering RNA
SLE	Systemic lupus erythromatosis

SNP	Single nucleotide polymorphisms
SSc	systemic sclerosis
STAT	Signal Transducer And Activator Of Transcription 1
Tc	T cytotoxic
T_{FH}	Splenic follicular Th
TGF-β	*Transforming growth factor-beta*
Th	T helper
TLR4	Toll-like receptor 4
Tm	melting temperature
TNF-α	Tumor necrosis factor-alpha
TNRC	Trinucleotide repeat containing 6
TPO-RAs	Thrombopoietin receptor agonists
TRAF6	Tumor necrosis factor receptor (TNFR)-associated factor 6
Tregs	Regulatory T Cells
UTR	untranslated region

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Introduction

Primary ITP is an autoimmune disorder characterized by isolated thrombocytopenia as a platelet count <100 × 10^9/L, in the absence of other causes or disorders that may be associated with thrombocytopenia. Primary ITP has a prevalence of up to 9.5/100,000 adults and an incidence of about 3.3/100,000 adults per year, and this increases with age **(Zufferey et al., 2017).**

The primary ITP diagnosis remains one of the exclusion cases; there are currently no strong clinical or laboratory criteria to determine the accuracy of the diagnosis **(Rodeghiero et al., 2018)**.

The pathogenesis of primary ITP remains incompletely understood, yet it appears to be highly multifactorial **(Zufferey et al.; 2017)**. ITP involves isolated thrombocytopenia as a result of anti-platelet antibody production by plasma cells that induce antibody-mediated platelet phagocytosis, T-cell mediated platelet destruction, and/or impairment of megakaryocyte function **(Khodadi et al.; 2016)**.

MicroRNAs (miRNAs) are small non-coding RNAs (19–24 nt) involved in gene expression regulation through direct binding to their target messenger RNA (mRNA) **(Qian**a et al.; 2018)**.** The primary role of miRNAs is being to regulate the translation of many genes, that are involved in a variety of cellular processes, including cell proliferation, differentiation, apoptosis, and immune processes **(Aalaei-Andabili & Rezaei.; 2016).**

One strand of the miRNA duplexes integrates into miRNA-induced silencing complex (miRISC) and becomes mature miRNA. The miRISC

contains proteins including AGO1- 4, GEMIN3, and GEMIN4 that participate in mRNA inhibition or shearing of target mRNA **(Arribas-Hernández et al.; 2016).**

AGO1 gene is located on 1p34.3 chromosome, encodes a member of the argonaute family of proteins, which associate with small RNAs and have important roles in RNA interference (RNAi) and RNA silencing (**Gutiérrez-Malacatt et al.; 2016)**.

The rs636832 SNP is located in the intron of AGO1 gene. Its expression level is correlated with the proportion of Th17 cells which is associated with the development and prognosis of Graves' disease. It has also been associated with a decreased risk for developing chronic hepatitis B and lung cancer **(Shang et al.; 2014).**

GEMIN 4 gene is located on chromosome 17p13.3 encoding the Gemin4 protein which is important component of the RISC complex. GEMIN proteins in miRNA ribonucleoprotein particles are involved in the processing of miRNA precursors through their interaction with the key components of the RNA-induced silencing complex (**Horikawa et al., 2008).**

The rs2740348 polymorphism is a C/G mutation located in the exon region of the *GEMIN4* gene **(Cheng et al., 2018).** It was studied on various researches. **Liu et al.; 2014**, demonstrate no prognostic value of rs2740348 on hepatocellular carcinoma. It has been associated with breast cancer risk and with a lower risk for prostate cancer development **(Liu et al.; 2012 & Bermisheva et al.; 2018).**

Aim of work

The aim of our study to demonstrate the relation between rs636832 & rs2740348 SNPs of AGO1 gene and Gemin4 gene respectively and the risk to primary ITP as well as response to therapy in a cohort of Egyptian Population.