Association between *Xmn*I γ^{G}-158 (C/T) gene polymorphism and Hemoglobin F level in Egyptian sickle cell disease patients

Thesis

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DEDICATION TO

My mother;

Mrs. Amal Khidir El-Sayed

and to the

memory of my dear beloved father;

Lieutenant. Babiker Mohamed Babiker

Former Lieutenant colonel pilot,

Sudanese Air Force

ABSTRACT

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Background Sickle cell disease (SCD) is a common form of hereditary disease of an autosomal recessive inheritance with a highly variable phenotype. Interindividual variation in Fetal hemoglobin (HbF) expression is a known and potentially heritable modifier of SCD severity. One of the genetic determinants that is thought to cause a modest increase in HbF level is the *Xmn*1 γ^{G}-158 C/T gene polymorphism. **Objectives**: This study aimed to investigate the prevalence of the X*mn*1 γ^{G}-158 (C/T) gene polymorphism in Egyptian SCD patients and the association between this polymorphism and HbF level .

Design and setting: A cross-sectional case control study which was conducted on 111 SCD patients. Each patient was subjected to full medical history taking, through medical examination, routine laboratory investigation and genotyping of *Xmn*1 γ^{G}-158 (C/T) genetic polymorphism by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method.

Results: *Xmn*1 positive site was found in nine 9 (8.1%) of SCD patients. The wild genotype *Xmn*1(-/-) was the predominant state; found in 102 (91.9%) of SCD patients. The heteromutant genotype *Xmn1*(+/-) was detected in 8 (7.2%) while the homomutant genotype *Xmn*1(+/+) was found in 1(0.9%) of the SCD patients. Fetal Hb level, total hemoglobin and mean corpuscular volume were significant higher in the mutant genotypes harboring patients with p value <0.05. No statistical significant different was encountred as regards clinical history (p>0.05).

Conclusions: The study demonstrated that Egyptian SCD patients have low frequency of mutant genotypes for X*mn*1 γ^{G}-158 (C/T) gene polymorphism whether in heterozygous (+/-) or homozygous (+/+) state .The presence of X*mn*1 γ^{G}-158 (C/T) gene polymorphism has positive influence on Hb F level.

Key Words: X*mn*1 γ^{G}-158 (C/T) gene polymorphism, Sickle cell disease, Fetal hemoglobin , PCR-RFLP.

CONTENTS

List of Abbreviations

ACS	Acute chest syndrome
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ALT	Alanine transaminase
ANC	Absolute neutrophilic count
AST	Aspartate Transamianse
AVN	Avascular necrosis
BABY HUG	Pediatric Hydroxyurea Phase III Clinical Trial
BMD	Bone mineral density
BMI	Body mass index
bp	Base pair
$\chi 2$	Chi square
CBC	Complete Blood Count
CDC	U.S. Centers for Disease Control and Prevention
CI	Confidence Interval
CS	Complement system
CSSCD	Cooperative study of sickle cell disease
DAT	Antiglobulin test
DHTR	Delayed hemolytic transfusion reaction
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleosides triphosphates
EDTA	Ethylene di-amine tetra acetic acid
EPO	Erythropoietin
HbF	Fetal hemoglobin
G6PD	Glucose-6-phosphate dehydrogenase
GAG	Guanine Adenine Guanine
GAWA	Genome wide association study

GH	Growth hormone
GTG	Guanine Thiamine Guanine.
Hb	Hemoglobin
Hb SS	Homozygous sickle cell disease
HbS	Sickle hemoglobin
Hct	Hematocrite
HDAC	Histone deacetylase
HPFH	Hereditary persistence of fetal hemoglobin
HPLC	High-performance liquid chromatography
HSCT	Hematopoietic stem cell transplant
HU	Hydroxyurea
HUG-KIDS	Phase I/IIMulticenter pediatric hydroxyurea trial
HUSOFT	The Hydroxyurea Safety and Organ Toxicity study
HUSTLE	Hydroxyurea Study of Long-Term Effects
ISCs	Irreversibly sickled cells
LCR	Locus Control Region
LD	Linkage disequilibrium
LDH	Lactate dehydrogenase
MSH	The Multicenter Study of Hydroxyurea in adult
MCV	Mean corpuscular volume
MTD	Maximum tolerated dose
NO	Nitric Oxide
NSAIDs	Nonsteroidal anti-inflammatory drugs
OM	Osteomyelitis
OP	Osteoporosis
OR	Odds ratio
p value	Probability value
PAP	Pulmonary artery pressure

PCR	Polymerase Chain Reaction
PCV	packed cell volume
PHT	Pulmonary hypertension
PLT	Platelets
QTL	Quantitative Trait Locus
RA	Rheumatoid arthritis
RBCs	Red blood cell count
RHC	Right heart catheterization
Sβ	Sickle beta-thalssemia
SCA	Sickle cell anemia
SCD	Sickle cell disease
SD	Standard Deviation
SNP	Single nucleotides polymorphism
SPSS	Statistical Package for the Social Science
TCD	Transcranial Doppler
TRJV	Tricuspid jet velocity
UVP	Ultra violet transilluminator
VOC	Vasoocclusive crisis
WBCs	White blood cell counts
β	Beta
β^s	Sickle beta
γ	Gamma

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INTRODUCTION

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Sickle hemoglobinopathies are a related group of common and rare hemoglobin genotypes where all affected individuals are either homozygotes for the sickle hemoglobin (HbS) mutation or compound heterozygotes for the HbS and another globin gene mutation ***(Steinberg et al., 2009)***. Vasoocclusion and hemolytic anemia are the major features of this Mendelian disease that is notable for its clinical and hematologic variability ***(Steinberg et al., 2012)***. Pediatric mortality is primarily due to bacterial infection and stroke. In adults, specific causes of mortality are more varied, but individuals with more symptomatic disease may exhibit early mortality ***(Koch et al., 2000).***

Fetal hemoglobin (HbF), is a tetramer of 2 α- and 2γ -globin chains (HbF $\alpha_2\gamma_2$), inhibits the HbS polymerization that leads to erythrocyte damage and dysfunction ***(Solovieff et al., 2010).*** Fetal hemoglobin concentration and α thalassemia are the major modifiers of disease but are unlikely to be the only ***(Steinberg et al., 2012)***. Interindividual variation in HbF expression is a known and potentially heritable modifier of SCD severity ***(Lettre et al., 2008).***

One of the genetic determinants that is thought to cause a modest increase in HbF level is the C→T substitution at −158 of the γ^G globin gene (*Xmn*1 γ^G-158 C/T gene polymorphism) ***(Depke et al., 2013).*** It has been shown to be associated with the increased production of HbF and can strongly influences heterogeneity of sickle cell anemia ***(Peri et al., 1997).***