



Association between $XmnI \gamma^G$ -158 (C/T) gene polymorphism and Hemoglobin F level in Egyptian sickle cell disease patients

Thesis

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*Nada Babiker
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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 *Xmn1* γ^G -158 C/T gene polymorphism. **Objectives:** This study aimed to investigate the prevalence of the *Xmn1* 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 *Xmn1* γ^G -158 (C/T) genetic polymorphism by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method.

Results: *Xmn1* positive site was found in nine 9 (8.1%) of SCD patients. The wild genotype *Xmn1*(-/-) 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 *Xmn1*(+/+) 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 encountered as regards clinical history (p>0.05).

Conclusions: The study demonstrated that Egyptian SCD patients have low frequency of mutant genotypes for *Xmn1* γ^G -158 (C/T) gene polymorphism whether in heterozygous (+/-) or homozygous (+/+) state .The presence of *Xmn1* γ^G -158 (C/T) gene polymorphism has positive influence on Hb F level.

Key Words: *Xmn1* γ^G -158 (C/T) gene polymorphism, Sickle cell disease, Fetal hemoglobin , PCR-RFLP.

CONTENTS

Subject	Page No
List of Abbreviations	8
List of Tables	11
List of Figures	13
Introduction	14
Aim of the work	16
Review of literature	18
CHAPTER 1: An overview on Sickle cell disease	19
CHAPTER 2: Genetic background on Sickle cell disease	56
CHAPTER 3: Fetal hemoglobin	66
CHAPTER 4: <i>Xmn1</i> γ^G -158 (C/T) gene polymorphism	78
Patients and Methods	82
Results	93
Discussion	112
Summary	123
Conclusions and Recommendation	127
References	130
Arabic Summary	148

List of Abbreviations

ACS	Acute chest syndrome
ACS	Acute chest syndrome
ALT	Alanine transaminase
ANC	Absolute neutrophilic count
AST	Aspartate Transaminase
AVN	Avascular necrosis
BABY HUG	Pediatric Hydroxyurea Phase III Clinical Trial
BMD	Bone mineral density
BMI	Body mass index
bp	Base pair
χ^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/II Multicenter 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-thalassemia
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

List of tables

Table No.	Title	Page No.
Table 1	Gene frequency and common disease pattern of sickle cell haemoglobin (HbS) in the Middle Eastern Arab countries	
Table 2	Clinical features of SCD.	
Table 3	Laboratory Values in Sickle Cell Syndromes Among Those Older Than 5 Years	
Table 4	Approaches to the management of sickle cell disease and its complications	
Table 5	Indications for blood transfusion in sickle-cell disease:	
Table 6	Sickle-cell disease genotypes	
Table 7	Relationship of α thalassemia to common clinical features of SCD	
Table 8	Candidate Genes Associated with Subphenotypes of SCD	
Table 9	Conditions affecting hemoglobin F levels .	
Table 10	Effect of HbF on some clinical complications of sickle cell disease.	
Table 11	HbF-inducing drugs in childhood SCD	
Table 12	Demographic data of sickle cell disease patients	
Table 13	Clinical data of sickle cell disease patients	
Table 14	Baseline Descriptive laboratory data of sickle cell patients	
Table 15	Comparison of laboratory data between (SS) patients (n=77) & S β patients (n=34)	
Table 16	Comparison between sickle cell patients and the control group as regards sex distribution and Xmn1 genotyping results	

Table No.	Title	Page No.
Table 17	Comparison of Xmn1 γ G158 (C/T) gene polymorphism genotyping results between (SS) and (S β) patients	
Table 18	Comparison of demographic data between Xmn1 positive patients and Xmn1 negative patients	
Table 19	Comparison of clinical data between Xmn1 positive patients and Xmn1 negative patients	
Table 20	Comparison of laboratory data between Xmn1 positive patients and Xmn1 negative patients	
Table 21	Baseline HbF% level among (SS) and (S β) patients who harboring Xmn1 γ G-158 (C $\rightarrow$ T) gene polymorphism	
Table 22	Baseline HbF% level of Xmn1(+/-) patient and Xmn1(+/-) patients with sickle homozygous genotype	
Table 23	Descriptive data of HU treatment of patients with increased HbF level	
Table 24	Hematological response of sickle cell patients with increased HbF level during hydroxyurea therapy: (n=53)	
Table 25	Correlation of HbF% after HU therapy and baseline variables in patients with increased HbF level : (n=53)	
Table 26	Regression analysis of Hb F% level after hydroxyurea therapy in patients with increased Hb F level	

List of figures

Figure No.	Title	Page No
Figure 1	Sickle cell disorder inheritance pattern	
Figure 2	The distribution of sickle-cell anemia haplotypes among nations with high prevalence of the disease	
Figure 3	Point mutation in sickle cell anaemia	
Figure 4	Pathophysiology of sickle cell disease and vaso-occlusion	
Figure 5	The Vicious Cycle of the Acute Chest Syndrome	
Figure 6	Elongated RBC with tapering of ends in the peripheral blood smear of a patient with sickle-cell trait	
Figure 7	Bone marrow aspirate showing erythrocyte hyperplasia	
Figure 8	The pathophysiology of sickle cell disease and sites where drug treatment could be focused	
Figure 9	The beta globin locus and interacting loci based on genetic epidemiologic data in normal and sickle cell adult populations	
Figure10	Analysis of SNPs in candidate genes suggest associations with subphenotypes of sickle cell anemia	
Figure11	Multiple beneficial effects of hydroxyurea for SCA	
Figure12	Showing QIAamp Spin procedure (Adapted from QIAamp DNA Mini Kit Handbook).	
Figure13	Schematic drawing of the PCR cycle.	
Figure14	<i>Xmn1</i> γ^G -158 (C→T) genotyping by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method	
Figure15	Distribution of SS patients and S β patients	
Figure16	Clinical data of sickle cell patients	
Figure17	Sex distribution in the SCD cases and controls	
Figure 18	Comparison of genotyping of <i>Xmn1</i> polymorphism in sickle patients and control	
Figure19	Comparison of clinical data between <i>Xmn1</i> positive patients and <i>Xmn1</i> negative patients	
Figure18	Comparison of laboratory data between <i>Xmn1</i> positive patients and <i>Xmn1</i> negative patients	
Figure19	correlation of HbF% after HU with HbF before HU therapy	
Figure20	Comparison of laboratory data between <i>Xmn1</i> positive patients and <i>Xmn1</i> negative patients	
Figure21	Correlation of HbF% after HU with HbF before HU therapy	

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 2 γ -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 $\rightarrow$ T substitution at -158 of the γ^G globin gene (*Xmn1* 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*).