



Medical Studies Department
Institute of Postgraduate Childhood Studies

Phenotype-Genotype Variation In a Sample of Egyptian Patients with Uncommon Beta Thalassemia Mutations

Thesis submitted for fulfillment of requirements of Ph.D. degree in Medical
Childhood Studies

Submitted by
Mohamed Hamdy Abd El-Latif Ghandour
M.Sc. in Pediatrics, Cairo University

Supervised By

Prof. Dr. Magdy Karam Eldin Aly	Prof. Dr. Randa Kamal Abd El Raouf
Professor of Public Health	Professor of Pediatrics
Department of Medical Studies	Department of Medical Studies
Institute of Postgraduate Childhood Studies	Institute of Postgraduate Childhood Studies

Prof. Dr. Ghada Yousef Elkamah	Prof. Dr. Khalda Elsayed Mohamed
Professor of Clinical Genetics	Professor of Molecular Genetics
National Research Center	National Research Center

2015

DEDICATION

*This thesis is dedicated to all
Thalassemic patients with lots of
love and lots of prayers.*

ACKNOWLEDGMENTS

- I would like to acknowledge the **Medical Studies department Institute of Postgraduate Childhood Studies** as well as the **Clinical Genetics department, National Research center** for their great support during this study.
- In order to accomplish this study, I had support from many people. Therefore I would like to express my sincere thanks to them.
- First of all I want to express my sincere thanks to **Prof. Dr Magdy Karam Eldin Aly** Professor of Public Health -The Department of Medical Studies - Institute of Postgraduate Childhood Studies for his Guidance and supervision.
- I will be always grateful to **Prof. Dr Randa Kamal Abd El Raouf** Professor of Pediatrics -Department of Medical Studies - Institute of Postgraduate Childhood Studies. There are no words can express my deep appreciation and thanks for her guidance, providing facilities, and continuous encouragement to bring the best in me.
- My deepest thanks and appreciation to **Prof. Dr Ghada Yousef El kamah** Professor of Clinical Genetics -National Research Center for her helpful discussion, guidance, close supervision and massive input.
- I will be always grateful to **Prof. Dr Khalda Elsayed Mohamed** of the molecular Genetics department at the national research center for making me feel welcomed and for providing a kind, stress free, helpful work environment.
- My thanks extend also to **Prof. Dr Ahmed Ibrahim Samy Al-Katoury** Professor of Clinical Genetics -National Research Center for his Guidance and supervision
- My thanks extend also to A. Professor of Pediatrics at the Institute of Postgraduate Childhood Studies **Dr.Ayman Mohamed Nada** for his great support.
- I am also thankful for all those who helped me in collecting the blood sample and clinical data from thalassamia patients In NRC
- Finally I want to thank my family for being a source of continuous help and support and my gratitude for their endless patience that enabled me to complete this project.
- My deepest appreciation goes to all my dear friends for their kindness, help and wonderful friendship.

ABSTRACT

Background: Beta-thalassemias represent a group of hereditary blood disorders characterized by anomalies in the synthesis of the beta chains of hemoglobin resulting in variable phenotypes ranging from severe anemia to clinically asymptomatic individuals. Three main forms have been described: Thalassemia major, Thalassemia intermedia and Thalassemia minor. In each population only a few common mutations in the β globin have been found to be responsible for β -thalassemia, which indicate population differences. In Egypt β -thalassemia occurs in high frequency and premarital screening has been implemented for this disorder. Few comprehensive mutational screening for the β -globin gene locus has been carried so far in Egypt.

Objective: This study was designed with the objective to sequence the β -globin gene in β -thalassemia patients with uncommon mutations among Egyptian population and to investigate the genotype phenotype correlation.

Subjects and Methods: 30 β -thalassemia patients were enrolled in the study and hematological and biochemical data were recorded after full clinical evaluation, DNA was extracted and the β -globin gene was sequenced.

Results: Direct sequencing of the β -globin gene identified a total of 9 previously reported point mutations. β -thalassemia patients were either homozygous, heterozygous or compound heterozygous for those mutations,

Conclusion: this study characterizes some of the uncommon mutations and variants in the β -globin gene in Egypt and correlate between the genotypes and the phenotypes of the cohort of patients enrolled.

Recommendations: Sequencing of more Beta thalassemia Patients whose mutations couldn't be identified by Regular methods to establish a beta globin gene database specific for Egyptian population

Keywords: Beta Thalassemia –DNA sequencing –Molecular Characterization

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ABBREVIATIONS

- **ALT:** Alanine Aminotransferase
- **ARMS :**Amplification refractory mutation system
- **AST:** Aspartate Aminotransferase
- **BMT :** Bone Marrow Transplantation
- **BP:** Base pair
- **CO:** Carbon monoxide
- **CO₂ :** Carbon dioxide
- **DBil :** Direct bilirubin
- **DNA:** DeoxyribonucleicAcid
- **DNase :** Deoxyribonuclease
- **dNTP:** Deoxy Nucleotide Triphosphate
- **G-WAS :**Genome-wide association studies
- **H₂A:** Ascorbic Acid
- **Hb :**Haemoglobin
- **HbA:** Normal Adult Hemoglobin
- **HbA₂:** Minor Adult Hemoglobin
- **HBB:**human hemoglobin β gene
- **HbF:** Fetal hemoglobin
- **HCT:** Hematocrit
- **HGMD:** Human Genome Mutation Database
- **HLA:** Human Leukocyte Antigen
- **hnRNA :**Heterogeneous nuclear RNA
- **HPFH :** Heterocellular persistence of fetal hameoglobin
- **HPLC:** High performance liquid chromatography
- **HS1-5:** Hypersensitive site 1-5
- **IVS:** Intervening Sequence
- **LCR:** Locus Control Region
- **MCH:** Mean corpuscular hameoglobin
- **MCHC:**Mean corpuscular hameoglobin concentration
- **MCV:** Mean corpuscular volume
- **mRNA :** Messenger RNA
- **NESTROF:** Naked eye single tube Red cell Osmotic fragility test
- **NO:** Nitric oxide
- **O₂ :** Oxygen
- **OMIM :** Online mendelian inheritance in man
- **PCR :** Polymerase chain reaction
- **PCV:** Packed cell volume
- **PRE:** Positive regulatory element

- **QTLs** :Quantitative trait loci
- **RBC** : Red blood cells
- **RDB**: Reverse Dot Blot
- **RDW**: Red Cell Distribution Width
- **RE**: Restriction Enzyme
- **RFLP** Restriction Fragments Length Polymorphism
- **RNA**: Ribonucleic Acid
- **Rpm**: Revolution per Minute
- **SCD**: Sickle Cell Disease
- **SNP**: Single Nucleotide Polymorphisms
- **TBE**: Tris Borate EDTA buffer
- **TBil** :Total bilirubin
- **UTR**: Untranslated Region

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INTRODUCTION

Introduction

Beta-thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent beta globin chain synthesis, resulting in reduced Hb in red blood cells (RBC), decreased RBC production and anemia. Most thalassemias are inherited as recessive traits. According to *Galanello et al, (2010)* beta-thalassemias can be classified into:

- Thalassemia major
- Thalssemia intermedia
- Thalssemia minor

Haemoglobinopathies represent the most common genetic disorders Worldwide (*Bournazos et al., 2007 ; Sankaran et al., 2010*).

Thalassemia is the world's most common monogenic disorder. In 2005 it has been estimated that about 1.5% of the global population (80 to 90 million people) were carriers of beta thalassemia, with about 60,000 symptomatic individuals born annually, (*Vichinsky , 2005*). In Egypt, it is the most common chronic haemolytic anemia (85.1%) (*El-Beshlawy et al., 1999*) among cases of anemia.

El-Beshlawy & Youssry (2009) estimated a carrier rate varying from 5.3 to 9%.

Thalassaemia is classified according to the chain of the globin molecule that is affected. Beta thalassemia major is an autosomal recessive disorder, (*Weatherall & Clegg 1996*). It is known to occur due to the mutations in the Beta-globin gene (HBB) on chromosome 11 (*Marengo-Rowe, 2007*). There are over 200 known mutations in Beta Globin Gene found to be associated with thalassaemia Beta-thalassemias are also very heterogeneous at the molecular

level. A complete updated list is available at the Globin Gene Server Web Site - <http://globin.cse.psu.edu/> (*Cao & Galanello 2010*).

It is believed that each population has its own spectrum of mutations The World Health Organization has highlighted the importance of characterization of the spectrum of Beta thalassemia mutations as one of the ways for community control of Beta thalassemia (*Thong & Soo 2005*). Thus, characterization of the patients mutations in this study is essential for the management of Egyptian patients, particularly as related to premarital carrier detection and prenatal diagnosis.

AIM OF THE STUDY

Aim of the Study

- 1) Proper Diagnosis of 30 unrelated Egyptian beta-thalassemia patients harboring uncommon mutations through molecular characterization and phenotypic evaluation.
- 2) The Determination of a relation between the Pehnotype of our studied Beta thalassemia patients and their causative mutation.
- 3) Studying the genetic background of this disease among patients to offer proper genetic counseling which is considered the primary step in carrier detection and disease prevention.