Medical Studies Department
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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

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DEDICATION

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

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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 specicifc for Egyptian population

Keywords: Beta Thalassemia –DNA sequencing –Molecular Charcterization

CONTENTS

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 Leukocytoe Antigen
- **hnRNA :**Heterogeneous nuclear RNA
- **HPFH** : Heterocellular persisitence 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

LIST OF FIGURES

LIST OF TABLES

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

Haemoglopinopathies 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

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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.