THE ROLE OF MOLECULAR GENETICS IN DIAGNOSIS AND THERAPY OF HUMAN INHERITED DISEASES

Essay

Submitted for partial fulfillment

for M.Sc.Degree in

Pediatrics

Presented by

Mona Mounir Eskarous

M.B.,B.Ch.

Supervised by

Dr. Rabah Mohamed Shawky

Professor Of Pediatrics

Faculty Of Medicine

Ain-Shams University

Dr. Heba Hassan El-Sedafy

Lecturer Of Pediatrics

Faculty Of Medicine

Ain-Shams University

Faculty Of Medicine

Ain-Shams University

1994

ACKNOWLEDGEMENT

I would like to offer my deepest gratitude to Professor ***Dr. Rabah Mohamed Shawky*** *, Professor Of Pediatrics And Genetics , Faculty Of Medicine , Ain-Shams University , for her valuable guidance , patience , endless time in helping me and for supplying me with all the medical and moral support , without which , this essay would not have come out in the way it is .*

I wish to express my deep thanks to ***Dr. Heba Hassan El-Sedafy*** *, Lecturer In Pediatrics , Faculty Of Medicine , Ain-Shams University , for her kindness , great help and continuous encouragement .*

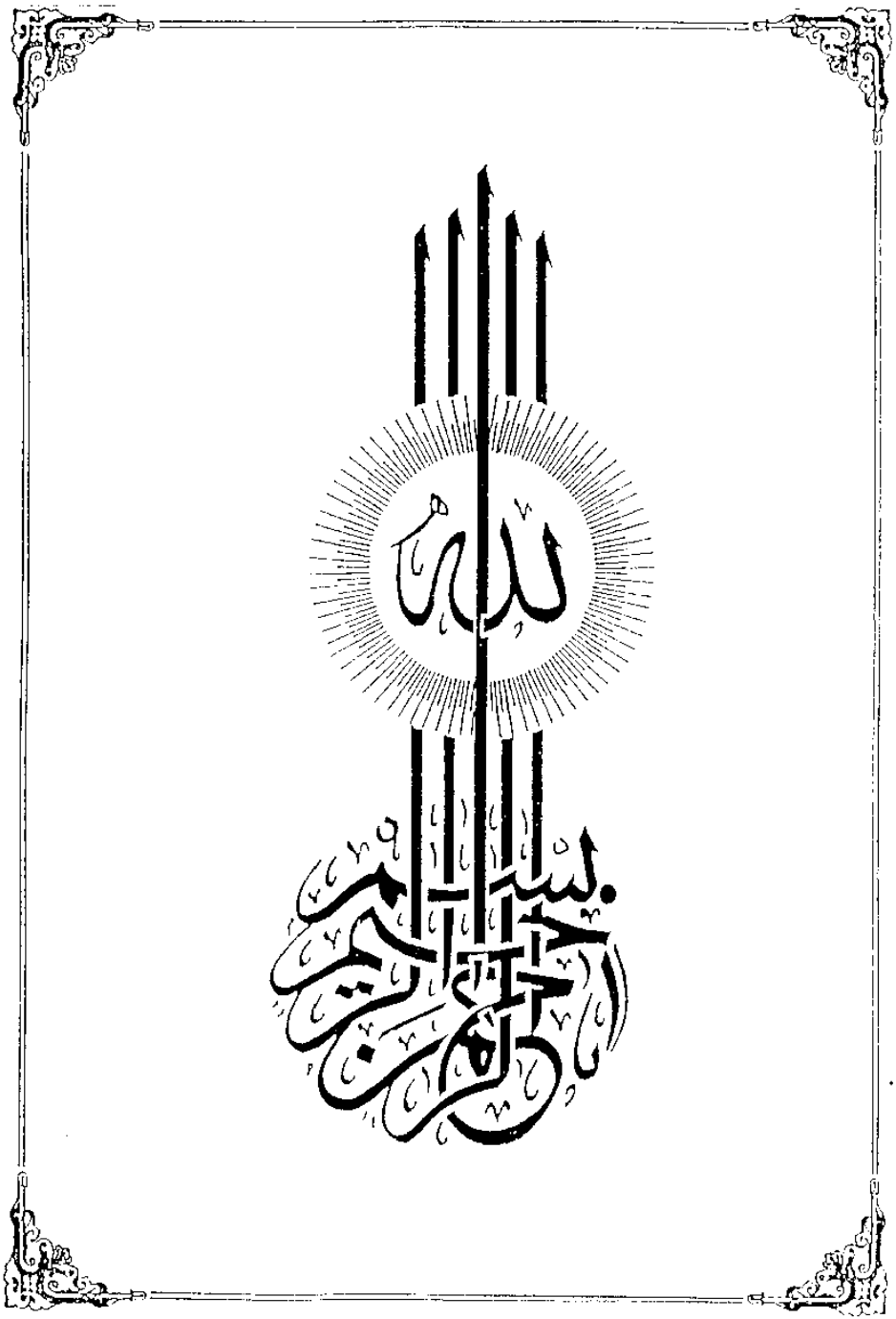

Table of contents

List Of Abbreviations

AAF : Acetylamine flurene .

AAV : Adeno-associated virus .

ABM : Adult bone marrow .

ADA : Adenosine deaminase deficiency .

AIDS : Acquired immuno-deficiency syndrome .

A.S. : Angleman Syndrome .

B.W.S. : Beckwith Widemann Syndrome .

C.F. : Cystic fibrosis .

CFTR : Cystic fibrosis transmembrane regulator gene .

CGD : Chronic granulometous disease .

CM : CentiMorgan .

CMV : Cytomegalovirus .

CISS : Chromosomal in situ suppression hybridization .

CVS : Chorionic Villus Sample .

DGS : DiGeorge Syndrome .

DMD : Duchenne and Becker Muscular Dystrophy .

DNA : Desoxy ribonucleic acid .

ESD : Estrase D enzyme .

FDA : Food and drug administration .

FLS : Family Linkage Studies .

FISH : Fluorescence in situ hybridization .

FITC : Fluorescein Isothiocyanate .

HAT : Hypoxanthine-aminopterin-thymidine .

HGH : Human growth hormone .

HIV : Human immuno-deficiency virus .

HPRT : Hypoxan-thine-guanine phosphoribosyl transferase .

HSV : Herps simplex virus .

LDL : Low density lipoprotein .

ISH : In situ hybridization .

LGS : Langer-Giedion syndrome .

MDS : Miller Dieker Syndrome .

OTD : Ornithine transcarbamylase .

PCR : Polymerase chain reaction .

PFGE : Pulsed field gel electrophoresis .

PWS : Prader Willi Syndrome .

REFLP : Restriction fragment length polymorphisms .

RG : Retinoblastoma gene .

RSV : Respiratory syncytial virus .

SCH : Somatic Cell Hybridization .

T-PA : Tissue plasminogen activator .

TIL : Tumour infiltrating lymphocytes .

TH : Tyrosine hydroxylase

TRITIC : Tetramethyl rhodomine isothiocyanate .

UDP : Uniparental disomy .

VDEPT : Viral directed enzyme prodrug therapy .

WEGRS : Wilms Tumour-Anirdia-Gonadoblastoma, Genitourinary tract malformation-Mental retardation syndrome .

YACs : Yeast artificial chromosomes .

List Of Figures

INTRODUCTION
&
AIM OF THE ESSAY

Introduction

Molecular genetics deals with the study of heredity through the methods of cytology and genetics. This science is concerned with the structure, number, function and movement of chromosomes and the numerous variations of these properties as they relate to transmission and expression of the genes *(Jurgan , 1980)*.

The scope of molecular genetics extends from the structure of the genes to the functioning of their products in a cell. This field is dominated by powerful and rapidly changing technology involving manipulation of DNA, RNA and protein resulting in interchange between new insights in basic science and application to medical problems. A fundamental goal of molecular genetics is to identify a heritable disease at the level of the affected gene and to chemically define the precise mutation *(Zasloff, 1992)* .

An understanding of mechanisms involved in the expression and regulation of any eukaryotic gene will ultimately require a determination of the location of its genetic elements within the genome of the corresponding organism. A first step in this determination is the localization of the gene to a particular chromosome and if possible to a particular subchromosomal region *(Gerhard et al, 1981)*.

Clinical genetics and molecular genetics can provide the essentials for the discovery of causes of developmental defects. The explanation of genetic

diseases by indirect methods is possible if we know that a certain gene at a given location (locus) is responsible for a given disease, we can isolate the DNA from that locus, examine it , translate it, examine the protein product, compare it with the same locus in a normal individual and use it for biologic test. Thus it is possible to learn about a disease and a normal process about which we had known only the genetic location, this known as reverse genetics *(Schmickel, 1986)*.

Since the introduction of chromosome analysis and the use of banding techniques most chromosomal aberrations can be detected. However many cases associated with very subtle deletions, duplications or translocations can not be detected even with all of the available technology *(Verma & Buba, 1989)*.

Aim of the essay

Aim of this essay is to show the important role of molecular genetics in diagnosis and treatment of pediatric inherited diseases

CHAPTER ONE

Chapter One

CHROMOSOMES AND CELL DIVISION

Human chromosomes consist of deoxyribonucleic acid (DNA) and specific proteins. In none dividing cells, chromosomes are tightly packaged in the nucleus. Chrmosomes contain most of the genetic information necessary for growth and differentiation *(Emery, 1983)*.

Although the correct number of chromosomes was established in 1956, it was not untill the early 1980s that newly developed molecular genetic techniques allowed the recognition of the more detailed characteristics of the chromosomes and the identification of a whole array of chromosome abnormalities *(Jurgen , 1980)* .

The nuclei of all human cells, with the exception of gametes, contain 46 chromosomes, consisting of 23 pairs. Of these, 22 pairs are called autosomes. They are numbered according to their sizes, chromosome one is the largest and chromosome 22 is the smallest. In addition there are 2 sex chromosomes : two x-chromosomes in females and one x and one y chromosome in males. The 2 members of a chromosome pair are called homologous chromosomes. One homologue of each chromosome pair is maternal in origin (from the egg), the second is paternal (from the sperm), The egg and the sperm each contain 23 chromosomes (haploid cell).During

the formation of zygote, they fuse into a cell with 46 chromosomes (deploid cell) *(Jurgan, 1980 ; Emery, 1983 ; Conner & Ferguson-Smith, 1987).*

Cell division

1- Mitosis :

It is somatic or body cell division which results in the growth of specific organs or the whole body. The process has different stages, during which DNA replication takes place and two daughter cells genetically identical to the original parent cell are formed.

These stages can be divided into the interphase stage during which DNA synthesis (and hence chromosome replication) occurs, and the mitotic stage during which the actual division process takes place. During interphase the chromosomes are not condensed and are invisible other than as a mass of chromatin. This is followed by prophase stage where there is gradual condensation of the chromatin. During metaphase, the phase after DNA replication but before cell division, individual chromosomes can be visualized. They consist of two arms : a short arm (p) and a long arm (q) separated by the centromere . Each arm consists of two identical parts, called chromatids. The chromatids of each chromosome arm are called sister chromatids. The centromere divides longitudinally and under the influence of the " spindle apparatus " homologous daughter chromatids move to opposite cell poles (anaphase), where they are included in separate daughter nuclei (telophase) *(Conner & Ferguson-smith, 1987)* .

2- Meiosis : (production of gametes)

During which eggs and sperms are formed. During meiosis, three unique processes take place: (i) crossing over of genetic material between two homologous chromosomes and this is preceded by the pairing of both members of each chromosome pair. (ii) random assortment of paternally and maternally derived homologous chromosomes to a particular daughter cell. The distribution of maternal or paternal chromosomes to a particular daughter cell is independent for each cell.(iii) two cell divisions, the first of which is reduction division, i.e a parental cell with 46 chromosomes divides into 2 daughter cells with 23 chromosomes each.

The second division is straight forward mitotic division in which the centromeres of the daughter chromosomes divide and homologous centromeres with their attached chromatids travel to opposite cell poles. The outcome of this two stage division process is a quarter of nuclei, each with half (n) the original number (2n) of chromosomes and with a new combination of parental genes *(Jurgen, 1980 ; Conner & Ferguson-smith, 1987).*

Types Of Chromosome Abnormalities

Chromosome abnormalities fall into two categories :- abnomalities of number and abnormalities of the structure, and may involve one or more autosomes, sex chromosomes or both *(Thompson & Thompson, 1986)* .