

# **Immunological Profile of Patients with Skeletal Dysplasia and Disproportionate Short Stature**

**Thesis**

**Submitted for partial fulfillment of the MD Degree  
in Clinical Pathology**

**By**

**Raghda Mohammed Mohammed Mostafa Ghorab**

*MB BCh, Ain Shams University*

*M.Sc. Clinical Pathology, Ain Shams University*

**Under supervision of**

**Prof. Nahla Mohamed Zakaria Yousef**

*Professor of Clinical Pathology,*

*Faculty of Medicine, Ain Shams University*

**Prof. Shams Mohamed Kholoussi**

*Professor of Clinical and Chemical Pathology,*

*Immunogenetics Department, Human Genetics & Genome Research*

*Division, National Research Centre*

**Prof. Rasha Mohamed Mamdouh Abdo**

*Professor of Clinical Pathology,*

*Faculty of Medicine, Ain Shams University*

**Prof. Samia Ali Temtamy**

*Professor of Human Genetics,*

*Department of Clinical Genetics, National Research Centre*

**Dr. Doaa Mohamed AbdElAziz**

*Assistant professor of Clinical Pathology,*

*Faculty of Medicine, Ain Shams University*

**Faculty of Medicine**

**Ain Shams University**

**2018**





*First and Foremost, thanks to “**ALLAH**” giving me the strength and power to complete this thesis.*

*I want to express my deep gratitude to Prof. Dr. Nahla Mohamed Zakaria Yousef, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University for her sincere and diligent supervision through her strict timing, valuable comments and unconditional support which contributed greatly to output this work in this final format.*

*I am greatly thankful to Prof. Dr. Shams Mohamed Kholoussi, Professor of Clinical Pathology at the Immunogenetics Department, Human Genetics and Genome Research Division, National Research Centre (NRC) for her continuous assistance and sincere effort she dedicated for this work,*

*I am profoundly indebted to Prof. Dr. Rasha Mohamed Mamdouh Abdo, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University for her genuine contribution in this work with her time and effort.*

*A special tribute is dedicated to Prof. Dr. Samia Ali Temtamy, Professor of Human Genetics, Department of Clinical Genetics, National Research Centre for suggesting ‘Disproportionate short stature’ as a starting point to begin with in the way of revealing Syndromic immunodeficiencies and for her continuous encouragement and meticulous guidance.*

*I wish to express my appreciation to Dr. Doaa Mohamed AbdEl Aziz, Assistant professor of Clinical Pathology, Faculty of Medicine, Ain Shams University for her helpful instructions and intellectual support.*

*My great thanks are directed to Dr. Haiam Abdel Raouf, Assistant professor at the Immunogenetics Department, NRC and Dr. Alaa Fayez, Researcher at the Department of Molecular Genetics and Enzymology, NRC for their great contribution in the practical part of this thesis. Also, deserved gratefulness is owed to Prof. Dr. Mona Aglan, professor at the Clinical Genetics*

*Department, NRC and Dr. Ghada Otaify, Researcher at the Clinical Genetics Department, NRC for their clinical guidance with patients.*

*I wish to acknowledge partial funding supported from STDF project 5253 for supplying Real-time PCR device used in this study and also internal NRC project 11010171 for partially funding chemical kits used in this study.*

*Finally, words alone cannot express the thanks I owe to my lovely family for their great support to me all through the way.*

*Raghda Ghorab*

## LIST OF CONTENTS

| <i>Title</i>                                                                             | <i>page No.</i> |
|------------------------------------------------------------------------------------------|-----------------|
| ❖ List of Abbreviations.....                                                             | <u>I</u>        |
| ❖ List of Figures.....                                                                   | <u>V</u>        |
| ❖ List of Tables.....                                                                    | <u>VII</u>      |
| ❖ Introduction .....                                                                     | <u>1</u>        |
| ❖ Aim of the work .....                                                                  | <u>5</u>        |
| ❖ Review of literature.....                                                              |                 |
| • Chapter 1:<br>Skeletal dysplasia syndromes with Syndromic<br>immunodeficiency          | <u>6</u>        |
| • Chapter 2:<br>Primary Immunodeficiency Disorders;<br>Overview and Laboratory Diagnosis | <u>18</u>       |
| ❖ Subjects and Methods.....                                                              | <u>48</u>       |
| ❖ Results .....                                                                          | <u>66</u>       |
| ❖ Discussion.....                                                                        | <u>97</u>       |
| ❖ Summary.....                                                                           | <u>113</u>      |
| ❖ Conclusion and Recommendations.....                                                    | <u>117</u>      |
| ❖ References .....                                                                       | <u>119</u>      |
| ❖ Appendices.....                                                                        | <u>133</u>      |
| ❖ Arabic Summary.....                                                                    | -               |



## LIST OF ABBREVIATIONS

| <i>Abbrev.</i> | <i>Full term</i>                                                                                           |
|----------------|------------------------------------------------------------------------------------------------------------|
| <b>ACP5</b>    | Acid phosphatase 5, tartrate resistant gene                                                                |
| <b>ADA</b>     | Adenosine deaminase                                                                                        |
| <b>AD</b>      | Autosomal dominant                                                                                         |
| <b>AIRE</b>    | Autoimmune regulator                                                                                       |
| <b>AK2</b>     | Adenylate kinase                                                                                           |
| <b>ALPS</b>    | Autoimmune lymphoproliferative syndrome                                                                    |
| <b>APECED</b>  | Autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy                                             |
| <b>APLAID</b>  | Autoinflammation and PLCG2-associated antibody deficiency and immune dysregulation                         |
| <b>AR</b>      | Autosomal recessive                                                                                        |
| <b>CANDLE</b>  | Chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature                       |
| <b>CASP</b>    | Caspase                                                                                                    |
| <b>CBC</b>     | Complete blood count                                                                                       |
| <b>CD</b>      | Cluster of differentiation                                                                                 |
| <b>CGD</b>     | Chronic granulomatous disease                                                                              |
| <b>cj</b>      | coding joint                                                                                               |
| <b>CHH</b>     | Cartilage-Hair Hypoplasia                                                                                  |
| <b>CHARGE</b>  | Coloboma, heart defects, atresia choanae, growth retardation, genital abnormalities, and ear abnormalities |
| <b>cm</b>      | Centimeter                                                                                                 |
| <b>CTRL</b>    | Control                                                                                                    |
| <b>CORO1A</b>  | Coronin 1A deficiency                                                                                      |
| <b>CVID</b>    | Common variable immunodeficiency                                                                           |
| <b>DBS</b>     | Dried blood spot                                                                                           |
| <b>DCLRE1C</b> | DNA cross-link repair enzyme 1C                                                                            |
| <b>DHR</b>     | Dihydrorhodamine                                                                                           |
| <b>DKC</b>     | Dyskeratosis Congenita                                                                                     |
| <b>DNA</b>     | Deoxyribonucleic acid                                                                                      |
| <b>EDTA</b>    | Ethylene diamine tetra-acetic acid                                                                         |

| <b><i>Abbrev.</i></b> | <b><i>Full term</i></b>                                        |
|-----------------------|----------------------------------------------------------------|
| <b>ESID</b>           | European Society of Immunodeficiency Disorders                 |
| <b>EVER</b>           | Epidermodysplasia veruciformis                                 |
| <b>FASL</b>           | Fas ligand                                                     |
| <b>FILS</b>           | Facial dysmorphism, immunodeficiency, livedo and short stature |
| <b>FITC</b>           | Fluorescein isothiocyanate                                     |
| <b>FMF</b>            | Familial Mediterranean Fever                                   |
| <b>FOXP3</b>          | Forkhead box protein 3                                         |
| <b>gDNA</b>           | genomic DNA                                                    |
| <b>G6PDH</b>          | Glucose-6-phosphate dehydrogenase                              |
| <b>Hb</b>             | Hemoglobin                                                     |
| <b>HIV</b>            | Human immunodeficiency virus                                   |
| <b>ID</b>             | Immunodeficiency                                               |
| <b>IDR</b>            | Immunodeficiency-related                                       |
| <b>IFN</b>            | Interferon                                                     |
| <b>Ig(s)</b>          | Immunoglobulin(s)                                              |
| <b>IPEX</b>           | Immunodysregulation polyendocrinopathy enteropathy X-linked    |
| <b>ITP</b>            | Idiopathic thrombocytopenic purpura                            |
| <b>IUIS</b>           | International Union of Immunological Societies                 |
| <b>IVIG</b>           | Intravenous immunoglobulin                                     |
| <b>JAK3</b>           | Janus kinase 3                                                 |
| <b>LIG4</b>           | Ligase                                                         |
| <b>LAD</b>            | Leukocyte adhesion deficiency                                  |
| <b>Max.</b>           | Maximum                                                        |
| <b>MHC</b>            | Major histocompatibility complex                               |
| <b>Min.</b>           | Minimum                                                        |
| <b>ml</b>             | Milliliters                                                    |
| <b>MoAbs</b>          | Monoclonal antibodies                                          |
| <b>MTHFD</b>          | Methylenetetrahydrofolate dehydrogenase                        |
| <b>MW</b>             | Molecular weight                                               |
| <b>NBS</b>            | New born screening                                             |
| <b>NCBI</b>           | National Center for Biotechnology Information                  |
| <b>NHEJ1</b>          | Nonhomologous end-joining protein 1                            |

| <b><i>Abbrev.</i></b> | <b><i>Full term</i></b>                                                                            |
|-----------------------|----------------------------------------------------------------------------------------------------|
| <b>NK</b>             | Natural Killer                                                                                     |
| <b>NRAS</b>           | Neuroblastoma RAS viral oncogene homolog                                                           |
| <b>NRC</b>            | National Research Centre                                                                           |
| <b>NS</b>             | Non-significant                                                                                    |
| <b>PBS</b>            | Phosphate buffered saline                                                                          |
| <b>PCR</b>            | Polymerase chain reaction                                                                          |
| <b>PE</b>             | Phytoerythrin                                                                                      |
| <b>PHA</b>            | Phytohaemagglutinin                                                                                |
| <b>PID EC</b>         | Primary Immunodeficiency Expert Committee                                                          |
| <b>PIDs</b>           | Primary immunodeficiencies                                                                         |
| <b>PLAID</b>          | PLCG2 associated antibody deficiency and immune dysregulation                                      |
| <b>PNP</b>            | Purine nucleoside phosphorylase                                                                    |
| <b>PRKDC</b>          | DNA-dependent protein kinase Cernunnos                                                             |
| <b>PTPRC</b>          | Protein tyrosine phosphatase receptor type C                                                       |
| <b>RAG</b>            | Recombination-activating gene                                                                      |
| <b>RMRP</b>           | Mitochondrial RNA-processing endoribonuclease gene                                                 |
| <b>RNase P</b>        | Ribonuclease P                                                                                     |
| <b>RNU4ATAC</b>       | small nuclear RNA, U4, AT-AC form gene                                                             |
| <b>rpm</b>            | Revolutions per minute                                                                             |
| <b>rs</b>             | Correlation coefficient                                                                            |
| <b>S</b>              | Significant                                                                                        |
| <b>SAC</b>            | Staphylococcus aureus Cowan antigen                                                                |
| <b>SCID</b>           | Severe combined immunodeficiency                                                                   |
| <b>SD</b>             | Standard deviation                                                                                 |
| <b>SEMD</b>           | Spondyloepimetaphyseal Dysplasia                                                                   |
| <b>SIOD</b>           | Schimke Immunoosseous Dysplasia                                                                    |
| <b>sj</b>             | Signal Joint                                                                                       |
| <b>SLE</b>            | Systemic lupus erythematosus                                                                       |
| <b>SMARCA1</b>        | SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A-like1 gene |
| <b>SMD</b>            | Spondylometaphyseal dysplasia                                                                      |
| <b>SPENCDI</b>        | Spondyloenchondrodysplasia with Immune Dysregulation                                               |

| <b><i>Abbrev.</i></b>                                                        | <b><i>Full term</i></b>                                                    |
|------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| <b>SPSS</b>                                                                  | Statistical Package for Special Sciences                                   |
| <b>SPUR</b>                                                                  | Serious, Persistent, Unusual (pathogens or site) and Recurrent             |
| <b>ST</b>                                                                    | Standard                                                                   |
| <b>TCL</b>                                                                   | T cell lymphopenia                                                         |
| <b>TCR</b>                                                                   | T cell receptor                                                            |
| <b>TCR-<math>\delta</math></b>                                               | T cell receptor delta                                                      |
| <b>TREC</b>                                                                  | T-cell receptor excision circle                                            |
| <b>TSH</b>                                                                   | Thyroid stimulating hormone                                                |
| <b>VDJ</b>                                                                   | variable, diversity and joining                                            |
| <b>VODI</b>                                                                  | Hepatic veno-occlusive disease with immunodeficiency                       |
| <b>WAS</b>                                                                   | Wiscott Adrich Syndrome                                                    |
| <b>WB</b>                                                                    | Whole blood                                                                |
| <b>WBCs</b>                                                                  | White blood cells                                                          |
| <b>WHIM</b>                                                                  | Warts, Hypogammaglobulinemia, Immuno-deficiency and Myelokathexis syndrome |
| <b>WHO</b>                                                                   | World Health Organization                                                  |
| <b><math>\alpha\beta</math>-TCR</b>                                          | alpha/beta T cell receptor                                                 |
| <b><math>\delta</math>REC</b>                                                | Delta recombination element                                                |
| <b><math>\delta</math>REC-<math>\psi</math>J<math>\alpha</math><br/>TREC</b> | Delta-Rec and psi-Joining segment-alpha Tcell receptor excision circle     |

## LIST OF FIGURES

| <b>Figure No</b> | <b>Title</b>                                                                                                                                                                   | <b>Page No</b> |
|------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <b>Fig. (1)</b>  | Depressed nasal bridge                                                                                                                                                         | 13             |
| <b>Fig. (2)</b>  | Bulbous nasal tip                                                                                                                                                              | 13             |
| <b>Fig. (3)</b>  | Flat face                                                                                                                                                                      | 14             |
| <b>Fig. (4)</b>  | High anterior hair line                                                                                                                                                        | 14             |
| <b>Fig. (5)</b>  | Patients with Sponastrime dysplasia                                                                                                                                            | 15             |
| <b>Fig. (6)</b>  | Brachydactyly                                                                                                                                                                  | 16             |
| <b>Fig. (7)</b>  | Fifth finger clinodactyly                                                                                                                                                      | 16             |
| <b>Fig. (8)</b>  | Percentage distribution of primary immunodeficiency disorders by different categories in Middle Eastern countries with high consanguinity compared to the registry of the ESID | 25             |
| <b>Fig. (9)</b>  | General clinical course of primary immunodeficiencies and different approaches in establishment of the diagnosis                                                               | 28             |
| <b>Fig. (10)</b> | Recombination processes leading to signal Joint (sj) TREC formation                                                                                                            | 35             |
| <b>Fig. (11)</b> | An algorithm of TREC analysis for NBS for SCID                                                                                                                                 | 39             |
| <b>Fig. (12)</b> | Nephelometry                                                                                                                                                                   | 53             |
| <b>Fig. (13)</b> | The principle and the basic components of flow cytometer                                                                                                                       | 55             |
| <b>Fig. (14)</b> | QIAamp DNA Blood Mini Kit Spin column extraction procedure                                                                                                                     | 60             |
| <b>Fig. (15)</b> | Box-Plot chart showing TRECs/ $\mu$ l DNA in control age groups                                                                                                                | 73             |
| <b>Fig. (16)</b> | Box-Plot chart showing TRECs/ml WB in control age groups                                                                                                                       | 74             |
| <b>Fig. (17)</b> | Box-Plot chart showing log TRECs/ml WB in control age groups                                                                                                                   | 74             |
| <b>Fig. (18)</b> | Patient (A) showing short limbed dwarfism with relatively shorter lower limbs                                                                                                  | 77             |
| <b>Fig. (19)</b> | Radiography showing mushroom shape appearance of metaphysis (A), absent carpal bones (B), thin narrow ribs (C) and platyspondyly (arrow)                                       | 77             |
| <b>Fig. (20)</b> | Patient (B) showing depressed nasal bridge, bilateral epicanthal folds and long philtrum (a). Disproportionate dwarfism is also illustrated (b)                                | 79             |
| <b>Fig. (21)</b> | Radiography showing wormian skull bones (A), metaphyseal cupping and widening (B)                                                                                              | 79             |

| <b>Figure No</b> | <b>Title</b>                                                                                                                                                                                                                                                                                                                                                                                            | <b>Page No</b> |
|------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <b>Fig. (22)</b> | Flow cytometric result of CD19 of patient (B)                                                                                                                                                                                                                                                                                                                                                           | 80             |
| <b>Fig. (23)</b> | Flow cytometric result of CD19 of a control in the same age group as patient (B)                                                                                                                                                                                                                                                                                                                        | 80             |
| <b>Fig. (24)</b> | Patient (F) showing short limbed dwarfism with significantly shorter lower limbs, camptodactyly and arachnodactyly                                                                                                                                                                                                                                                                                      | 81             |
| <b>Fig. (25)</b> | Radiography of patient (F) showing characteristic bowing of upper and lower limbs                                                                                                                                                                                                                                                                                                                       | 81             |
| <b>Fig. (26)</b> | Patient (M) showing disproportionate short limbed dwarfism with relatively shorter lower limbs                                                                                                                                                                                                                                                                                                          | 82             |
| <b>Fig. (27)</b> | Radiography showing bilateral hip dislocation and bilateral bowed femur                                                                                                                                                                                                                                                                                                                                 | 82             |
| <b>Fig. (28)</b> | Patient (J) showing disproportionate short limbed dwarfism (a), decreased height of vertebral bodies (b), epiphyseal dysplasia with mushroom like metaphysis (c&d) and absent carpal bones (d)                                                                                                                                                                                                          | 84             |
| <b>Fig. (29)</b> | Patient (O) showing disproportionate short limbed dwarfism (a), bowing of radii and femora with metaphyseal dysplasia (b&c)                                                                                                                                                                                                                                                                             | 85             |
| <b>Fig. (30)</b> | Patient (P) showing disproportionate dwarfism, facial asymmetry and bilateral mesomelia                                                                                                                                                                                                                                                                                                                 | 86             |
| <b>Fig. (31)</b> | Radiography of patient (P) showing metaphyseal widening and bowing in left femur, bilateral bowing of both radii                                                                                                                                                                                                                                                                                        | 86             |
| <b>Fig. (32)</b> | Patient (V) showing disproportionate short limbed dwarfism and right sided genu valgus (a), radiography showing bilateral bowing of tibia and fibula with metaphyseal widening and mild epiphyseal irregularity (b), hand x-ray showing cupping and fraying of carpal and metacarpal bones (c), dysplastic changes in lower thoracic and lumbar vertebrae (d) and skull x-ray showing wormian bones (e) | 88             |
| <b>Fig. (33)</b> | Patient (Y) showing disproportionate dwarfism (a), Radiographic survey showed shortening of long bones with metaphyseal widening (b&c) and platyspondyly (d)                                                                                                                                                                                                                                            | 89             |

## LIST OF TABLES

| <b>Table No</b>   | <b>Title</b>                                                                                                                                                                  | <b>Page No</b> |
|-------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <b>Table (1)</b>  | Syndromic immunodeficiency with skeletal dysplasia                                                                                                                            | 9              |
| <b>Table (2)</b>  | Primary immunodeficiencies associated with epiphyseal and metaphyseal dysplasia                                                                                               | 10             |
| <b>Table (3)</b>  | The 10 warning signs of primary immune deficiency in children                                                                                                                 | 26             |
| <b>Table (4)</b>  | Immune deficiency disease related (IDR) score                                                                                                                                 | 27             |
| <b>Table (5)</b>  | Immunophenotypic findings with correlated genetic defects in SCID                                                                                                             | 32             |
| <b>Table (6)</b>  | Main clinical and laboratory findings of immune dysregulation syndromes and causative genes                                                                                   | 43             |
| <b>Table (7)</b>  | Laboratory tests of immune function                                                                                                                                           | 47             |
| <b>Table (8)</b>  | Haematological values for normal infants                                                                                                                                      | 52             |
| <b>Table (9)</b>  | Haematological values for normal children                                                                                                                                     | 52             |
| <b>Table (10)</b> | Normal range of IgG, IgA and IgM compared with age                                                                                                                            | 54             |
| <b>Table (11)</b> | Sample real time PCR plate                                                                                                                                                    | 62             |
| <b>Table (12)</b> | Volume of reagents needed for indicated wells                                                                                                                                 | 63             |
| <b>Table (13)</b> | Sequence of primers and probes for the real time PCR assay                                                                                                                    | 63             |
| <b>Table (14)</b> | Comparison between patients and controls regarding age                                                                                                                        | 66             |
| <b>Table (15)</b> | Comparison between patients (n=25) and controls (n=20) regarding sex                                                                                                          | 67             |
| <b>Table (16)</b> | Perinatal risk factors among patients                                                                                                                                         | 67             |
| <b>Table (17)</b> | Initial manifestation of PID in the studied patients                                                                                                                          | 68             |
| <b>Table (18)</b> | Consanguinity rates among whole study population (n=45), control group (n=20), all patients with skeletal dysplasia (n=25) and patients with syndromic immunodeficiency (n=9) | 68             |
| <b>Table (19)</b> | Degree of consanguinity in those who are consanguineous (n=18)                                                                                                                | 69             |
| <b>Table (20)</b> | Comparison between consanguinity rates among patients with skeletal dysplasias and the control group                                                                          | 69             |
| <b>Table (21)</b> | Comparison between consanguinity rates among patients with syndromic immunodeficiency and the control group                                                                   | 69             |
| <b>Table (22)</b> | Paternal age of patients enrolled in the study                                                                                                                                | 70             |

| <b>Table No</b>   | <b>Title</b>                                                                                                                                                   | <b>Page No</b> |
|-------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|
| <b>Table (23)</b> | Pattern of inheritance and presence of affected sibling among skeletal dysplasias (n=25) and syndromic immunodeficiencies' patients (n=9)                      | 70             |
| <b>Table (24)</b> | Comparison between controls of group 1 (n=9) and group 2 (n=11) regarding the different laboratory parameters                                                  | 72             |
| <b>Table (25)</b> | Comparison between patients with syndromic immunodeficiency (n=5) and controls from 0-3 years (n=9) regarding studied laboratory parameters                    | 90             |
| <b>Table (26)</b> | Comparison between patients with syndromic immunodeficiency (n=4) and controls from >3-15 years (n=11) regarding studied laboratory parameters                 | 92             |
| <b>Table (27)</b> | Correlation between clinical immune manifestation of PID and laboratory presence of Immune Defect                                                              | 93             |
| <b>Table (28)</b> | Correlation between age and measured laboratory parameters in controls                                                                                         | 94             |
| <b>Table (29)</b> | Multiple regression analysis between age, absolute lymphocyte count, CD3% and absolute CD3 as predictor variables and TRECs/ $\mu$ l DNA as dependent variable | 95             |
| <b>Table (30)</b> | Multiple regression analysis between age, absolute lymphocyte count, CD3% and absolute CD3 as predictor variables and TRECs/ml WB as dependent variable        | 95             |
| <b>Table (31)</b> | Multiple regression analysis between age, absolute lymphocyte count, CD3% and absolute CD3 as predictor variables and Log TRECs/ml WB as dependent variable    | 96             |



---

# INTRODUCTION

---