

## TABLE OF CONTENTS

|                                                                                                                 | Page  |
|-----------------------------------------------------------------------------------------------------------------|-------|
| <b>ACKNOWLEDGEMENTS</b>                                                                                         | iii   |
| <b>ABSTRACT (THAI)</b>                                                                                          | v     |
| <b>ABSTRACT (ENGLISH)</b>                                                                                       | vii   |
| <b>LIST OF TABLES</b>                                                                                           | xiv   |
| <b>LIST OF FIGURES</b>                                                                                          | xv    |
| <b>ABBREVIATIONS AND SYMBOLS</b>                                                                                | xviii |
| <b>CHAPTER I INTRODUCTION</b>                                                                                   |       |
| <b>1.1 Rationale of the study</b>                                                                               | 1     |
| <b>1.2 Literature review</b>                                                                                    | 3     |
| 1.2.1 <i>p63</i> and its structure and functions                                                                | 3     |
| 1.2.2 Roles of <i>p63</i> in development                                                                        | 8     |
| 1.2.2.1 Knockout of <i>p63</i> <sup>-/-</sup> in mice                                                           | 10    |
| 1.2.3 Pathways of <i>p63</i>                                                                                    | 15    |
| 1.2.3.1 Upstream regulatory genes                                                                               | 17    |
| 1.2.3.1.1 Bone morphogenetic proteins gene ( <i>BMP</i> )s<br>and fibroblast growth factors gene ( <i>FGF</i> ) | 17    |
| 1.2.3.2 Direct downstream target genes                                                                          | 17    |
| 1.2.3.2.1 Distal-less homeobox 3 gene ( <i>DLX3</i> )                                                           | 18    |
| 1.2.3.2.2 IκB kinase alpha gene ( <i>Ikkα</i> )                                                                 | 21    |
| 1.2.3.2.3 Fibroblast-growth-factor-receptor-2 gene<br>( <i>FgfR2b</i> )                                         | 23    |

|                                                                                                      | Page |
|------------------------------------------------------------------------------------------------------|------|
| 1.2.3.2.4 $\beta$ -catenin gene/ wingless related protein gene ( <i>Wnt</i> )                        | 25   |
| 1.2.3.2.5 Ectodysplasin receptor gene ( <i>Edar</i> )                                                | 26   |
| 1.2.3.2.6 <i>Jag1</i> and <i>Notch1</i>                                                              | 27   |
| 1.2.4 <i>TP63</i> -associated human syndromic and non-syndromic malformations                        | 29   |
| 1.2.4.1 <u>E</u> ctrodactyly- <u>E</u> ctodermal Dysplasia- <u>C</u> left Lip/Palate (EEC) syndrome  | 31   |
| 1.2.4.2 <u>A</u> nkyloblepharon- <u>E</u> ctodermal Defects- <u>C</u> left Lip/Palate (AEC) syndrome | 32   |
| 1.2.4.3 <u>R</u> app- <u>H</u> odgkin <u>S</u> ndrome (RHS)                                          | 34   |
| 1.2.4.4 <u>L</u> imb- <u>M</u> ammary <u>S</u> ndrome (LMS)                                          | 36   |
| 1.2.4.5 Acro-Dermato-Ungual-Lacrimal-Tooth (ADULT) syndrome                                          | 37   |
| 1.2.4.6 Non-syndromic (Isolated) Split Hand/Foot Malformation (SHFM4)                                | 37   |
| 1.2.4.7 Non-Syndromic Cleft Lip (NSCL)                                                               | 38   |
| 1.2.5 Genotype-phenotype correlations of <i>TP63</i>                                                 | 38   |
| 1.2.5.1 Relationship between AEC syndrome and RHS                                                    | 40   |
| 1.2.5.2 Relationship between EEC, LMS and ADULT syndrome                                             | 40   |
| 1.2.6 Orofacial clefts and <i>TP63</i> -associated syndromes                                         | 41   |
| 1.2.7 Tooth development in mouse and human                                                           | 42   |
| 1.2.7.1 Stages of tooth morphogenesis                                                                | 42   |
| 1.2.7.2 Molecular interactions in odontogenesis                                                      | 44   |

|                                                                          | Page |
|--------------------------------------------------------------------------|------|
| 1.2.7.3 Hypodontia (Tooth agenesis)                                      | 46   |
| 1.2.7.4 Genes known to cause non-syndromic hypodontia in mice and humans | 47   |
| 1.2.8 p63 and tooth development                                          | 50   |
| 1.2.9 Facial development and palate development                          | 53   |
| 1.2.9.1 Facial development                                               | 53   |
| 1.2.9.2 Palate development                                               | 55   |
| 1.2.9.3 Orofacial clefts                                                 | 57   |
| 1.2.10 p63 and palate development                                        | 58   |
| <b>1.3 Objectives</b>                                                    | 61   |
| <b>1.4 Hypothesis</b>                                                    | 62   |
| <b>CHAPTER II RESEARCH DESIGN AND METHODS</b>                            |      |
| <b>2.1 Research design</b>                                               | 63   |
| <b>2.2 Methods</b>                                                       | 65   |
| 2.2.1 Selecting of population                                            | 65   |
| 2.2.2 Blood sample collection                                            | 68   |
| 2.2.3 Genomic DNA preparation                                            | 68   |
| 2.2.4 <i>TP63</i> mutation analysis                                      | 70   |
| 2.2.4.1 Primer designs                                                   | 70   |
| 2.2.4.2 PCR optimization                                                 | 70   |
| 2.2.4.3 Agarose gel electrophoresis                                      | 75   |
| 2.2.4.4 Purification of PCR products and DNA sequencing                  | 75   |
| 2.2.5 Data analysis                                                      | 76   |

## CHAPTER III RESULTS

|                                                                                 |    |
|---------------------------------------------------------------------------------|----|
| <b>3.1 The pathogenic mutation at codon 227 in exon 6 of the <i>TP63</i></b>    | 77 |
| 3.1.1 The affected girl                                                         | 78 |
| 3.1.1.1 Clinical findings                                                       | 78 |
| 3.1.1.2 Radiographic findings of the affected girl                              | 79 |
| 3.1.1.3 Scanning Electron Microgram (SEM) of the affected girl                  | 80 |
| 3.1.2 The affected father                                                       | 81 |
| 3.1.2.1 Clinical findings                                                       | 81 |
| 3.1.2.2 Oral manifestations of the affected father                              | 82 |
| 3.1.2.3 Radiographic findings of the affected father                            | 83 |
| 3.1.2.4 SEM of the affected father                                              | 84 |
| 3.1.3 <i>TP63</i> mutation analysis                                             | 85 |
| <b>3.2 The additional results of <i>TP63</i> mutation analysis in the study</b> | 87 |
| <b>3.3 The single nucleotide polymorphism (SNP) of the <i>TP63</i></b>          | 95 |

## CHAPTER IV DISCUSSION

|                                                                                     |     |
|-------------------------------------------------------------------------------------|-----|
| 4.1 <i>TP63</i> mutation and syndromic hypodontia with/without orofacial clefts     | 97  |
| 4.1.1 A novel R227P mutation of <i>TP63</i> gene in a Thai family with EEC syndrome | 97  |
| 4.1.1.1 Phenotype of a Thai family with EEC syndrome with the R227P mutation        | 99  |
| 4.1.1.1.1 Abnormalities of Limbs                                                    | 99  |
| 4.1.1.1.2 Ectodermal defects                                                        | 102 |

|                                                                                                                  | Page |
|------------------------------------------------------------------------------------------------------------------|------|
| 4.1.1.1.3 Orofacial clefts                                                                                       | 108  |
| 4.1.1.2. R227P mutation: mutation hotspot and highly conserved amino acid                                        | 110  |
| 4.1.1.3 Structural model of p63 protein affected with R227P mutation                                             | 112  |
| 4.1.1.4 Comparison of R227Q mutations and this R227P mutation                                                    | 114  |
| 4.1.1.4.1 p63 and micturition difficulties                                                                       | 116  |
| 4.1.1.4.2 p63 and extensive dental caries                                                                        | 117  |
| 4.1.2 The absence of <i>TP63</i> mutation in other 8 cases of syndromic hypodontia with/without orofacial clefts | 119  |
| <b>4.2 <i>TP63</i> mutation and non-syndromic hypodontia, non-syndromic orofacial clefts</b>                     | 119  |
| <b>4.3 Single nucleotide polymorphism (SNPs) of <i>TP63</i> in this study</b>                                    | 120  |
| <b>CHAPTER V CONCLUSIONS</b>                                                                                     | 122  |
| <b>REFERENCES</b>                                                                                                | 124  |
| <b>APPENDICES</b>                                                                                                |      |
| Appendix A Homo sapiens TAp63 alpha mRNA sequence                                                                | 136  |
| Appendix B Homo sapiens TAp63 alpha amino acid sequence                                                          | 137  |
| Appendix C The previously reported SNPs of <i>TP63</i> found in the study                                        | 141  |
| Appendix D List of chemicals and materials were used in the study                                                | 151  |
| Appendix E List of instruments were used in the study                                                            | 153  |
| Appendix F List of solutions and buffers were used in the study                                                  | 155  |
| <b>CURRICULUM VITAE</b>                                                                                          | 157  |

## LIST OF TABLES

| <b>Table</b> |                                                                                                                                                        | <b>Page</b> |
|--------------|--------------------------------------------------------------------------------------------------------------------------------------------------------|-------------|
| 1.1          | Incidence of orofacial clefts in <i>TP63</i> -associated syndromes                                                                                     | 42          |
| 2.1          | Summary of the relevant data of all patients                                                                                                           | 66          |
| 2.2          | Craniofacial Genetics Laboratory (CGL) DNA number and phenotype of all patients in this study                                                          | 67          |
| 2.3          | Intronal primer of <i>TP63</i>                                                                                                                         | 72          |
| 2.4          | Optimized protocol for the chemicals used in the PCR procedure                                                                                         | 73          |
| 2.5          | Optimized protocol for temperatures used in the PCR procedure                                                                                          | 74          |
| 3.1          | Summary of both <i>TP63</i> pathogenic mutation and single nucleotide polymorphisms (SNPs) with phenotype in all patients                              | 88          |
| 3.2          | Summary of single nucleotide polymorphisms (SNPs) in all patients                                                                                      | 92          |
| 3.3          | Summary of <i>TP63</i> single nucleotide polymorphisms (SNPs) in this study                                                                            | 96          |
| 4.1          | Comparison between 3 amino acids; arginine (R), glutamine (Q), proline(P)                                                                              | 111         |
| 4.2          | Reviewing of the clinical features of R227Q mutations in EEC and ADULT syndrome and comparing with R227P mutation in the Thai family with EEC syndrome | 115         |

## LIST OF FIGURES

| Figure                                                                                                                                                | Page |
|-------------------------------------------------------------------------------------------------------------------------------------------------------|------|
| 1.1 Structures of p63 proteins                                                                                                                        | 4    |
| 1.2 Transition of p63 isoforms at different stages of epidermal morphogenesis in mouse embryos                                                        | 8    |
| 1.3 Different concepts of the role of p63 in epidermal morphogenesis between two independent studies which generated <i>p63</i> knockout mouse models | 9    |
| 1.4 <i>p63</i> Knockout model of Mills et al.                                                                                                         | 12   |
| 1.5 <i>p63</i> Knockout model of Yang et al.                                                                                                          | 14   |
| 1.6 Pathways of p63                                                                                                                                   | 15   |
| 1.7 List of the published p63 target genes                                                                                                            | 16   |
| 1.8 Tricho-Dento-Osseous Syndrome (TDO) is caused by mutations in the <i>DLX3</i> gene                                                                | 20   |
| 1.9 Molar tooth phenotype of <i>Ikka</i> mutant mice                                                                                                  | 22   |
| 1.10 Expression pattern of <i>Ikka</i> during early tooth and whisker development                                                                     | 23   |
| 1.11 Hypohidrotic ectodermal dysplasia is caused by mutation in the <i>EDA</i> gene                                                                   | 27   |
| 1.12 Pathogenic <i>TP63</i> mutations in seven allelic diseases                                                                                       | 30   |
| 1.13 EEC syndrome is caused by mutations in the DNA-binding domain of the <i>TP63</i> gene                                                            | 31   |
| 1.14 AEC syndrome is caused by mutations in the SAM domain of <i>TP63</i> gene                                                                        | 33   |
| 1.15 Thai patient with RHS at ages 8 and 21 years                                                                                                     | 35   |

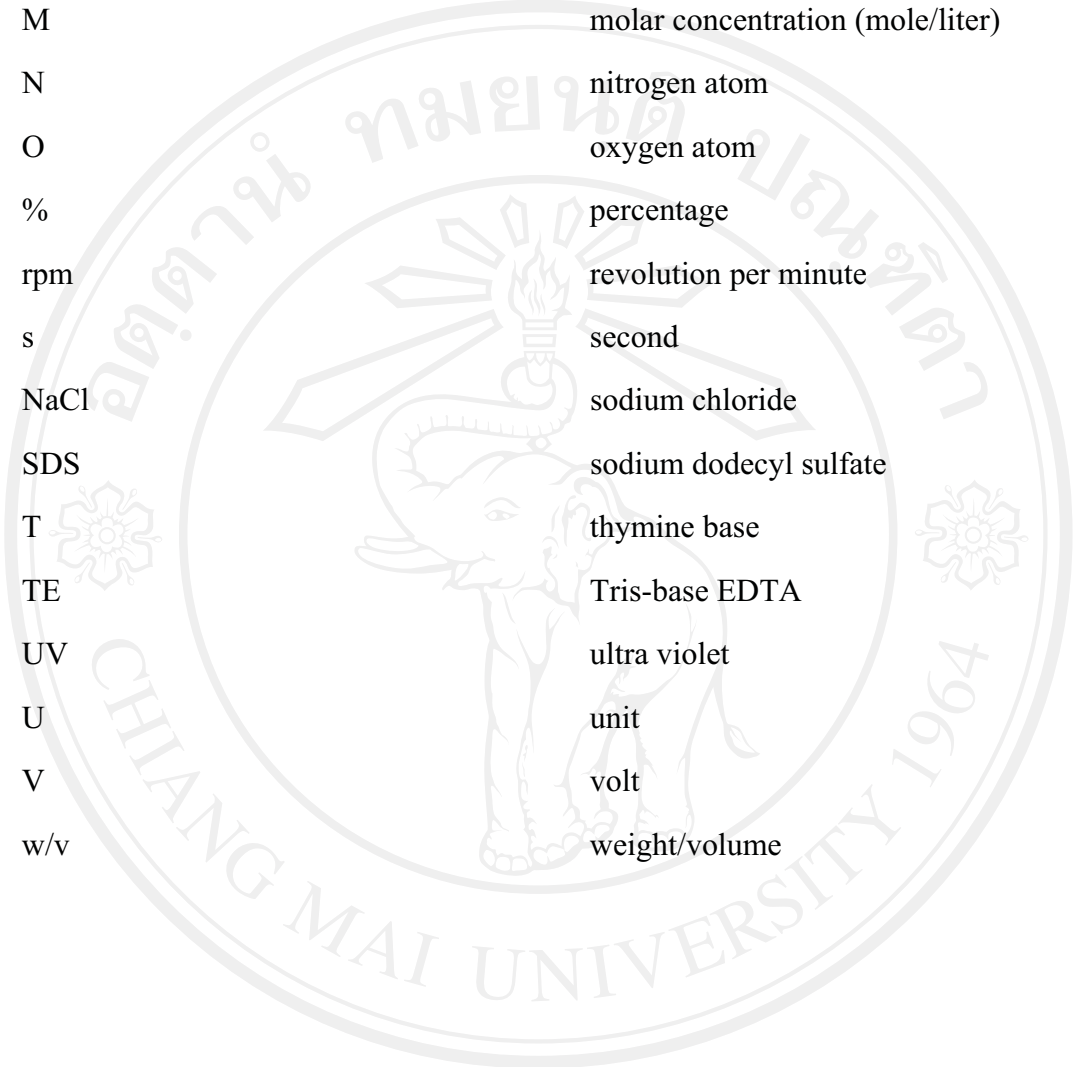
|      |                                                                                                           |    |
|------|-----------------------------------------------------------------------------------------------------------|----|
| 1.16 | Dr. Piranit Kantaputra, Thai patient with RHS, and Dr. Warissara Sripathomsawat                           | 35 |
| 1.17 | Genotype-Phenotype Correlations of <i>TP63</i>                                                            | 39 |
| 1.18 | Stages of tooth development                                                                               | 43 |
| 1.19 | Role of gene expression in early tooth development                                                        | 45 |
| 1.20 | Molecular signaling in tooth development                                                                  | 45 |
| 1.21 | pan-p63 hybridization signal in tooth development                                                         | 51 |
| 1.22 | p63 isoforms in tooth development                                                                         | 52 |
| 1.23 | Facial development                                                                                        | 54 |
| 1.24 | Palate development                                                                                        | 56 |
| 1.25 | Expression of p63 transcripts/proteins during facial morphology                                           | 59 |
| 1.26 | Bilateral cleft lip and complete cleft of the secondary palate phenotype of <i>p63<sup>-/-</sup></i> mice | 60 |
| 2.1  | Summary of all procedures in this study                                                                   | 64 |
| 2.2  | Diagram presents the genomic DNA preparation                                                              | 69 |
| 3.1  | A Thai EEC syndrome family with novel R227P mutation                                                      | 77 |
| 3.2  | Clinical findings of a 4-month-old Thai girl with EEC syndrome                                            | 78 |
| 3.3  | Radiographic findings of the affected girl                                                                | 79 |
| 3.4  | Renal ultrasound of the affected girl                                                                     | 80 |
| 3.5  | SEM of the affected girl's scalp hair                                                                     | 80 |
| 3.6  | Clinical findings of the affected father with EEC syndrome                                                | 81 |
| 3.7  | Oral manifestations of the affected father                                                                | 82 |
| 3.8  | Right hand radiographic findings of the affected father                                                   | 83 |
| 3.9  | SEM of the affected father's scalp hair                                                                   | 84 |



|      |                                                                                                                                                                      |     |
|------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 3.10 | <i>TP63</i> Mutation analysis of both daughter and father                                                                                                            | 85  |
| 3.11 | Comparison the altered nucleotide and altered amino acid with other species                                                                                          | 86  |
| 4.1  | Signaling pathways in the failure to maintain median AER, or in defective AER signaling, underlie SHFM                                                               | 101 |
| 4.2  | Some affected individuals of the largest previously reported family with EEC syndrome with the R227Q mutation are reported to have unusually extensive dental caries | 109 |
| 4.3  | Comparing structural models of p63 DNA-Binding Domain (DBD) between WT, R227P mutation, and R227Q mutation                                                           | 113 |
| C.1  | Chromatogram of RefSNP ID: rs28673064                                                                                                                                | 141 |
| C.2  | Chromatogram of RefSNP ID: rs62702062                                                                                                                                | 142 |
| C.3  | Chromatogram of RefSNP ID: rs34429985                                                                                                                                | 143 |
| C.4  | Chromatogram of RefSNP ID: rs2276792                                                                                                                                 | 144 |
| C.5  | Chromatogram of RefSNP ID: rs6789961                                                                                                                                 | 145 |
| C.6  | Chromatogram of RefSNP ID: rs6790167                                                                                                                                 | 146 |
| C.7  | Chromatogram of RefSNP ID: rs9840359                                                                                                                                 | 147 |
| C.8  | Chromatogram of RefSNP ID: rs9840360                                                                                                                                 | 148 |
| C.9  | Chromatogram of RefSNP ID: rs1554131                                                                                                                                 | 149 |
| C.10 | Chromatogram of RefSNP ID: rs1345186                                                                                                                                 | 150 |

## ABBREVIATIONS AND SYMBOLS

|                    |                                  |
|--------------------|----------------------------------|
| A                  | adenine base                     |
| $\alpha$           | alpha                            |
| bp                 | base pair                        |
| $\beta$            | beta                             |
| C                  | cytosine base                    |
| $^{\circ}\text{C}$ | degree Celsius                   |
| $\Delta$           | delta (upper-case letter)        |
| $\delta$           | delta (lower-case letter)        |
| dNTP               | deoxynucleotide triphosphate     |
| DNA                | deoxyribonucleic acid            |
| $\epsilon$         | epsilon                          |
| EDTA               | ethylenediaminetetra-acetic acid |
| $\gamma$           | gamma                            |
| g                  | gram                             |
| G                  | guanine base                     |
| H                  | hydrogen atom                    |
| kb                 | kilobase                         |
| L                  | liter                            |
| $\text{MgCl}_2$    | magnesium chloride               |
| mRNA               | messenger ribonucleic acid       |
| $\mu\text{L}$      | microliter                       |
| $\mu\text{M}$      | micromolar                       |
| mg                 | milligram                        |
| mL                 | milliliter                       |
| mm                 | millimeter                       |



|      |                                  |
|------|----------------------------------|
| mM   | millimolar                       |
| min  | minute                           |
| M    | molar concentration (mole/liter) |
| N    | nitrogen atom                    |
| O    | oxygen atom                      |
| %    | percentage                       |
| rpm  | revolution per minute            |
| s    | second                           |
| NaCl | sodium chloride                  |
| SDS  | sodium dodecyl sulfate           |
| T    | thymine base                     |
| TE   | Tris-base EDTA                   |
| UV   | ultra violet                     |
| U    | unit                             |
| V    | volt                             |
| w/v  | weight/volume                    |

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่  
Copyright© by Chiang Mai University  
All rights reserved