CHAPTER III

RESULTS

3.1 The pathogenic mutation at codon 227 in exon 6 of the *TP63*

The pathogenic mutation at codon 227 in exon 6 of the *TP63* gene was detected in a family with EEC syndrome. A 4-month-old Thai girl (CGL. number 181) and her affected father (CGL. number 182) were seen at the Department of Pediatrics, Faculty of Medicine, Chiang Mai University **(Figure 3.1**).

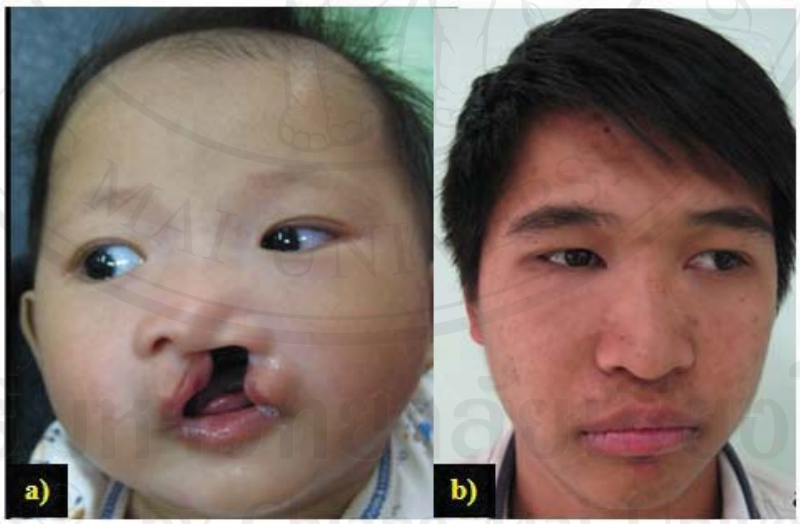

Figure 3.1 A Thai EEC syndrome family with novel R227P mutation; (a) a 4-month-old Thai girl, and (b) her affected father.

3.1.1 The affected girl

3.1.1.1 Clinical findings

The girl was the only child of a 22-year-old mother and a 24-year-old father. Consanguinity was denied. The pregnancy was uneventful. There was no history of prenatal drug use or toxic exposure. The patient was born at term, delivered normally. At four months, her body weight, length and head circumference were within normal percentiles for age. Developmental milestones were normal. Her clinical findings included dry and sparse, dark hair, left cleft lip and palate, and depressed nasal bridge **(Figure 3.2a, g)**. Ectrodactyly of both hands and the right foot were observed. Syndactyly of the 4th and 5th toes of the right foot was noted **(Figure 3.2b-e)**. She had slightly dry skin, and thin nails. Nipples were normal **(Figure 3.2f)**.

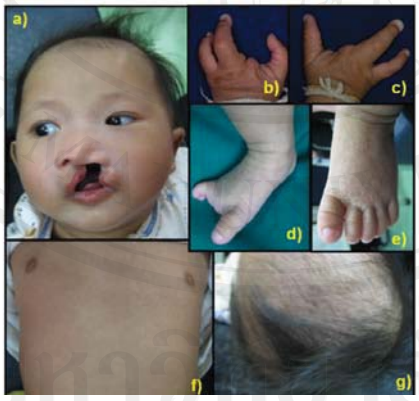

Figure 3.2 Clinical findings of a 4-month-old Thai girl with EEC syndrome; (a) Unilateral left cleft lip and palate with depressed nasal bridge, (b, c, d) Ectrodactyly of both hands and the right foot, syndactyly of the 4th and 5th toes of the right foot with slightly dry skin, and thin nails, (e) Normal left foot, (f) Normal nipples, (g) Dry and sparse, dark hair.

3.1.1.2 Radiographic findings of the affected girl

Radiographic findings of the girl showed absence of proximal, middle and distal phalanges of the 2nd and 3rd digits of both hands. The right foot also was characterized by absence of proximal, middle and distal phalanges of the 2nd and 3rd toes, a rudimentary bone at the clefting area of the right foot **(Figure 3.3a, b)**. A chest radiograph and renal ultrasound of the kidneys and bladder were unremarkable **(Figure 3.4)**.

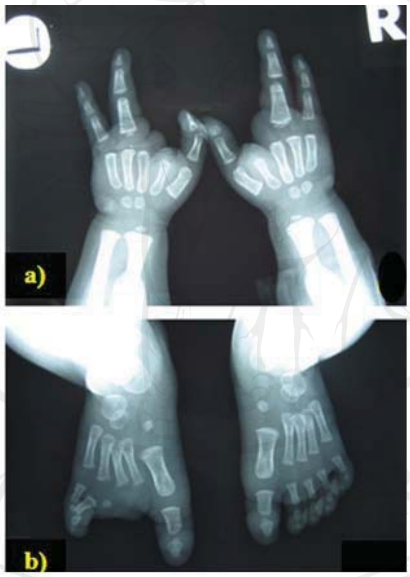

Figure 3.3 Radiographic findings of the affected girl; (a) Absence of proximal, middle and distal phalanges of the 2nd and 3rd digits of both hands. (b) Absence of proximal, middle and distal phalanges of the 2nd and 3rd toes, a rudimentary bone at the clefting area of the right foot (Courtesy of Dr. Pranoot Tanpaiboon, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand).

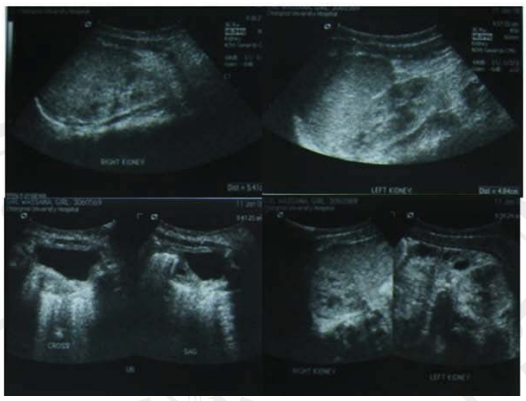

Figure 3.4 Renal ultrasound of the affected girl shows normal kidneys and bladder (Courtesy of Dr. Pranoot Tanpaiboon, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand).

3.1.1.3 Scanning Electron Microgram (SEM) of the affected girl

The SEM of the scalp hair of the affected girl showed a small hair bulb, thin hair shafts, and hypoplastic cuticles **(Figure 3.5).**

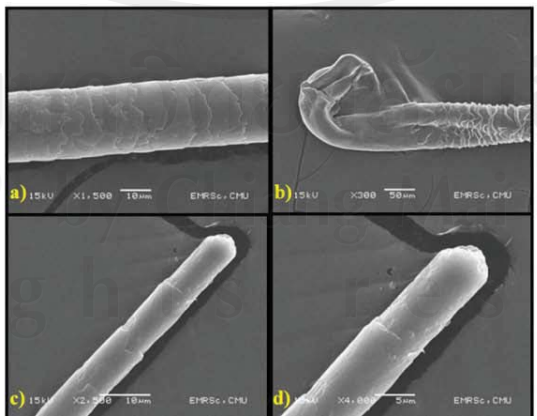

Figure 3.5 SEM of the affected girl's scalp hair; (a) hypoplastic cuticles, (b) small hair bulb, and (c, d) thin hair shafts.

3.1.2 The affected father

3.1.2.1 Clinical findings

Her affected father presented with normal dark hair and nipples **(Figure 3.6a, b)**. Skin was dry. He had ectrodactyly of the right hand, bifid right thumb, and flexion contracture of the distal phalanx of the left index finger **(Figure 3.6c)**. Ectrodactyly of the feet was not observed. The 2nd toes appeared small and narrow **(Figure 3.6d)**. Toenails were hypoplastic, while the fingernails appeared normal.

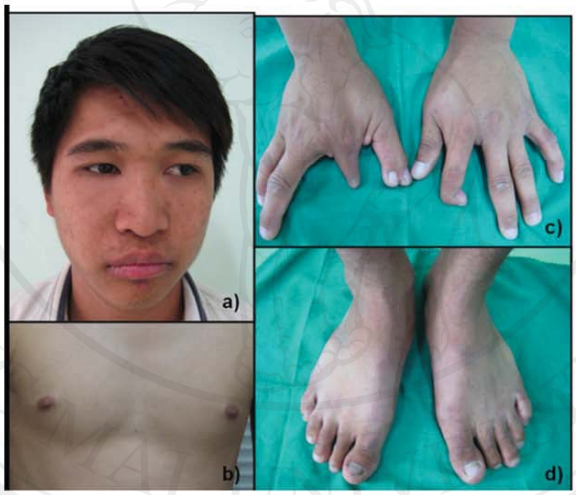

Figure 3.6 Clinical findings of the affected father with EEC syndrome; (a) normal dark hair, dry skin, (b) normal nipples, (c) ectrodactyly of the right hand, bifid right thumb, and flexion contracture of the distal phalanx of the left index finger, (d) Absence of ectrodactyly of feet, the 2nd toes appear small and narrow. Toenails are hypoplastic, while the fingernails appear normal.

3.1.2.2 Oral manifestations of the affected father

Oral manifestations included congenital absence of the permanent mandibular canines, generalized microdontia, prominent marginal ridges of permanent maxillary incisors, round-shaped permanent molars, barrel-shaped permanent maxillary central incisors, enamel hypoplasia of permanent mandibular first premolars, and extensive dental caries **(Figure 3.7)**.

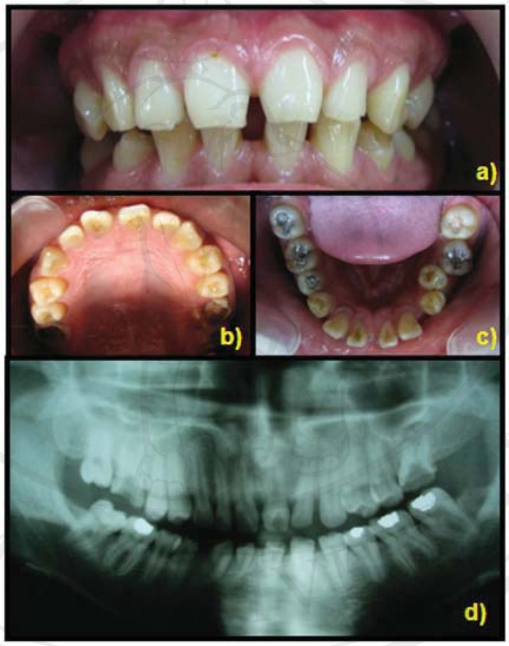

Figure 3.7 Oral manifestations of the affected father; barrel-shaped permanent maxillary central incisors, generalized microdontia, (b) prominent marginal ridges of permanent maxillary incisors, and (c) congenital absence of the permanent mandibular canines, enamel hypoplasia of permanent mandibular first premolars, round-shaped permanent molars, and extensive dental caries.

3.1.2.3 Radiographic findings of the affected father

Right hand radiographic findings showed duplication of the proximal and triangular-shaped distal phalanges of the 1st digit. Absence of the middle and distal phalanges of the 2nd digit and also hypoplasia of the proximal phalanx, which appeared tapered-ended and dislocated from the distal end of the 2nd metacarpal, were observed. There were no proximal, middle, or distal phalanges of the 3rd digits. The malformed proximal end of the 4th phalanx extended to articulate with the 3rd and 4th metacarpals **(Figure 3.8)**.

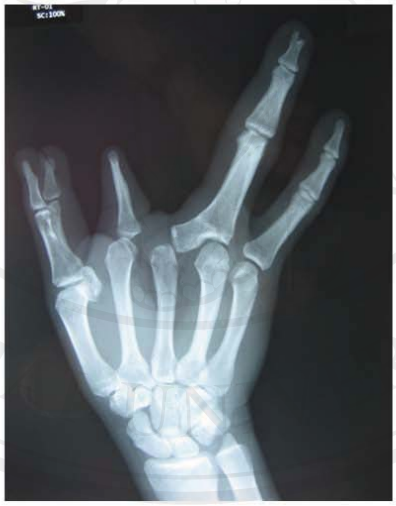

Figure 3.8 Right hand radiographic findings of the affected father; duplication of the proximal and triangular-shaped distal phalanges of the 1st digit, absence of the middle and distal phalanges and hypoplasia of the proximal phalanx of the 2nd digit. There are no proximal, middle, or distal phalanges of the 3rd digit. The proximal phalanx of the 4th digit is articulated with the 3rd metacarpal (Courtesy of Dr. Pranoot Tanpaiboon, Department of Pediatrics, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand).

3.1.2.4 SEM of the affected father

The SEM of the scalp hair of the affected father showed a small hair bulb, thin hair shaft, and hypoplastic cuticles **(Figure 3.9)**.

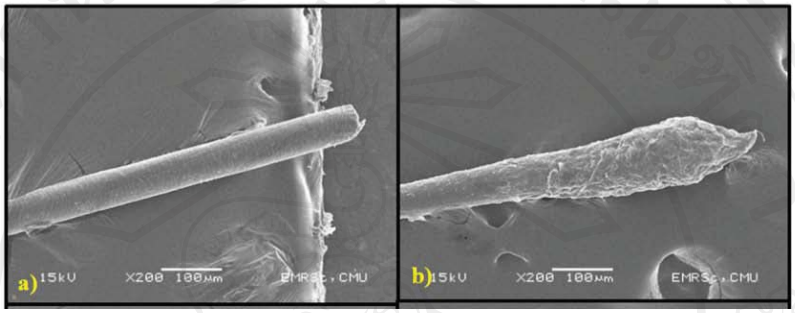

Figure 3.9 SEM of the affected father's scalp hair; (a) hypoplastic cuticles and thin hair shaft, (b) small hair bulb.

3.1.3 *TP63* mutation analysis

Mutation analysis of both the affected girl and her affected father revealed a heterozygous missense mutation of G>C at nucleotide position 680 within exon 6 (+/-c.680 G>C), which is located in the DNA-binding domain (DBD) of *TP63* **(Figure 3.10)**.

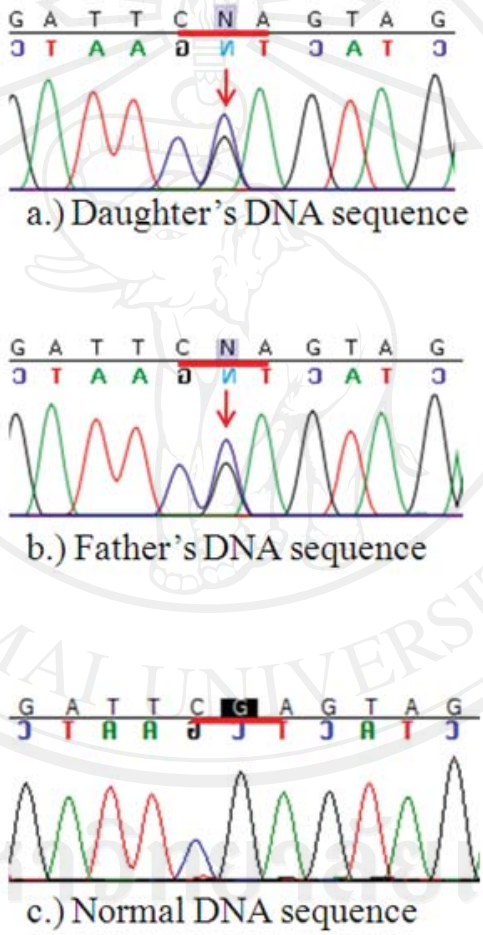

Figure 3.10 *TP63* Mutation analysis of both daughter and father revealed a heterozygous mutation of G>C at nucleotide position 680 within exon 6 (+/-c.680 G>C). Chromatograms of; (a) the affected daughter, (b) the affected father, (c) control.

The mutation changed an amino acid from arginine (CGA) to proline (CCA) at position 227 (p.R227P). This mutation was not found in DNA from the mother or from 200 control chromosomes. The R227 is located in the DBD of *TP63* and is highly conserved in many species, such as human, rhesus, tarsier, mouse, dog, opossum, platypus, chicken, X_tropicalis and stickleback **(Figure 3.11)**.

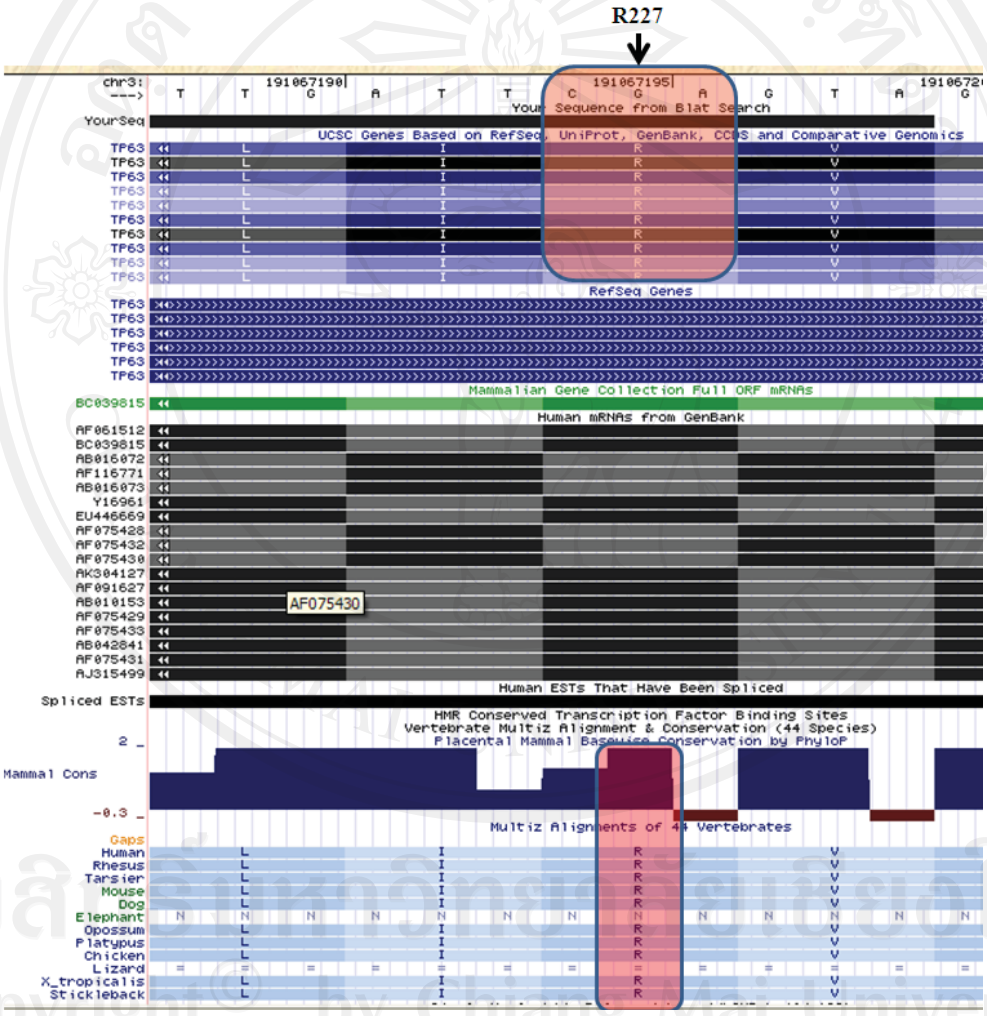

Figure 3.11 Comparison the altered nucleotide and altered amino acid with other species. Arginine at position 227 (arrow) is highly conserved in many species, such as human, rhesus, tarsier, mouse, dog, opossum, platypus, chicken, X_tropicalis and stickleback (http://genome.ucsc.edu).

3.2 The additional results of *TP63* mutation analysis in the study

Thirty genomic DNA samples were extracted from peripheral blood. The summary of both *TP63* pathogenic mutation and single nucleotide polymorphisms (SNPs) in all patients using the direct gene sequencing technique including 16 exons and their flanking introns are shown in the **Tables 3.1** and **3.2**.

Table 3.1 Summary of both *TP63* pathogenic mutation and single nucleotide polymorphisms (SNPs) with phenotype in all patients

CGL. No.	Phenotype	DNA variant	Ref SNP ID (dbSNP Database)									
			rs28673064	rs62702062	rs34429985	rs2276792	rs6789961	rs6790167	rs9840359	rs9840360	rs1554131	rs1345186
001	Hypodontia, Ectodermal dysplasia: blond hair	-	+								+	+
004	Acrocardiofacial syndrome	-	+								+	+
006	Hypodontia (#18)	-	+				+	+	+		+	+
007	Hypodontia (#38,48)	-	+					+	+		+	+
008	Hypodontia (#38)	-									+	+
017	Hypodontia, Ectodermal dysplasia: uncombable hair	-	+									
022	Hypodontia (#31, 41), Peg-shaped lateral incisors	-				+	+	+	+		+	+
023	Bilateral CL/P and Polydactyly	-				+	+	+	+		+	+

NOTE: +, present; -, no *TP63* pathogenic mutation

Table 3.1 (continued) Summary of both *TP63* pathogenic mutation and single nucleotide polymorphisms (SNPs) with phenotype in all patients

CGL. No.	Phenotype	DNA variant	Ref SNP ID (dbSNP Database)									
			rs28673064	rs62702062	rs34429985	rs2276792	rs6789961	rs6790167	rs9840359	rs9840360	rs1554131	rs1345186
024	Hypodontia (#13,23)	-	+					+	+		+	+
029	Hypodontia (#14,15,24,25,34,35, 44,45)	-	+								+	+
039	Hypodontia (#13,22,23,31,41,17)	-				+	+	+	+	+	+	+
040	Mammary hypoplasia, Eye anomalies	-									+	+
181	EEC syndrome	c.680G>C									+	+
182	EEC syndrome	c.680G>C									+	+
184	Hypodontia, Ectodermal dysplasia: blond hair	-									+	+

NOTE: +, present; -, no *TP63* pathogenic mutation

Table 3.1 (continued) Summary of both *TP63* pathogenic mutation and single nucleotide polymorphisms (SNPs) with phenotype in all patients

CGL. No.	Phenotype	DNA variant	Ref SNP ID (dbSNP Database)									
			rs28673064	rs62702062	rs34429985	rs2276792	rs6789961	rs6790167	rs9840359	rs9840360	rs1554131	rs1345186
210	Hypodontia (#12,22)	-		+					+			+
220	CL/P	-		+								
239	CL/P	-		+				+	+			+
249	CP and Ankyloglossia	-						+				+
253	CP and Ankyloglossia	-		+	+		+	+	+			+
289	CL/P	-		+								
294	CL	-		+	+							
306	CL/P	-	+	+							+	+
307	CL/P	-	+								+	+
311	CL/P	-	+	+								

NOTE: +, present; -, no *TP63* pathogenic mutation

Table 3.1 (continued) Summary of both *TP63* pathogenic mutation and single nucleotide polymorphisms (SNPs) with phenotype in all patients

CGL. No.	Phenotype	DNA variant	Ref SNP ID (dbSNP Database)									
			rs28673064	rs62702062	rs34429985	rs2276792	rs6789961	rs6790167	rs9840359	rs9840360	rs1554131	rs1345186
316	CL/P	-	+	+							+	+
319	CL/P	-										
320	CL/P	-		+				+	+			
323	Hypodontia (#42)	-	+	+								
431	Hypodontia (#13,23)	-		+					+			

NOTE: +, present; -, no *TP63* pathogenic mutation

Table 3.2 Summary of single nucleotide polymorphisms (SNPs) in all patients

Ref SNP ID	CGL. No.																														
	001	004	006	007	008	017	022	023	024	029	039	040	181	182	184	210	220	239	249	253	289	294	306	307	311	316	319	320	323	431
rs28673064	+	+	+	+		+			+	+													+	+	+	+			+	
rs62702062																+	+	+		+	+	+	+		+	+		+	+	+
rs34429985																				+		+								
rs2276792							+	+			+																			
rs6789961			+				+	+			+									+										
rs6790167			+	+			+	+	+		+							+	+	+								+		
rs9840359			+	+			+	+	+		+					+		+		+								+		+
rs9840360											+																				
rs1554131	+	+	+	+	+		+	+	+	+	+	+	+	+	+								+	+		+				
rs1345186	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+		+	+	+			+	+		+				

NOTE: +, present

In 10 patients with non-syndromic hypodontia, we found

- 5 patients with RefSNP ID: rs28673064; dbSNP Database (50%)
- 3 patients with RefSNP ID: rs62702062; dbSNP Database (30%)
- 2 patients with RefSNP ID: rs2276792; dbSNP Database (20%)
- 3 patients with RefSNP ID: rs6789961; dbSNP Database (30%)
- 5 patients with RefSNP ID: rs6790167; dbSNP Database (50%)
- 7 patients with RefSNP ID: rs9840359; dbSNP Database (70%)
- 1 patient with RefSNP ID: rs9840360; dbSNP Database (10%)
- 7 patients with RefSNP ID: rs1554131; dbSNP Database (70%)
- 8 patients with RefSNP ID: rs1345186; dbSNP Database (80%)

In 10 patients with non-syndromic orofacial clefts, we found

- 4 patients with RefSNP ID: rs28673064; dbSNP Database (40%)
- 8 patients with RefSNP ID: rs62702062; dbSNP Database (80%)
- 1 patient with RefSNP ID: rs34429985; dbSNP Database (10%)
- 2 patients with RefSNP ID: rs6790167; dbSNP Database (20%)
- 2 patients with RefSNP ID: rs9840359; dbSNP Database (20%)
- 3 patients with RefSNP ID: rs1554131; dbSNP Database (30%)

- 4 patients with RefSNP ID: rs1345186; dbSNP Database (40%)

In 10 patients with syndromic hypodontia with/without orofacial clefts, we found

- 3 patients with RefSNP ID: rs28673064; dbSNP Database (30%)
- 1 patient with RefSNP ID: rs62702062; dbSNP Database (10%)
- 1 patient with RefSNP ID: rs34429985; dbSNP Database (10%)
- 1 patient with RefSNP ID: rs2276792; dbSNP Database (10%)
- 2 patients with RefSNP ID: rs6789961; dbSNP Database (20%)
- 3 patients with RefSNP ID: rs6790167; dbSNP Database (30%)
- 2 patients with RefSNP ID: rs9840359; dbSNP Database (20%)
- 7 patients with RefSNP ID: rs1554131; dbSNP Database (70%)
- 9 patients with RefSNP ID: rs1345186; dbSNP Database (90%)

3.3 The single nucleotide polymorphism (SNP) of the *TP63*

All single nucleotide polymorphisms (SNPs) of *TP63* were also analyzed in this study **(Appendix C)**. These summarized data are exhibited in **Table 3.3**.

Table 3.3 Summary of *TP63* single nucleotide polymorphisms (SNPs) in this study

Nucleotide position	Exon/Intron	RefSNP Alleles	HGVS Names NG_007 550.1	HGVS Names NM_003722.4	Reference SNP Cluster Report:	Frequency	
						Heterozygous	Homogygous
-58	UTR-5	A/T	g.5032A>T	c.-58A>T	rs28673064	5	7
325-18542 _325-18541	Intron 3	-/AGAG	g.163304 _163305ins4	c.325-18542 _325-18541ins4	rs62702062	8	4
579+39	Intron 4	A/T	g.182139T>A	c.579+39G>T	rs34429985	2	0
766+42	Intron 5	G/A	g.238034G>A	c.766+42G>A	rs2276792	2	1
1130-22	Intron 8	A/G	g.242876A>G	c.1130-22C>A	rs6789961	5	0
1212+79	Intron 9	A/G	g.243059A>G	c.1212+79A>G	rs6790167	8	2
1349+40	Intron 10	C/G	g.246609G>C	c.1349+40A>G	rs9840359	9	2
1349+41	Intron 10	A/G	g.246610G>A	c.1349+41A>G	rs9840360	1	0
1350-34	Intron 10	A/C	g.259934T>G	c.1350-34G>T	rs1554131	9	8
1350-23	Intron 10	A/G	g.259945T>C	c.1350-23G>T	rs1345186	9	12