CHAPTER IV

RESULTS

Thirty Thai patients with non-syndromic hypodontia, 28 with non-syndromic orofacial clefts and two with syndromic orofacial cleft were screened for *MSX1* mutations. Six heterozygous missense variations were detected in nine patients with orofacial clefts and hypodontia. Two novel mutations are suspected to be pathogenic (Family I and II), and four are known non-pathogenic polymorphisms (Jezewski et al., 2003; Suzuki et al., 2004; Tongkobpetch et al., 2006). The author did not find any variation in 200 chromosomes from 100 unaffected controls. The descriptions of the patients who carried pathogenic mutations are as follows.

The following sections provide the pedigree, clinical evaluations and mutation analysis of Family I, in which the heterozygous missense mutation (p.P241S) was found.

Family I

Pedigree

The pedigree of Family I with p.P241S mutation is presented in Figure 4.1.

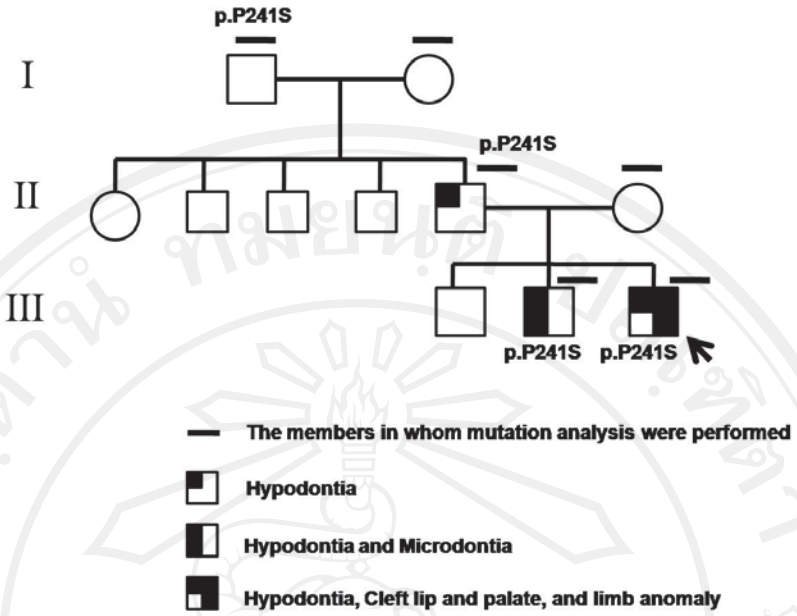

Figure 4.1 Pedigree of the family with p.P241S indicating clinical status ("Circles" indicate female, "Squares" indicate male, "Square with the arrow head" indicate proband I).

Clinical evaluations

Six members of Family I were examined. Proband I presented with repaired bilateral cleft lip and palate, hypodontia of the left and right maxillary permanent lateral incisors, the left maxillary permanent second premolar and the right mandibular permanent third molar, and preaxial-polydactyly of the left thumb (Figure 4.2). The other members of this family were healthy. The father of proband I had hypodontia of the right mandibular permanent lateral incisor (Figure 4.3). The brother of proband I had hypodontia of right and left mandibular permanent third molars, and microdontia and dens invaginatus of left and right maxillary permanent lateral incisors (Figure 4.3). The grandfather and grandmother of proband I had unclear dental histories. The mother of proband I had hypodontia of all permanent third molars.

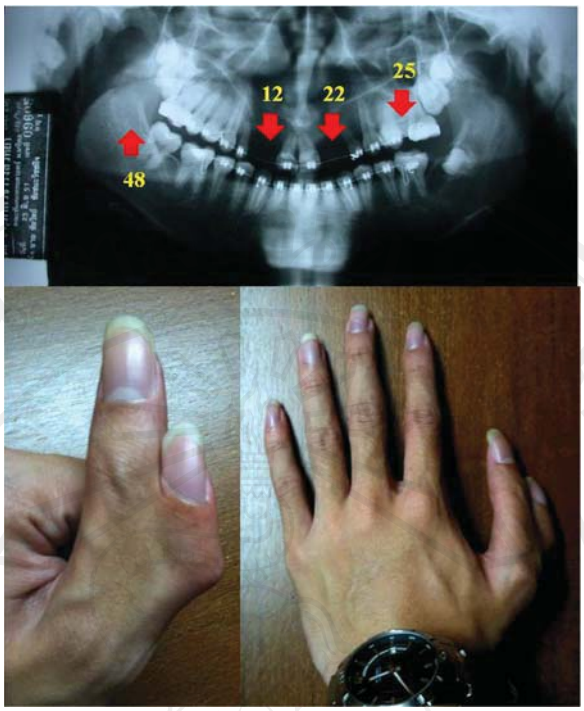

Figure 4.2 Clinical and radiographic findings of proband I. The panoramic radiograph shows hypodontia of teeth 12, 22, 25 and 48. Tooth 37 was extracted because of caries. The left hand shows preaxial-polydactyly of the left thumb.

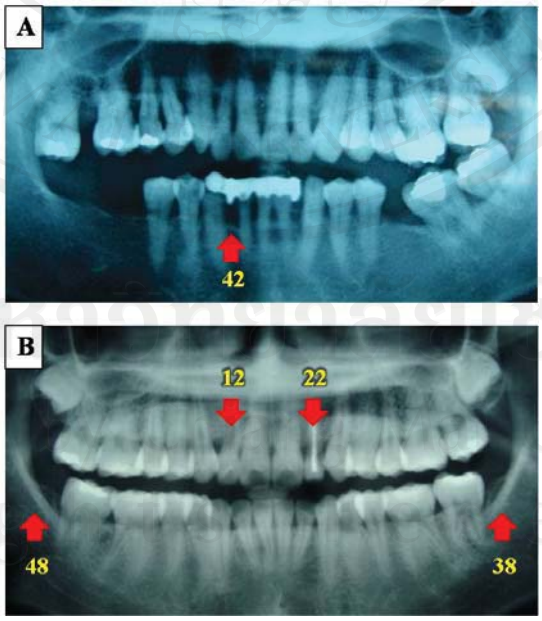

Figure 4.3 Panoramic radiographs of the members of Family I who also had the same amino acid mutation as proband I. A) The father of proband I had hypodontia of tooth 42. B) The brother of proband I had hypodontia of teeth 38 and 48, and microdontia and dens invaginatus of teeth 12 and 22.

Mutation analysis

In Family I, sequence analysis revealed a novel heterozygous missense mutation c.721C>T localized in exon 2 (Figure 4.4), which results in proline to serine substitution at amino acid 241 (p.P241S). The alignment of the protein sequence of human MSX1 and the sequences of chimpanzee (Pan_troglodytes), house mouse (Mus_musculus), brown rat (Rattus_norvegicus), cow (Bos_taurus), dog (Canis_familiaris), chicken (Gallus_gallus), African clawed frog (Xenopus_laevis) and zebrafish (Danio_rerio), revealed that this mutation is located in the highly conserved region. In addition, this novel mutation was presented not only in the proband but also in his father, brother and grandfather. This mutation was not detected in 100 healthy, unrelated Thai controls who neither had orofacial cleft or hypodontia.

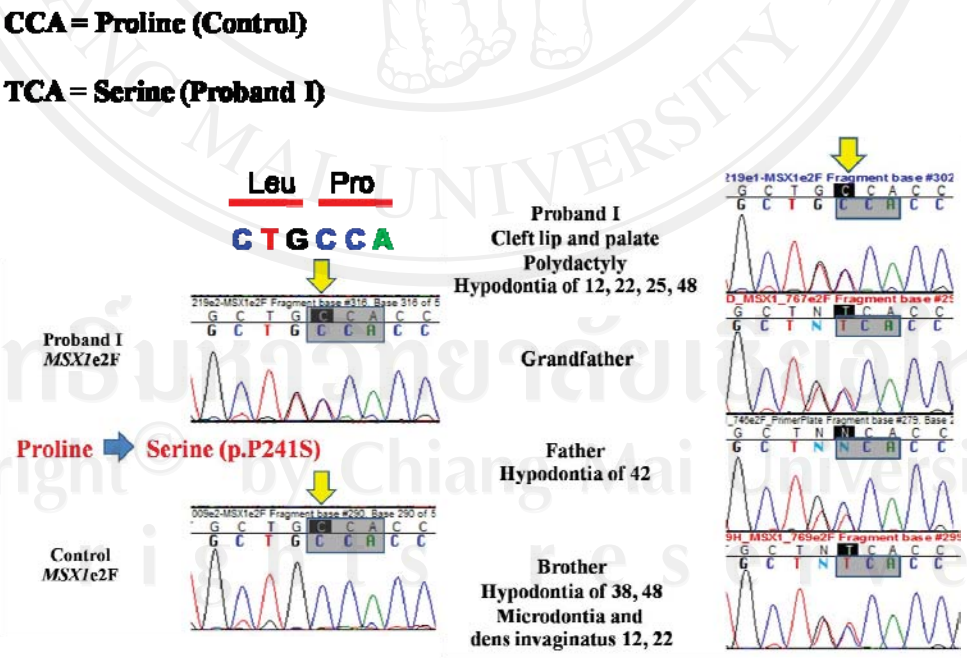

Figure 4.4 Mutation analysis of proband I shows the heterozygous missense mutation c.721C>T, located in exon 2, which results in p.P241S. This mutation was also detected in his grandfather, father and brother.

The next sections provide the pedigree, clinical evaluations and mutation analysis of Family II, which the heterozygous missense mutation (p.A197T) was found.

Family II

Pedigree

The pedigree of Family II with p.A197T mutation is presented in Figure 4.5.

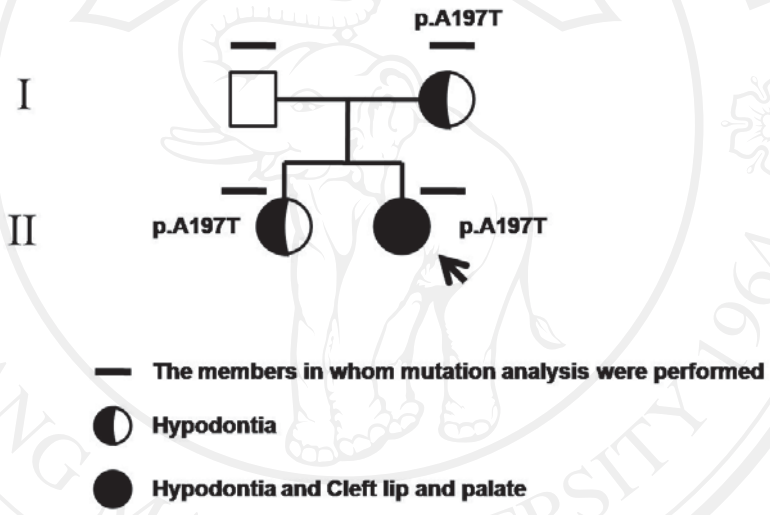

Figure 4.5 Pedigree of the family with p.A197T indicating clinical status ("Circles" indicate female, "Square" indicates male, "Circle with the arrow head" indicates proband II).

Clinical evaluations

Four members of Family II were investigated. Proband II was affected with unilateral right cleft lip and palate and hypodontia of the right maxillary permanent lateral incisor (Figure 4.6). The other members of this family were not affected with medical problems. The mother of proband II had microdontia of the right maxillary permanent lateral incisor (Figure 4.7). The sister of proband II had hypodontia of the right mandibular permanent third molar (Figure 4.7).

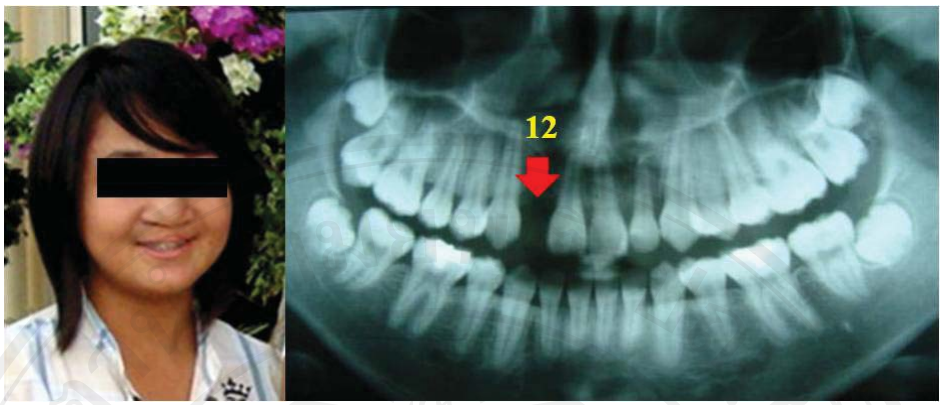

Figure 4.6 Clinical and radiographic findings of proband II. Clinical features include unilateral right cleft lip and palate. The panoramic radiograph shows hypodontia of tooth 12. Teeth 35 and 45 were extracted for orthodontic reason.

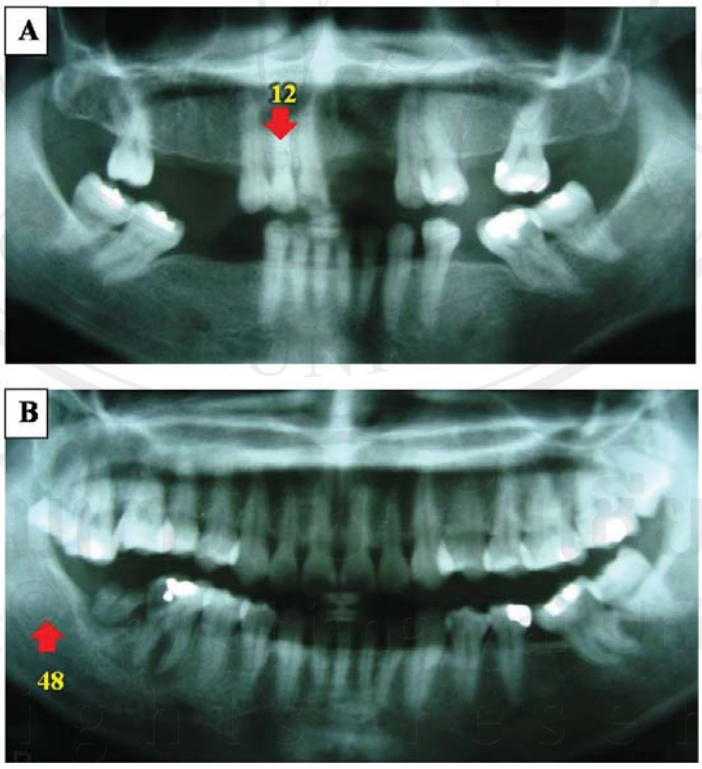

Figure 4.7 Panoramic radiographs of the members of Family II who had the same amino acid mutation as proband II. A) The mother of proband II had microdontia of tooth 12. B) The sister of proband II had hypodontia of tooth 48.

Mutation analysis

The novel heterozygous single base substitution c.589G>A that is located in exon 2 and in the homeodomain, was found in Family II (Figure 4.8). The G to A transversion results in the substitution of alanine to threonine at amino acid 197 (p.A197T). The ClustalX amino acid homology quality score revealed that this substitution is located in the highly conserved region (Figure 4.9). This mutation was also found in the mother and sister of the proband, but not in 100 control subjects same as family I.

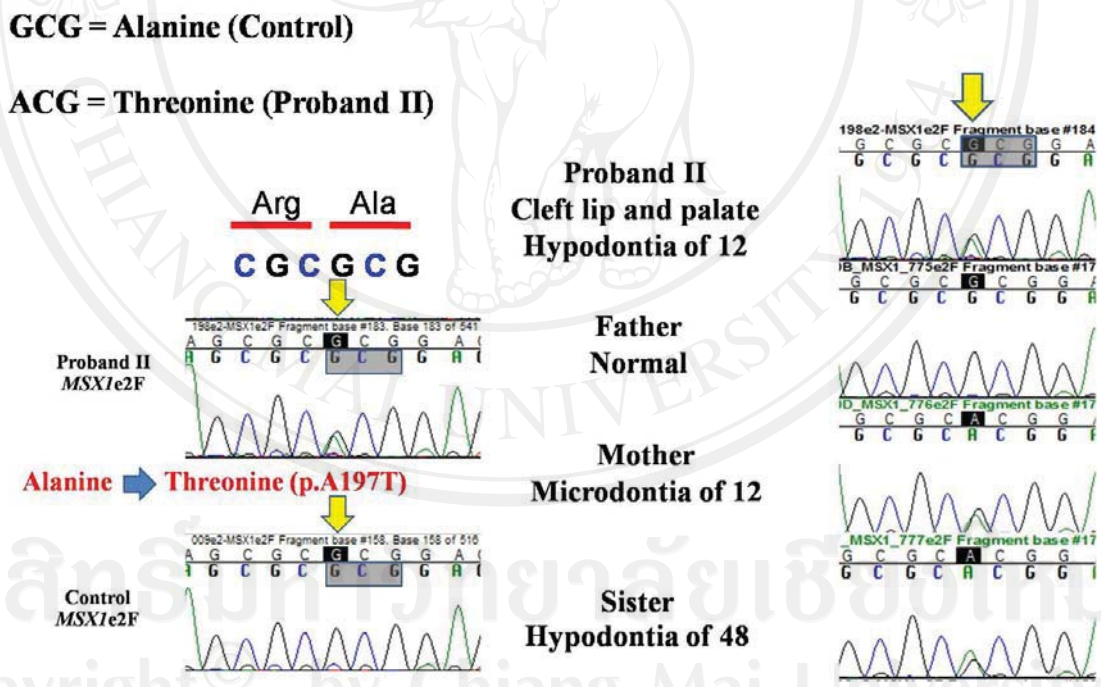

Figure 4.8 Mutation analysis of proband II shows the heterozygous missense mutation c.589G>A, located in exon 2, which results in p.A197T. This mutation was also detected in her mother and sister.

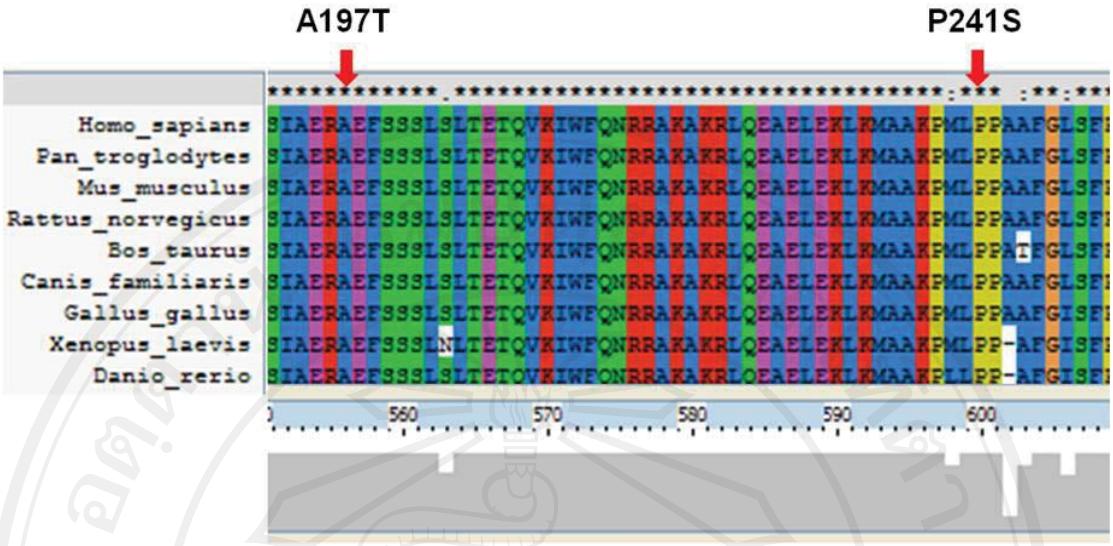

Figure 4.9 Sequence alignment of the Msx1 protein across various species. The highly conserved amino acid mutations, p.A197T and p.P241S, are indicated by the arrows. The amino acid homology quality score is shown by the level of the gray bar at the bottom of this figure. The high level of this bar shows the significance of the alanine and proline that are conserved among various species.

In addition to the two variations in which mutations were assumed, we also detected four single nucleotide polymorphisms (SNPs) in the coding regions (Table 4.1 and Figure 4.10). Two synonymous coding variants were detected, p.G24G (c.72C>A) in the patient with hypodontia of the right mandibular lateral incisor, and p.G110G (c.330C>T) (reference SNP number; rs 34165410) in one of the normal control subjects. Two non-synonymous coding variants were detected, p.A34G (reference SNP number; rs 36059701) and p.P147Q. The p.A34G (c.101C>G) was detected in three unrelated patients (one with cleft lip and palate, one with hypodontia of right and left maxillary permanent canines, and one with hypodontia of nine permanent teeth), this non-synonymous coding variant was a change within amino acid class, previously reported in controls, and previously suggested to be a non-pathogenic polymorphism (Jezewski et al., 2003; Suzuki et al., 2004). The p.P147Q

(c.440C>A) was detected in the patient with unilateral cleft lip and palate (Figure 4.11) and in his healthy mother. The p.P147Q has previously been reported to be associated with cleft lip and palate in seven Vietnamese and two Philippine patients (Suzuki et al. 2004; Vieira et al. 2005). However, Tongkobpetch and coworkers have suggested that the p.P147Q variant is not pathogenic because they found this variant in eight of 100 Thai controls (Tongkobpetch et al., 2006).

Table 4.1 *MSX1* single nucleotide polymorphisms (SNPs) in this study.

Nucleotide position	Exon	Nucleotide change	Expected amino acid change	dbSNP rs# cluster id	Number of samples	Phenotypes
72	1	C>A	p.G24G		1	-Hypodontia of tooth 42
101	1	C>G	p.A34G	rs 36059701	3	-Cleft lip and palate -Hypodontia of teeth 13 and 23 -Hypodontia of nine permanent teeth
330	1	C>T	p.G110G	rs 34165410	1	-Healthy
440	1	C>A	p.P147Q		2	-Cleft lip and palate -Healthy

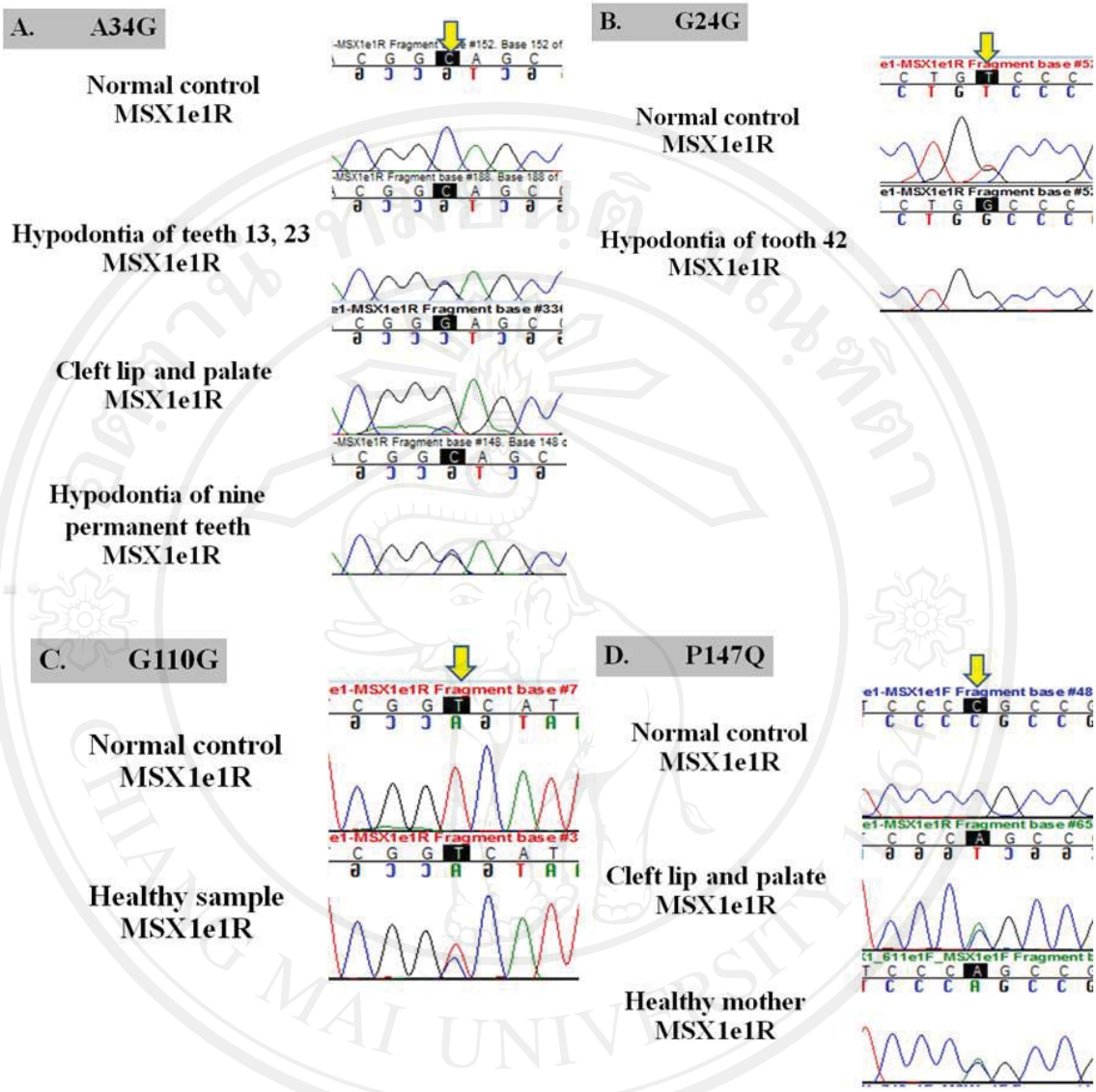

Figure 4.10 SNPs in coding regions compared with those in a normal sequence. A) The p.A34G (c.101C>G) in three patients (one with cleft lip and palate, one with hypodontia of teeth 13 and 23, and one with hypodontia of nine permanent teeth). B) The p.G24G (c.72C>A) in the patient with hypodontia of tooth 42. C) The p.G110G (c.330C>T) in one of the healthy control subjects. D) The p.P147Q (c.440C>A) in the patient with cleft lip and palate and in his healthy mother.

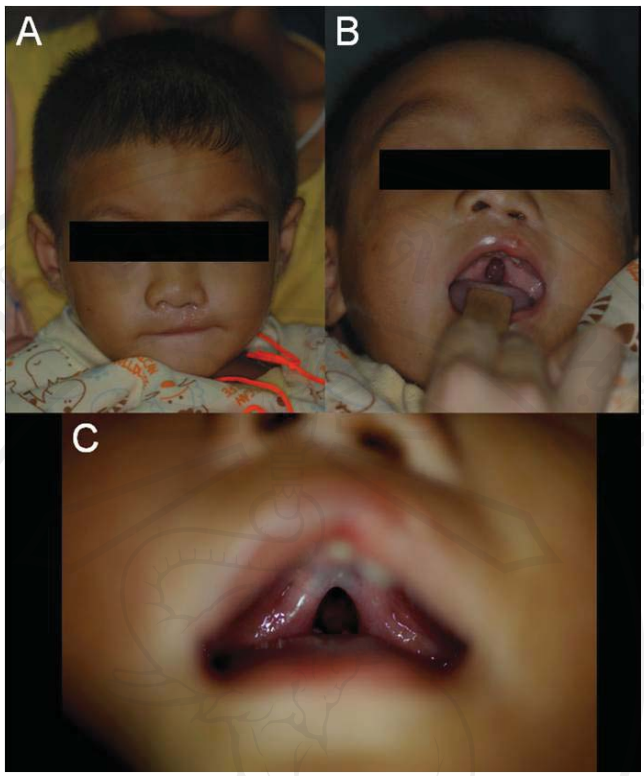

Figure 4.11 Clinical findings of the patient with p.P147Q. A) Frontal view shows left unilateral cleft lip and palate. B) and C) Intraoral examination shows cleft lip and palate.