CHAPTER IV

RESULTS

Sixty three patients with orofacial clefts, twenty three patients with isolated ankyloglossia and six patients with isolated hypodontia were screened for *TBX22* mutation. One heterozygous missense mutation was detected in the proband, a boy, and his unaffected mother and grandfather who lived in Nan province. The boy was affected with unilateral cleft lip and palate, hypodontia of a maxillary left permanent lateral incisor and a maxillary left second premolar, ankyloglossia, carpal bone anomalies, and hypoplastic right thumb (Figure 4.1A-B). A single base substitution (452G→T) was identified in exon 3 (Figure 4.2) which is located in the DNA binding domain of *TBX22*. The protein sequence for human TBX22 was aligned to the sequences of mouse, rat, chicken, horse, monkey and chimpanzee (Figure 4.3). The variation of arginine (R) to leucine (L) at the 151 position of polypeptide (R151L) revealed that this mutation occurred in the48 highly conserved region. The father of the affected boy had ankyloglossia with no mutation in *TBX22*. A distant female cousin of the proband, whose maternal grandmother was a half sister of that of the proband, had cleft lip and palate and syndactyly of fingers 2 and 3 of the left hand, but the TBX22 mutation was not found. (Figure 4.2). This mutation was not detected in 100 unrelated normal Thai controls.

In addition to the pathogenic mutation, we also found two novel SNPs in non-coding regions (Figure 4.4). The novel 799-61T→C in intron 5 was identified in three unrelated samples (one cleft palate, one cleft lip and palate with syndactyly, and

one isolated ankyloglossia) from a total of 91 patients (3.29%) and 949+57delC in intron 7 was identified in 18 samples (19.8%). Mutations in the coding regions were not detected in patients with isolated ankyloglossia and hypodontia.

Table 4.1 Characteristics of patients and genetic variants in this study.

	Total	**Mutation**	**SNP in intron 5**	**SNP in intron 7**
Orofacial clefts	63	1	2	8
Cleft palate	10	-	1	1
Cleft palate with ankyloglossia	6	-	-	-
Cleft lip and palate	31	-	-	3
Cleft lip	5	-	-	1
Cleft lip with ankyloglossia	1	-	-	1
Cleft lip and palate with ankyloglossia	4	-	-	-
Cleft lip and palate with hypodontia	1	-	-	-
Cleft lip and palate with ankyloglossia and hypodontia	1	-	-	-
Cleft lip and palate with ankyloglossia, hypodontia and upper limb anomalies	1	1	-	-
Cleft lip and palate with hemifacial microsomia	1	-	-	-
Cleft lip and palate with oblique facial cleft	1	-	-	1
Cleft lip and palate with syndactyly	1	-	1	1
Isolated ankyloglossia	23	-	1	6
Isolated hypodontia	6	-	-	1

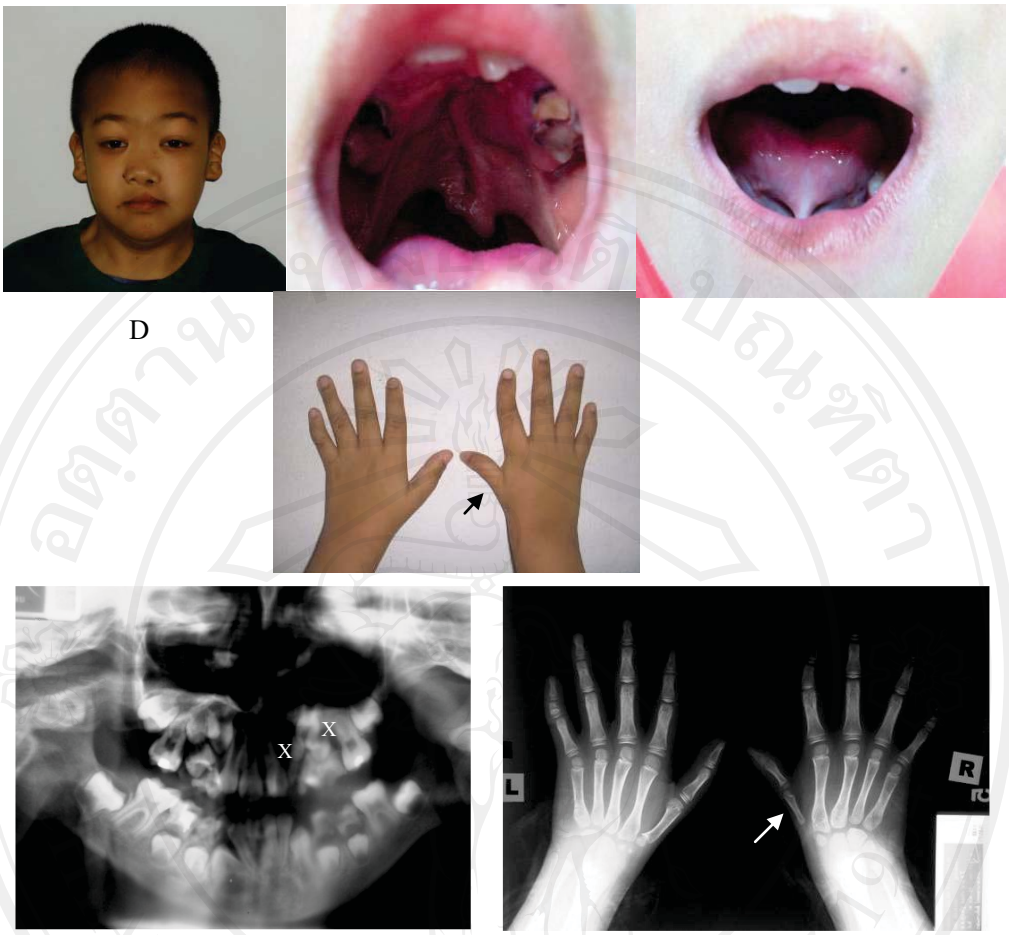

Figure 4.1 Clinical and radiographic findings of affected patient with *TBX22* mutation. A-D, Clinical features of the patient with cleft lip and palate with ankyloglossia and upper limb abnormalities with the lingual frenum attached from alveolar ridge almost to the tip of the tongue, a thick fibrous frenum, short length of frenum (<1 cm). E, Panoramic radiograph shows teeth 22 and 25 missing. F, Left and right hand radiograph of the proband; the left hand shows tiny trapezoid, trapezium and scaphoid. The right hand shows small size of the first proximal phalange and the first metacarpal, and lack of trapezium and scaphoid.

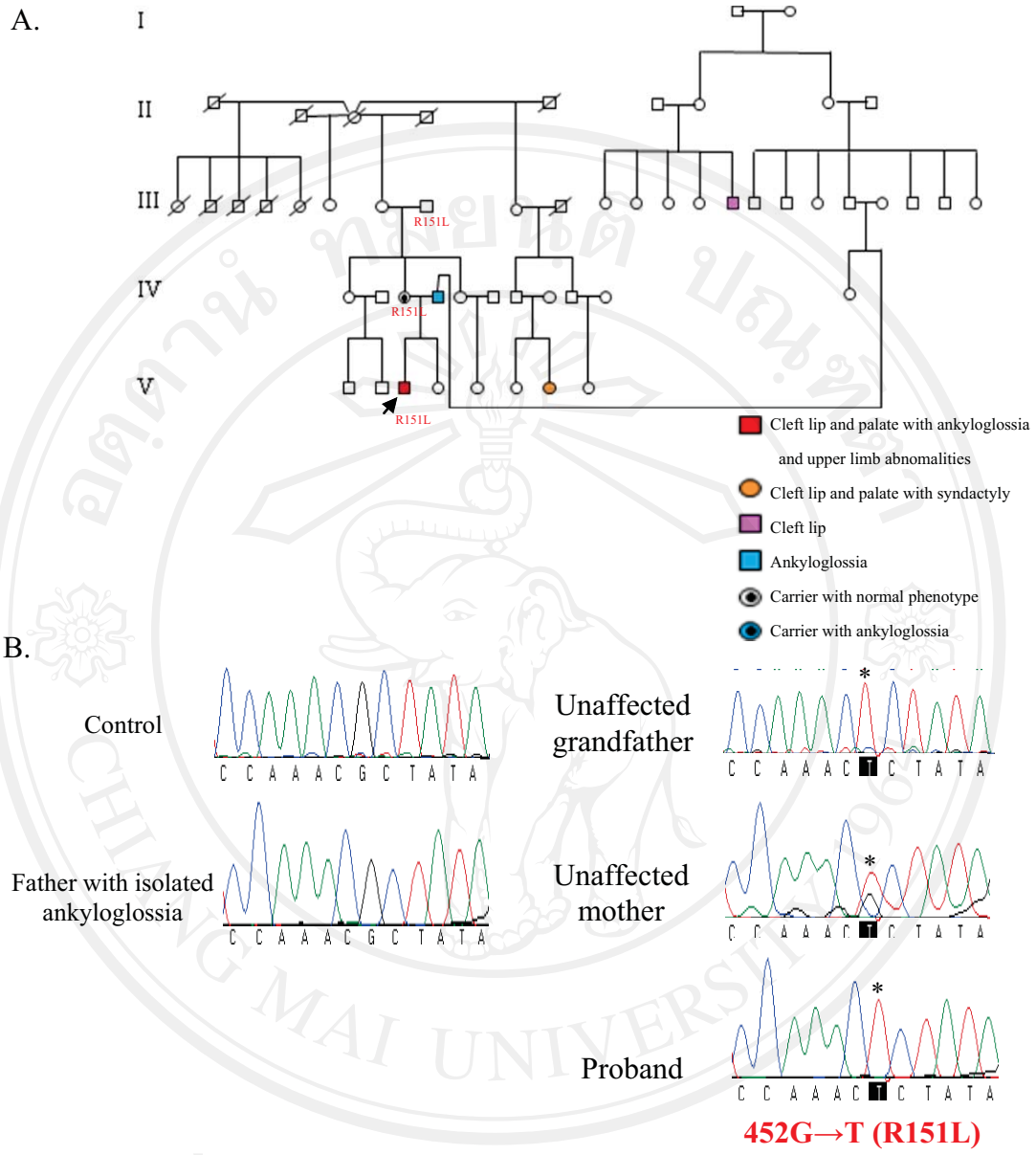

Figure 4.2 Mutation analysis. A, Pedigree of the patient with *TBX22* mutation. Proband has a cousin with cleft lip and palate with syndactyly and a grandfather's cousin with cleft lip. B, Electropherograms of the proband, his unaffected mother and grandfather, and his father with isolated ankyloglossia compared with the controls, show a hemizygous 452G→T mutation in the proband and a heterozygous mutation in his unaffected mother and grandfather.

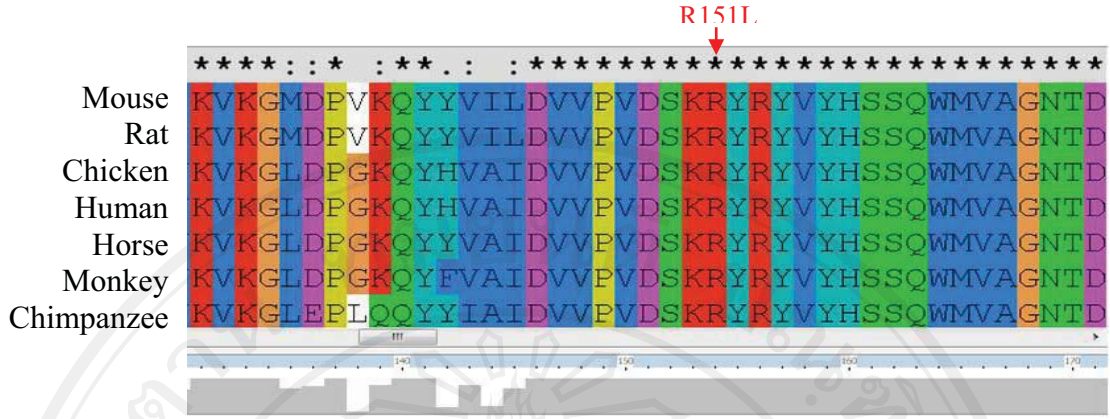

Figure 4.3 TBX22 protein sequence comparison between species. The highly conserved amino acid variant from arginine (R) to leucine (L) is indicated by the arrow. The gray bar at the bottom of the figure represents the amino acid homology quality score. The steady vertical bar shows the significance of amino acid alignment.

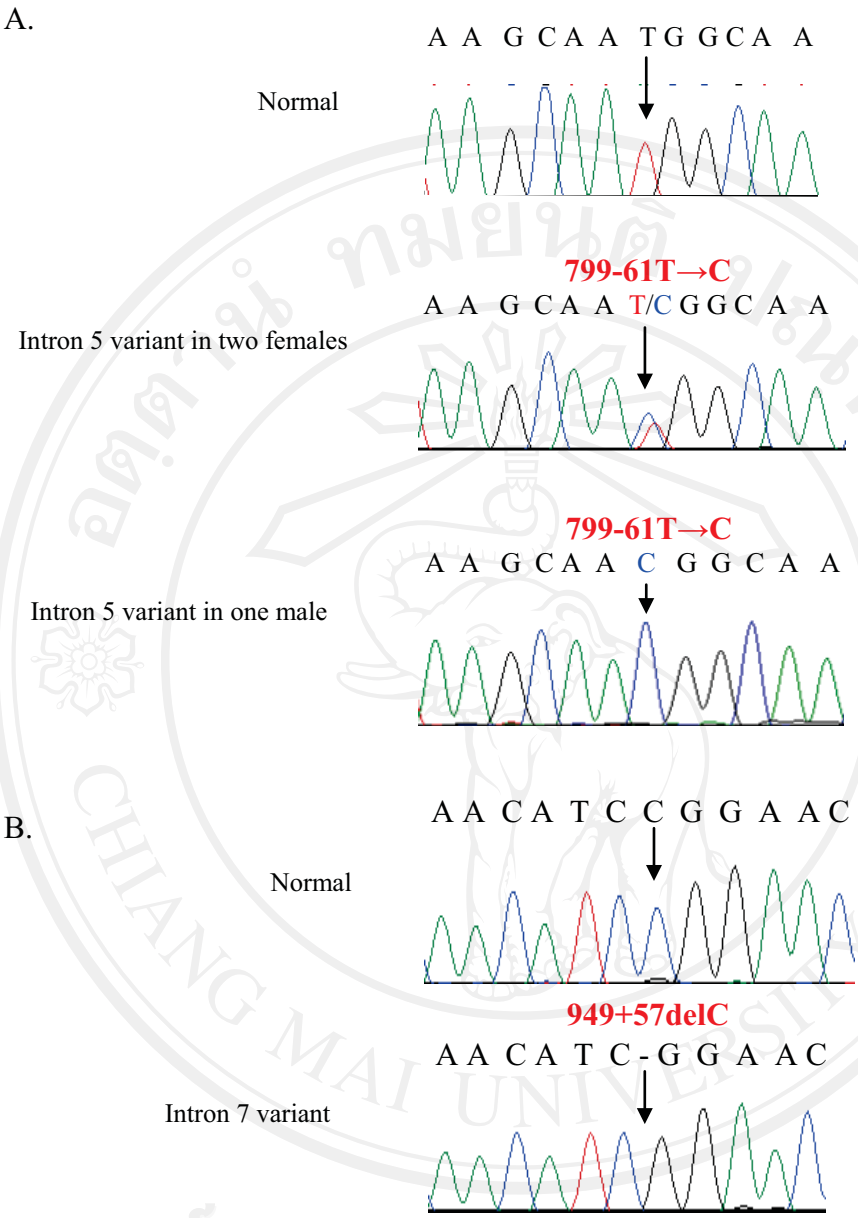

Figure 4.4 *TBX22* single nucleotide polymorphism (SNP) in non-coding regions. A, A novel SNP in intron 5 (799-61T→C) shows heterozygous change in two females (middle), hemizygous change in one male (lower) compared with a normal sequence (upper). B, A SNP in intron 7 (949+57delC) (lower) compared with a normal sequence (upper).