

CHAPTER V

DISCUSSION

The author reports a hemizygous missense 452G→T (R151L) mutation in *TBX22* in a Thai boy who was affected with unilateral cleft lip and palate, ankyloglossia, hypodontia of a maxillary left permanent lateral incisor and of a maxillary left second premolar, carpal bones anomalies and a hypoplastic thumb on the right hand. The unaffected mother and maternal grandfather also carried the same mutation. The mutation was not found in the proband's father who had isolated ankyloglossia. His ankyloglossia was likely to be caused by a different gene. This particular mutation has previously been reported to be associated with a cleft of the soft palate in an unrelated Thai male (Suphapeetiporn *et al.*, 2007). It is interesting to note that this mutation was found for the second time in the Thai population. This may imply the influence of founder effect that can be verified by performing haplotype analysis. It is noteworthy that this mutation has been reported twice only in Thailand. It has not been reported anywhere else in the world. This might have been due to the small number of populations studied. It has only been studied in patients from Iceland, Canada, Brazil, America, Tunisia, and Thailand (Braybrook *et al.*, 2001, 2002; Marçano *et al.*, 2004; Chabouni *et al.*, 2005; Suphapeetiporn *et al.*, 2007).

The existence of a different phenotype might have been caused by the effects of modifier genes, by incomplete penetrance, and/or by the modulation of gene-environment interaction. Moreover, haplotype variants in *TBX22* promoter have recently been described to be the causes of a variety of phenotype (Pauws *et al.*, 2009).

The phenotype associated with *TBX22* mutations has been reported to be quite variable, ranging from non penetrance, cleft palate, cleft palate with ankyloglossia, to ankyloglossia alone. The combination of cleft lip and palate, ankyloglossia, hypodontia of a maxillary left permanent lateral incisor and of a maxillary left second premolar, and upper limb anomalies in the proband is quite remarkable. To my knowledge, this is the first time that the *TBX22* mutation may be described in association with cleft lip and palate, hypodontia, and upper limb anomalies. This is not too surprising, as *Tbx22* has been reported to be highly expressed in the developing paraxial mesoderm, especially in the myotome, in the developing craniofacial structures, limbs (Haenig *et al.*, 2002), and tooth buds (Braybrook *et al.*, 2002). Hypodontia and limb anomalies might have been overlooked in previously reported cases. Most of the previous reports did not demonstrate panoramic radiographs of teeth.

How this R151L mutation contributes to cleft lip and palate, ankyloglossia, hypodontia of a maxillary left permanent lateral incisor and of a maxillary left second premolar, and upper limb anomalies is worth discussing. Firstly, this mutation occurs in the highly conserved region in mouse, rat, chicken, monkey, chimpanzee and horse *TBX22* protein sequences (Figure 4.3). This implies the significance of this specific nucleotide for living organisms. Additionally, the crystallographic structure of the T-box domain demonstrates that this mutation causes deleterious amino acid substitution. Arginine at the 151 position, which is located on strand c, which extends to the DNA during DNA binding, forms a polar interaction with the DNA backbone (Müller and Herrmann, 1997). The substitution of positive polar arginine by non-polar leucine can affect DNA binding ability by interfering with polar interaction to DNA.

Subsequently, the lack of DNA binding causes notable loss of *TBX22* function (Andreou *et al.*, 2007). Polyphen (<http://genetics.bwh.harvard.edu/pph/>) predicted this variant to be damaging on the structure and function of *TBX22* protein.

Lastly, the regulation of gene transcription in developmental processes requires balance between transcriptional activation and repression. *TBX22* functions as a transcriptional repressor, and repressive activity of *TBX22* requires sumoylation, a post-translational modification with a small ubiquitin-like modifier protein (SUMO). SUMO1 attaches to K63 within the N-terminal region, which contains lysine. Missense mutations in the T-box domain can produce either an obvious downregulation or a lack of SUMO-1 conjugation, which can lead to subsequent loss of *TBX22* activity (Andreou *et al.*, 2007). The disability of the DNA binding domain to conjugate with SUMO1 has a major effect on the regulation of downstream target interaction, resulting in a cleft phenotype (Andreou *et al.*, 2007). It has been reported that haploinsufficiency of *SUMO1* causes cleft lip and palate (Alkuraya *et al.*, 2006). Other proteins, which are known to be associated with cleft lip and palate, that need sumoylation include *MSX1*, *SATB2*, and *P63* (Andreou *et al.*, 2007).

Having limb anomalies in the proband is quite interesting; however, it corresponds to *Tbx22* expression in the forelimbs of the chick embryo (Haenig *et al.*, 2002). Mutations in *TBX5* have been known to cause Holt-Oram syndrome which is characterized by upper limb anomalies and congenital heart defects (Li *et al.*, 1997). Upper limb anomalies found in my patient might reflect the interaction between *TBX22* and *TBX5*. However, it is possible that the upper limb anomalies were caused by a different mutation in a different gene. Perhaps it is more likely that a polymorphism acts as a modifier for the *TBX22* mutation. It is less likely that

environmental factors during pregnancy had the influences on the phenotype, as the pregnancy history was unremarkable.

Regarding orofacial clefts, *TBX22* mutations have been reported to be associated with cleft palate. This association might have been due to the small numbers of patients with cleft lip and palate in the studies. The association between mutation in *TBX22* and cleft lip and palate should be confirmed in the larger cleft lip and palate population. To my knowledge, this is the first time that cleft lip and palate, hypodontia, and limb anomalies have been found in association with mutation in *TBX22*. The hypodontia and limb anomalies in the proband may not be that surprising, as *Tbx22* has been found to be expressed in the developing tooth buds of the mouse embryo (Braybrook *et al.*, 2002), and the developing forelimbs of the chick embryo (Haenig *et al.*, 2002). Why they have not been found in previous studies is worth discussing. These phenotypes may be rare, possibly because of the ethnic background or because of modifier genes. This rarity deserves further study in much larger populations and in different ethnic backgrounds.

The heterozygous mutation in the unaffected mother may be the consequence of either incomplete penetrance or X-inactivation. It has been reported that carrier females have a range of phenotypes from a complete phenotype to absolutely asymptomatic. This range shows incomplete penetrance in families with X-linked cleft palate (Marçano *et al.*, 2004). Approximately 50% of carrier females have an active X chromosome with normal allele; the other 50% have a mutant allele. The normal cells are sufficient for normal function, that results in a normal phenotype in female carriers of an X-linked disorder (Puck and Willard, 1998). The palates of the mother and grandfather appeared normal, but they may have had incomplete bone

formation similar to that found in *Tbx22*^{null} mice which have been reported to have complete fusion of the hard palate but severely reduced bone formation (Pauws *et al.*, 2009). Furthermore, there is high intrafamilial and interfamilial phenotypic variation in families with cleft palate with ankyloglossia (Marçano *et al.*, 2004). However, there is possibility that this mutation may not be associated with phenotype found in the proband. The only way to verify if the mutation has effects on gene transcription is by performing luciferase reporter assay.

Interestingly, the ankyloglossia found in patients, in this and other studies, with *TBX22* mutations appeared to share some characteristics. The ankyloglossia features of the present case and those in previous reports (Stanier and Moore, 2004; Suphapeetiporn *et al.*, 2004) appeared to have the lingual frenum attached from the alveolar ridge to almost the tip of the tongue. Isolated short lingual frenum is not associated with *TBX22* mutation.

Cleft lip has been reported once in a member of a family with *TBX22* mutation (IVS4+1G→A), who had cleft palate with ankyloglossia (Braybrook *et al.*, 2001). This study has demonstrated a newly-recognized syndrome of cleft lip and palate, ankyloglossia, hypodontia, and upper limb anomalies caused by a *TBX22* mutation.

Mutation of *TBX22* appears to cause more than just cleft palate and ankyloglossia. I would like to propose an appropriate name “Cleft lip/palate-Ankyloglossia-Hypodontia-Limb anomalies Syndrome”. This name certainly embraces the broader spectrum of this genetic disorder. Furthermore, this study has demonstrated that mutations in *TBX22* may cause mixed types of orofacial clefts similar to those caused by mutations in *P63* (van Bokhoven *et al.*, 2001) and *IRF6* (Kondo *et al.*, 2002).

In this study *TBX22* mutation was identified in the coding region. However the disruption of gene may be caused by abnormalities in the promoter (Pauws *et al.*, 2009), enhancer, intron, exon deletion, whole gene deletion, and methylation which is the addition of methyl group to substrate, such as, protein, DNA, for regulation of gene expression or regulation of protein function and RNA metabolism.

The author can not identify the association between *TBX22* mutation and isolated ankyloglossia or isolated hypodontia. Most of the cases of isolated ankyloglossia in this study had a short lingual frenum. But ankyloglossia in the proband, who carried the *TBX22* mutation, was more severe than in the other cases. The most frequently missing tooth in the cases of hypodontia in this study was the third molar. But mutation in the *TBX22* gene may be associated with other missing teeth. Therefore, this hypothesis might be feasible if the sample were increased in size and if more specific inclusion criteria were applied in sample selection. *TBX22* may be a very uncommon cause of isolated ankyloglossia and hypodontia.

In conclusion, this study demonstrates for the first time that the cleft lip and palate with ankyloglossia, hypodontia of a maxillary left permanent lateral incisor and a maxillary left second premolar and upper limb anomalies may be associated with a hemizygous 452G→T (R151L) mutation in *TBX22*.