CHAPTER V
DISCUSSION

Amelogenesis imperefecta is an inherited defect of enamel formation with clinical heterogeneity which causes by variety of gene mutations (Masuya et al., 2005; Wright et al., 2006). The literature review established that both Amelogenin (*AMEL*) and Enamelin (*ENAM*) are associated with hypoplastic AI. Both genes code important enamel proteins in amelogenesis. Amelogenin helps controlling the direction of crystallite growth and forming the scaffold, whereas enamelin helps controlling the crystallite growth regulation and elongation in developing enamel (Robinson et al., 1990; Fincham and Simmer, 1997; Hu et al., 1997; Wright, 2006).

AMEL is another interesting candidate gene for hypoplastic AI, which presents the phenotype of hypoplastic and hypomaturation AI in *AMEL* mutations (Lench et al., 1994; Lench and Winter, 1995; Collier et al., 1997; Kindelan et al., 2000; Ravassipour et al., 2000; Greene et al., 2002; Hart et al., 2002; Kim et al., 2004; Kida et al., 2007). The mode of transmission of *AMEL* is X-linked. The mutant allele is expressed in males, whereas the mosaic pattern (Lyonization) shows in females, with discoloration or vertical bands in enamel due to X chromosome inactivation (Witkop, 1967). Several reports of *AMEL* mutation with hypoplastic AI describe the phenotype of probands as thin, smooth hypoplastic enamel mutations (Lench and Winter, 1995; Kindelan et al., 2000; Greene et al., 2002; Hart et al., 2002; Kim et al., 2004; Kida et al., 2007). Those reports also describe the vertical ridges and grooves on enamel of the heterozygous females in families (Lench and Winter, 1995; Kindelan et al., 2000; Greene et al., 2002; Hart et al., 2002; Kim et al., 2004; Kida et al., 2007). In this study, the history taking and the clinical evaluations of each proband revealed that the vertical ridges and grooves were not shown on the enamel of any relatives in the studied families. *ENAM* seems to be a more likely candidate gene than *AMEL*.

Proband 1 showed generalized hypoplastic and hypocalcified AI, fusion of the mandibular incisors, unerupted supernumerary premolar, ectrodactyly and ectodermal dysplasia. The teeth affected by hypocalcified AI may show the phenotype similar to hypoplastic AI when the soft uncalcified enamel wear off leaving the yellow exposed dentine (Witkop, 1988). The morphology of the developing crowns from the panoramic radiograph is similar to the normal crowns but the radiodensity is decreased. It is possible to suspect that this phenotype may suggest to hypocalcified AI.

Dental anomalies associated with AI have been published in several studies (Collins et al., 1999; Poulsen et al., 2008). Dental anomalies that have been reported to be associated with AI consists of delayed eruption, others retention or impaction of teeth, and yet others follicular cysts, taurodontism, reduced crown size, enlarged pulp chambers, intrapulpal calcification, crown resorption and tooth agenesis (Collins et al., 1999; Poulsen et al., 2008). Anterior open bite, gingival enlargement, gingivitis and periodontitis have also been reported to be associated with AI (Poulsen et al., 2008). Interestingly, the fusion of the mandibular left central and lateral incisors and the unerupted supernumerary upper left premolar in this proband were not anomalies commonly associated with AI in the referenced studies (Collins et al., 1999; Poulsen et al., 2008). Moreover, according to the term of amelogenesis imperfecta which represents the condition of hereditary enamel defects of the entire dentitions without other systemic manifestations, the ectrodactyly and ectodermal dysplasia of this proband are not associated with AI (Ooya et al., 1988; Witkop, 1988; Aldred and Crawford, 1995). The author suspects that ectrodactyly and ectodermal dysplasia of this proband might have been caused by mutation in other genes.

The association between AI and gingival hyperplasia has been published in several studies (Atasu et al., 1999; Brennan et al., 1999; Macedo et al., 2005). Those studies of unusual gingival hyperplasia with hypoplastic and hypocalcification AI suggested that the plaque and calculus deposition on the teeth due to the difficulty in cleaning caused by dentine hypersensitivity seemed to be the etiological factor for gingival hyperplasia in patients with AI (Brennan et al., 1999; Macedo et al., 2005). The histologic examination of gingival biopsies in those studies showed dense fibrous connective tissue, numerous dystrophic calcifications and islands of odontogenic

epithelium with infiltration of the inflammatory cells (Brennan et al., 1999; Macedo et al., 2005). Those studies also reported favorable periodontal treatment outcomes in cases of AI with gingival hyperplasia (Brennan et al., 1999; Macedo et al., 2005). A notable feature in proband 2 of this study was AI associated with gingival overgrowth without history of systemic disease and drug taking, a feature which is similar to the findings in the subjects in the referenced studies. The histopathologic examination from the overgrowth of masticatory gingiva in this proband also shows the similar finding as the referenced studies. Plaque and calculus do not reasonably seem to be the cause of gingival hyperplasia in this proband. The author supports the opinion that it is unknown if the non-enamel manifestations of this abnormal gingival overgrowth and hyperplasia of the alveolar bone may result directly from the mutated genes which are expressed in the gingiva and alveolar bone, or from secondary effect which result from abnormal enamel formation that effects other malformations of the surrounding tissues. Alternatively, it has also been suggested that some AI associated with non-enamel manifestations may result from other modifying genes, or from environmental effects (Collins et al., 1999).

Proband 2 also had delayed eruption of second molars compared to her age which is similar to other reports of AI associated with dental anomalies described above (Poulsen et al., 2008; Aren et al., 2003). It is suspected that the overgrowth of the gingival in this case may impede the eruption of these teeth which leads to the delayed eruption. It is interesting to note that this proband had alveolar bone loss at the mandibular left first molar before this tooth was crowned. The vertical bone loss after stainless steel crowns were placed on the mandibular first molars may have been caused by traumatic occlusion after treatment or from plaque and calculus accumulation at the margin of the crowns. It is suspected that the distal surfaces of the mandibular first molar crowns were hard to clean, which may have led to the progression of the periodontal disease. Periodontal bacterial cultures and further investigations could help to identify whether aggressive periodontitis might be a cause of such vertical bone loss.

There are some studies associating AI with gingival enlargement and pericoronal lesions which are similar to the interesting findings of proband 3 (Peters et al., 1992; Feller et al., 2006; Martelli-Junior et al., 2008; Roquebert et al., 2008). There was no

history of systemic disease and drug taking associated with the gingival overgrowth of this proband. The pericoronal radiolucencies surrounding the impacted and inferiorly displaced mandibular second molars on both sides of proband 3, displacing the inferior alveolar canal were similar to those previous reports (Peters et al., 1992; Feller et al., 2006; Roquebert et al., 2008). The author supports the opinion of the previous reports that the large unilocular pericoronal lesions involving the mandibular molars are the probable cause of the impacted teeth and related to the displacement of the teeth to the inferior borders of the mandible and the clinically gingival enlargement may represent the hamartomatous growth of those intraosseous lesions rather than plaque-induced gingival overgrowths (Feller et al., 2006; Martelli-Junior et al., 2008). It is interesting to note that this proband did not experience any symptoms of mandibular paresthesia which is similar to the previously reported case (Feller et al., 2006).

The large pericoronal lesions associated with AI and multiple unerupted teeth in several previous reports was diagnosis as WHO-type central odontogenic fibroma-like lesion (van Heerden et al., 1990; Peters et al., 1992; Raubenheimer and Noffke, 2002; Feller et al., 2006).

Central odontogenic fibroma, a benign fibroblastic odontogenic neoplasm, usually present as the well-defined unilocular radiolucent lesion but, it may appear as multilocular lesions and mixed radiolucent and radiopaque appearance (Hirshberg et al., 1996; Feller et al., 2006). Previous reports of central odontogenic fibromas reported that this neoplastic lesion can cause bony expansion and tooth displacement and also associated with the impacted teeth (Peters et al., 1992; Kaffe and Buchner, 1994; Hirshberg et al., 1996). The histopathologic features of this lesion include odontogenic epithelial strands in association with cellular fibrotic proliferation with some calcified material (Feller et al., 2006). Proband 3 had supernumerary tooth and root dilacerations as the dental anomalies associated with AI. It is interesting to note that the supernumerary tooth located between mandibular right molars shown in the panoramic radiographic of this proband was not anomalies commonly associated with AI, whereas the root dilacerations of maxillary molars were similar to several cases of AI in some previous reports (Gutierrez et al., 2007; Martelli-Junior et al., 2008).

Proband 3 also had vertical bone loss at both mandibular first molars which is similar to the finding of proband 2 after the stainless steel crowns were placed. It is suspected that the vertical bone loss from both cases may have been caused by traumatic occlusion after treatment or by plaque and calculus deposition at the margins of the crowns.

The author supports the opinion that it is unknown if the non-enamel manifestations, such as hyperplastic alveolar bone, gingival overgrowth, root dilacerations, supernumerary teeth, impacted teeth and pericoronal lesions, of this proband may result directly from the mutated genes, which are expressed in specific tissues, or from secondary effects which results from abnormal enamel formation that effects other malformations of the surrounding tissues. Alternatively, it has also been suggested that some AI associated with non-enamel manifestations may result from other modifying genes, or from environmental effects (Collins et al., 1999). Whether the non-enamel features are the result from the direct consequence of the molecular defect affecting enamel or from unknown secondary factors remains to be determined (Collins et al., 1999).

Hypocalcified AI shows soft consistency of enamel which easily lost by masticatory force and attrition rapidly (Lee et al., 2008; Hyun et al., 2009). Probands 4 and 5 showed hypoplastic enamel with several islands of normal-looking enamel in the cervical part of their deciduous teeth. The radiographic examination of the probands' teeth showed normal thickness with reduction of radiodensity enamel of developing permanent tooth similar to the previous reports of hypocalcified AI (Kim et al., 2008; Hyun et al., 2009). The masticatory force seems to be the cause of the soft uncalcified enamel removal which finally shows the similar phenotype like hypoplastic AI. Therefore it is possible to suspect that both probands 4 and5 may be affected with hypocalcified AI rather than hypoplastic AI. The author supports the opinion that some different AI types show the similar clinical features which lead to the difficulties in clinical diagnosis (Stephanopoulos et al., 2005).

Proband 6 showed generalized yellow-brown discoloration, labially vertical ridges, nearly normal size and morphology of teeth without interdental spacing like other phenotype of generalized hypoplastic AI. There was no radiographic examination of this proband. The radiographic examination can guide to the more

precise diagnosis. The phenotype of vertical ridges on labial surfaces of his teeth is similar to the phenotype of vertical grooves on enamel of heterozygous female affected with *AMEL* mutation. However, it is interesting that the vertical grooves associated with *AMEL* mutation in male have not been reported. It is suspected that the hypoplastic AI in this proband may cause from other unknown gene associated with hypoplastic AI.

A study with negative findings in two Brazilian families with AI identified six major candidate genes for AI was reported (Santos et al., 2007). The exon-intron boundaries of those six candidate genes, *ENAM*, *AMBN*, *AMEL*, *MMP-20*, *KLK4* and *Amelotin*, were amplified and sequenced (Santos et al., 2007). The study suggested that the etiology of AI may involve many additional genes (Santos et al., 2007).

Another study of mutational analysis of the AI candidate genes in 24 families with isolated enamel defects was reported (Kim et al., 2006). *ENAM*, *AMBN*, *AMEL*, *MMP-20*, *KLK4*, *TUFT1* and distal-less 3 (*DLX3*) were sequenced (Kim et al., 2006). Only six disease-causing mutations were identified (2/24 were *AMEL* mutations, 3/24 were *ENAM* mutations and 1/24 was *MMP-20* mutation) (Kim et al., 2006). That study suggested that the list of known AI candidate genes was not enough to identify the cause of AI (Kim et al., 2006). The author supports the opinion that there are more unknown associated genes that may help in identifying the causes of AI.

In this study, the author suggests that amelogenesis is a complex process, which requires many genes and proteins to play their crucial roles in the formation of dental enamel. The current candidate genes in AI are insufficient to identify the causes of AI. Further studies of yet-to-be discovered candidate genes will help explaining the complexity of enamel formation and identifying the causes of AI.