CHAPTER IV

RESULTS

DNA from a total of 16 unrelated Thai patients, four of whom had hypohidrotic ectodermal dysplasia and 12 of whom had non-syndromic hypodontia, was analyzed for *EDA* mutations by direct sequencing. Mutation analysis revealed a total of six variants of the *EDA* gene (p.Arg156Cys, p.Glu164Ala, p.Arg334His, p.Ala349Thr, c.646-663del18 and IVS5+11_12delCT), which were detected in five of the 16 unrelated patients. Of these, the 18-bp in-frame deletion located in the coding sequences of exon 5 from a family with XLHED is a novel mutation. Moreover, the p.Glu164Ala mutation which has been previously reported to contribute to XLHED, can also contribute to non-syndromic hypodontia. Contrastingly, this study found that the p.Arg334His variant was detected in three unrelated healthy controls with the same ethnic background. This suggests that the p.Arg334His variant is nonpathogenic. The results of this study are shown in Table 4.1.

Family I

Clinical report

A six-year-old sporadic Thai boy (CGL DNA No.738) (Figure 4.1) presented with typical phenotypes of HED, including hypohidrosis, hypotrichosis and hypodontia. His parents were healthy and non-consanguineous. He was born at full-term gestation. He and his parents came to see a dentist with a chief complaint of

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

severe tooth loss. A panoramic radiograph was made to explore the numbers of teeth and other abnormalities. As the radiographic examination demonstrated, he had multiple absences of permanent tooth buds and only five teeth present (Figure 4.1). Both upper central deciduous incisors were conical in shape and first erupted at the age of two years. The other three teeth were permanent teeth, classifying as left and right mandibular canines and left maxillary first molar. All those permanent teeth seemed to be normal in shape, but smaller than normal. Upon physical examination, the patient demonstrated dry and soft skin with periorbital hyperpigmentation and wrinkling, sparse, fine and very slow growing scalp hair, lack of eyebrows and eyelashes and an obviously HED facial appearance with prominent forehead and lips,

Table 4.1 *EDA* mutations identified from the Thai individuals in this study.

Family No.	CGL DNA No.	Sex	Phenotypes	Results
I	738	M	XLHED	p.Arg156Cys, IVS5+11_12delCT
	740	F	Carrier	p.Arg156Cys, IVS5+11_12delCT
II	779	M	XLHED	c.646-663del18, p.Arg334His
	785	M	XLHED	c.646-663del18, p.Arg334His
	786	M	XLHED	c.646-663del18, p.Arg334His
	806	F	Carrier	c.646-663del18, p.Arg334His
	814	M	XLHED	c.646-663del18, p.Arg334His
	815	F	Carrier	c.646-663del18, p.Arg334His
III	813	M	XLHED	p.Ala349Thr
	807	F	Carrier	p.Ala349Thr
IV	431	F	Non-syndromic hypodontia	p.Glu164Ala
	811	M	Non-syndromic hypodontia	p.Glu164Ala
V	728	F	Non-syndromic hypodontia	p.Arg334His
	810	M	Non-syndromic hypodontia	p.Arg334His
	704	F	Normal	p.Arg334His
	712	F	Normal	p.Arg334His
	717	F	Normal	p.Arg334His

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

hyperthermia but no unknown infections or intellectual problems. His stature and weight were appropriate for his age. His mother (CGL DNA No.740) (Figure 4.2), an obligate carrier, was healthy without any manifestations of ectodermal defects. Excluding this affected boy, there was no evidence of another case of HED in this family.

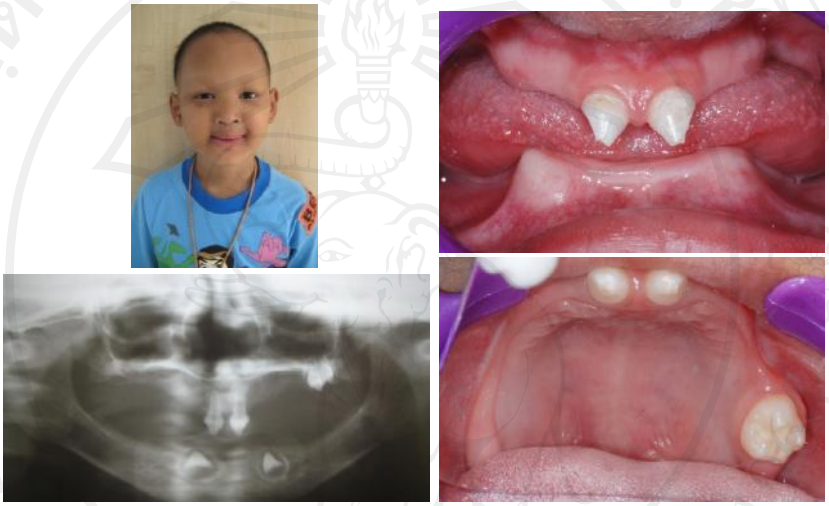

Figure 4.1 Facial and oral appearance of patient CGL DNA No.738. He manifested the full typical phenotypes of HED. He had severe tooth loss and conical maxillary central incisors.

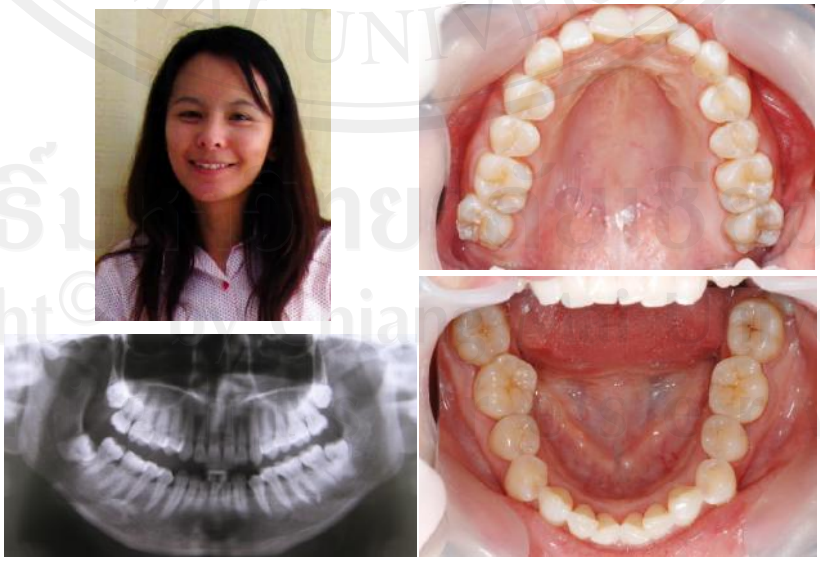

Figure 4.2 Mother (CGL DNA No.740) of patient 738. Clinical and radiographic examination did not reveal any ectodermal abnormalities, which are seen in most female carriers.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Mutation analysis

In case of patient 738, c.466C>T transition was detected in exon 3 (Figure 4.3). This mutation results in a change of codon CGC (Arginine) to TGC (Cysteine) at the amino acid position 156. Because this subject was sporadic and his mother was healthy without any signs that she might be an obligate carrier of HED, and to confirm the pattern of transmission and diagnose the type of HED, the DNA sample of the mother was analyzed by direct sequencing. The c.466C>T variant was also detected in the mother, identified a heterozygous carrier of XLHED. In addition to the p.Arg156Cys mutation, one 2-bp deletion (IVS5+11_12delCT) was detected in the proband and in his mother (Figure 4.4). However, this variant has been reported as a single nucleotide polymorphism (reference SNP number; rs 10579679).

The Homo sapiens EDA amino acid sequence (NP_001390.1) was aligned with sequences from twelve other species by using the ClustalX 2.0 alignment software. The alignment revealed that the arginine position is located in the highly conserved region among various species (Figure 4.5).

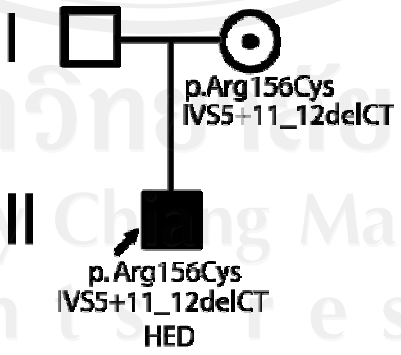

Figure 4.3 Pedigree of Family I. The diagram represents that the putative mutations were transmitted from the obligate-carrier mother to the affected proband.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

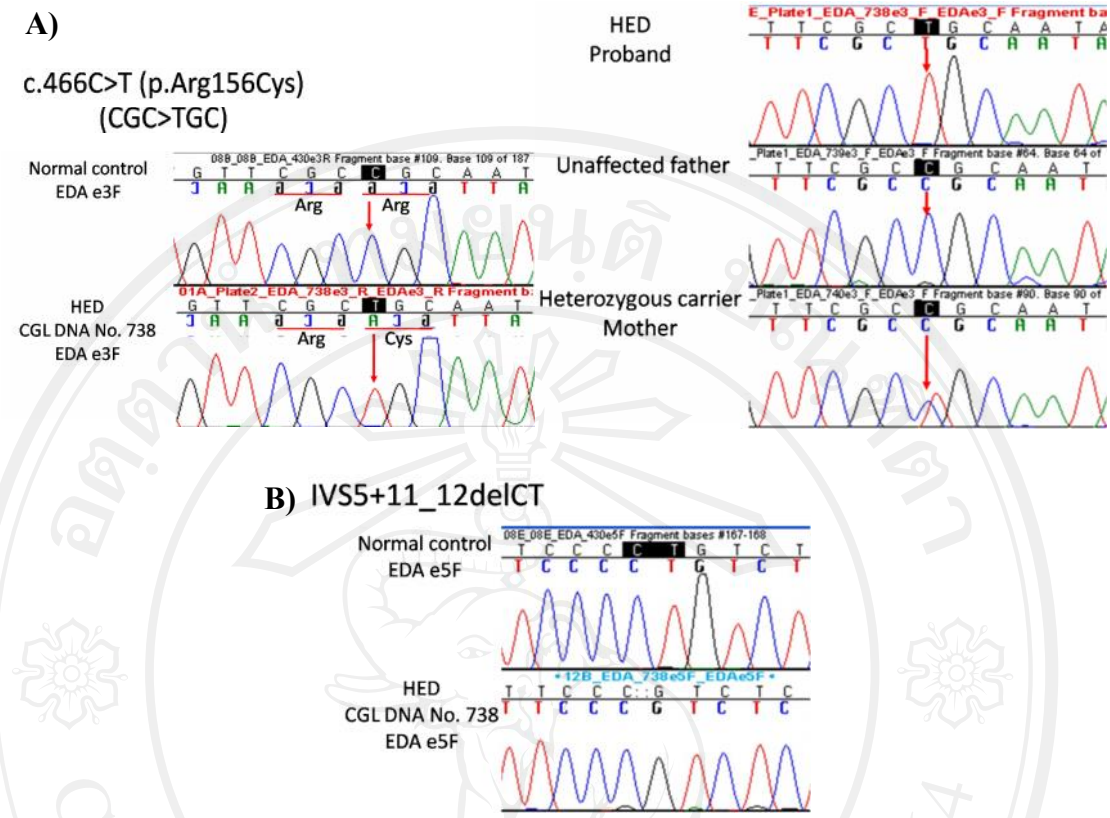

Figure 4.4 Identification of *EDA* mutations in the proband affected with HED (CGL DNA No.738). A, Chromatograms display a nucleotide change (c.466C>T) resulting in p.Arg156Cys. Red arrows represent the positions in which the single nucleotide substitution occurred. B, Chromatograms show the 2-bp deletion variation in the proband, compared with that of a normal control (black highlight).

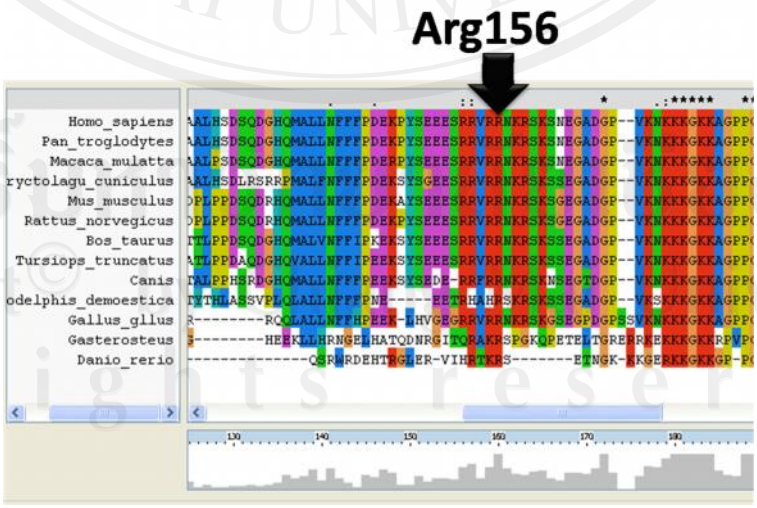

Figure 4.5 Multiple sequence alignments of the amino acid position 156 of EDA. The highly conserved amino acid R156 position across various species is indicated by the black arrow.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Family II

Clinical report

The author reports a five-generation Thai family. The pedigree of this large family showed an apparent X-linked recessive pattern of inheritance (Figure 4.6). Four (IV6, IV19, V14, and V15) affected males, two female (IV25 and V12) carriers and one normal male (IV16) participated in this study. The phenotypic appearance of affected males in this family comprised sparse scalp and body hair, very scant eyelashes and eyebrows, reduced ability to sweat, and congenital absence of teeth (Figure 4.7). The mustache and beard appeared unremarkable in the adult patients. Frequent episodes of hyperthermia were observed. Affected males reported to use towels which were soaked with water to reduce the heat during summer time. They had neither nail nor immune abnormalities. Most teeth were absent, but maxillary permanent central incisors, which were abnormally cone-shaped, appeared in all affected members. Most affected males (three out of the four) had at least one first permanent molar. An unusual face was remarkable in affected males. Interestingly, most of them had a chubby appearance with more fat deposition than that seen in previously reported XLHED patients.

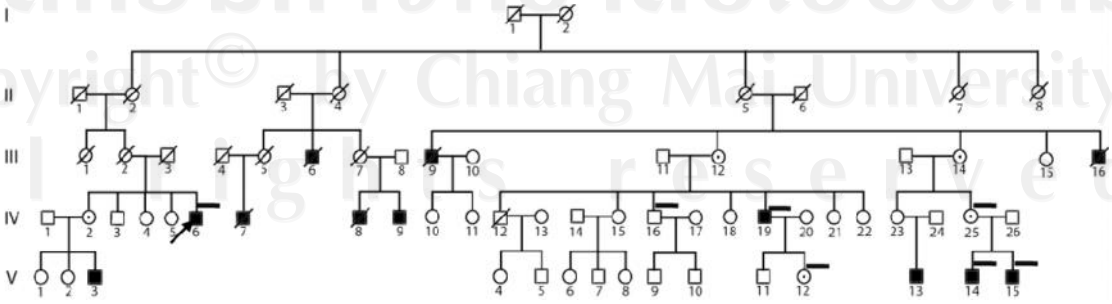

Figure 4.6 Pedigree of Family II, affected with XLHED. This family comprised 67 members, spanning five generations.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Figure 4.7 Phenotypes of members in Family II, affected with XLHED. Four males (IV6, IV19, V14 and V15) were affected with XLHED whose features were complete manifestations of this syndrome, but whose hands were normal (shown only IV6). The panoramic radiographs of two young affected patients (V14, V15) show that they had multiple missing teeth and the maxillary central incisors were conical in shape. Two obligate female carriers of XLHED seemed to have unusual faces. Female carrier IV25 had hypodontia, whereas V12 was healthy without hypodontia, hypohidrosis or hypotrichosis.

Both female carriers had mild phenotypes of HED, which were different in character. One (IV25) female carrier had slight and thin scalp hair, missing teeth, but no complaints about intolerance to heat. She has had diabetes mellitus for 7 years. Otherwise she was healthy. The other carrier (V12) had a phenotype with abnormally shaped teeth and a face slightly recognizable as having HED. She had no hypodontia but the maxillary central incisors and lateral incisors seemed to be conically shaped and reduced in size. In addition, she also had prolonged retention of the left maxillary deciduous canine.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Mutation analysis

Analysis of the *EDA* gene revealed c.646-663del18, located in exon 5, found in the affected participants and in female carriers (Figure 4.8). This region is located within the collagen domain and is highly conserved among various species (Figure 4.9). This particular deletion was not found in any of the controls and was first detected in this study, indicating to a novel mutation. In addition to the 18 bp in-frame deletion, chromatograms of some coding sequences of exon 9 showed a G to A transition at nucleotide 1001 (c.1001G>A), resulting in a p.Arg334His missense mutation (Figure 4.10). The heterozygous transition at the same nucleotide was found in both participating females. However, one hundred unrelated ethnically-matched, normal individuals were analyzed to exclude any polymorphisms. Three healthy female controls were detected the p.Arg334His variant (Figure 4.11). Because this variant found in female controls, there were possibilities that p.Arg334His might be either a single nucleotide polymorphism or a pathogenic mutation.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

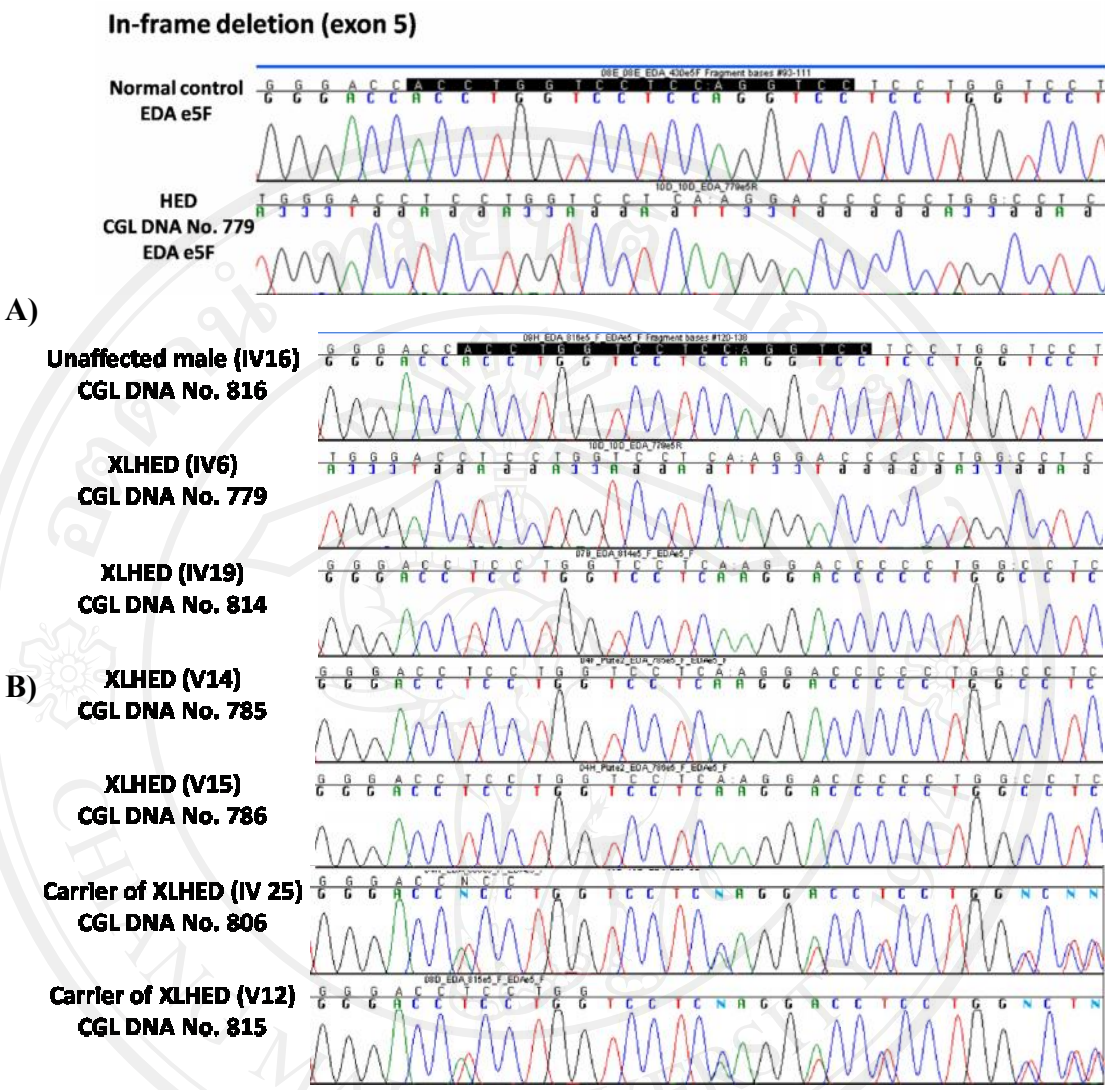

Figure 4.8 Mutation analysis (c.646-663del18) of Family II, affected with XLHED. A, Chromatograms show c.646-663del18 in the affected proband (CGL DNA No779), compared with that of normal individuals (black highlight). B, The same 18-bp deletion of all affected and carrier individuals was detected, but not shown in a normal male (CGL DNA No.816) in family II, representing by the black highlight.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright © by Chiang Mai University
All rights reserved

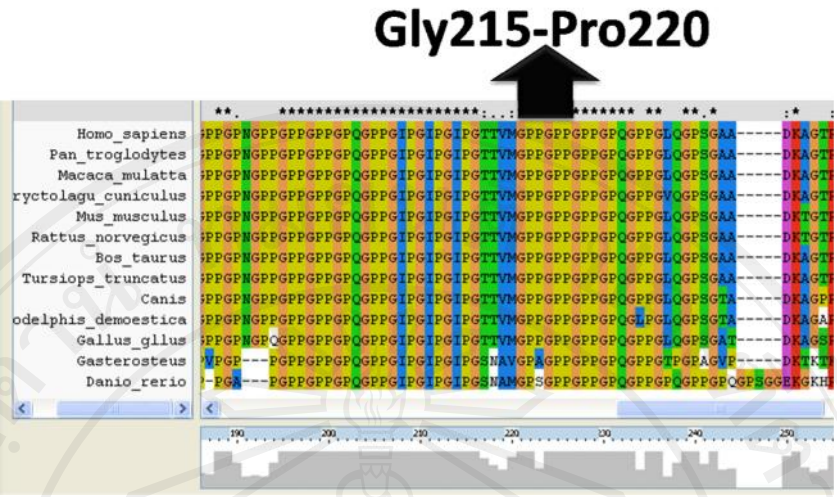

Figure 4.9 Multiple sequence alignments of the amino acid position 215 to position 220. The black arrow demonstrates high conservation in six amino acids of EDA.

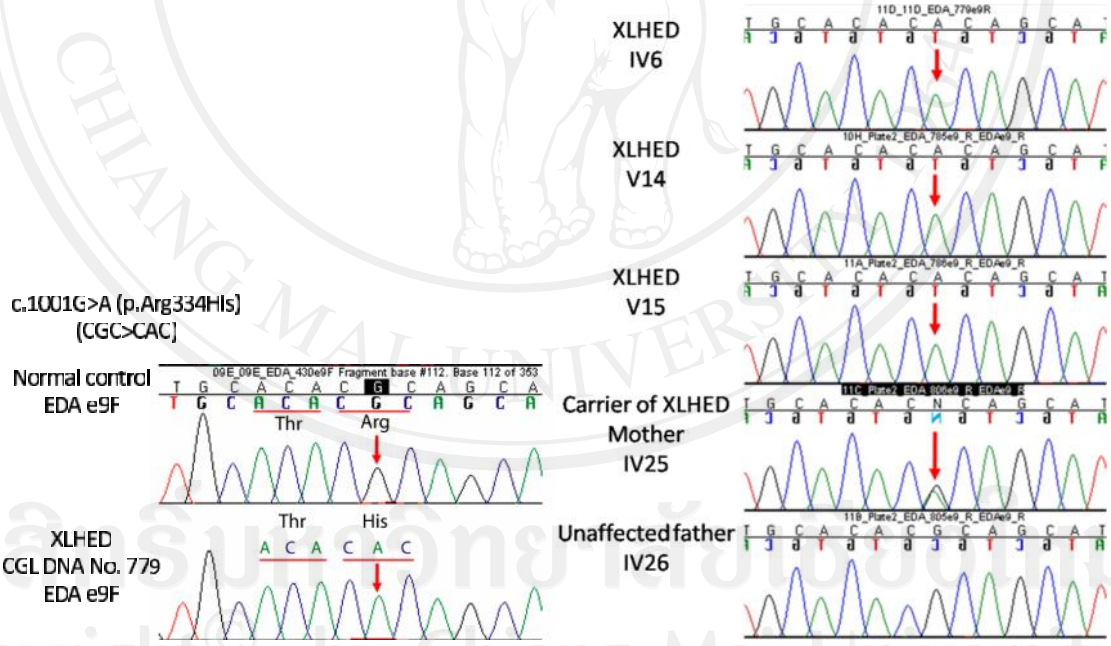

Figure 4.10 Mutation analysis (p.Arg334His) of participating members in Family II, affected with XLHED. The left chromatograms demonstrate the p.Arg334His mutation in Patient CGL DNA No. 779. This mutation (indicated by the red arrows) was also found in the proband as well as three other affected males and two carriers.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

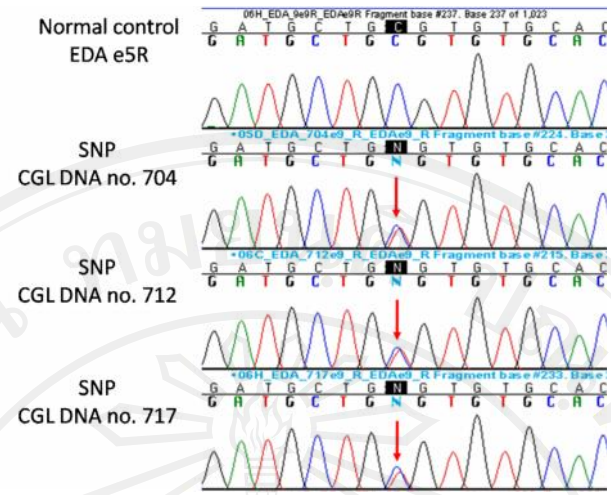

Figure 4.11 Detection of p.Arg334His in controls. This variant was detected in three normal controls, suggesting that it is nonpathogenic, and thus clarifying the genetic etiology of XLHED in Family II.

Family III

Clinical report

A two-year-old Thai boy (CGL DNA No.813) was born from non-consanguineous healthy parents at full-term of gestation. He had three typical features of HED, sparse scalp and body hair, reduced sweating ability and severe absence of teeth (Figure 4.12). Eyebrows and eyelashes were almost absent. Fingernails and toenails were normal. There were no erupted teeth in his mouth. Because the patient was too young to have a panoramic radiograph made, the actual number of teeth could not be established. He had suffered from recurrent hyperthermia but had no experience of recurrent infection. He usually wore sport T-shirts to reduce body heat and to freshen body skin. He manifested the distinctive face of HED with protrusive forehead and lips, depressed nasal bridge, and periorbital wrinkles. His mother had a complete permanent dentition with microdontia of both upper lateral incisors. Otherwise she was clinically normal. This family history was negative for HED.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

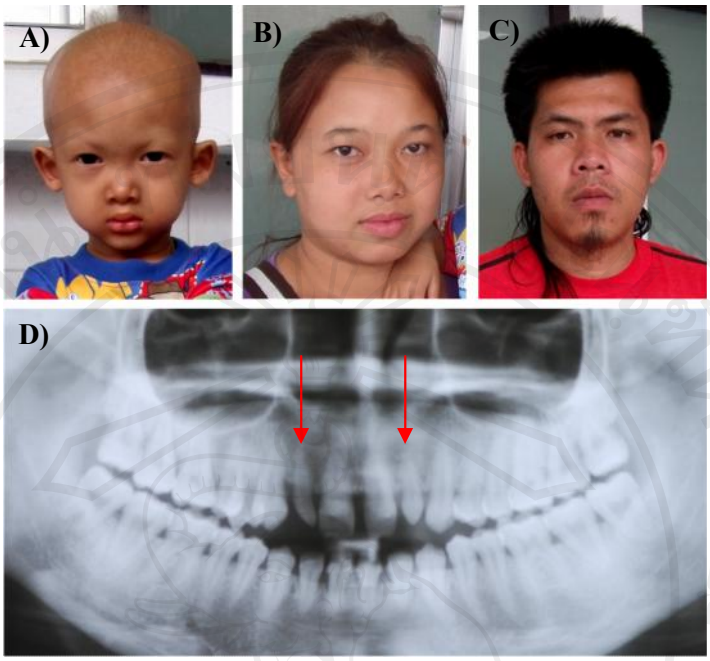

Figure 4.12 Phenotypes of members in the Family III. Facial appearance of A, proband (CGL DNA No.813) B, mother of the proband (CGL DNA No.807) and C, father of the proband (CGL DNA No.808). D, panoramic radiograph of the proband's mother shows two peg-shaped teeth.

Mutation analysis

In this family, direct sequencing detected a missense mutation involving c.1045G>A located in exon 9 of the *EDA* gene, which causes alanine to threonine substitution at amino acid 349 (p.Ala349Thr) in the TNF homology domain (Figure 4.13). This missense mutation was also detected in his mother in a heterozygous pattern, indicating a carrier of the *EDA* mutation. The clustalX alignment of the alanine position 349 of the human EDA protein with its corresponding position in others species revealed that Ala349 was completely conserved in all species (Figure 4.14).

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

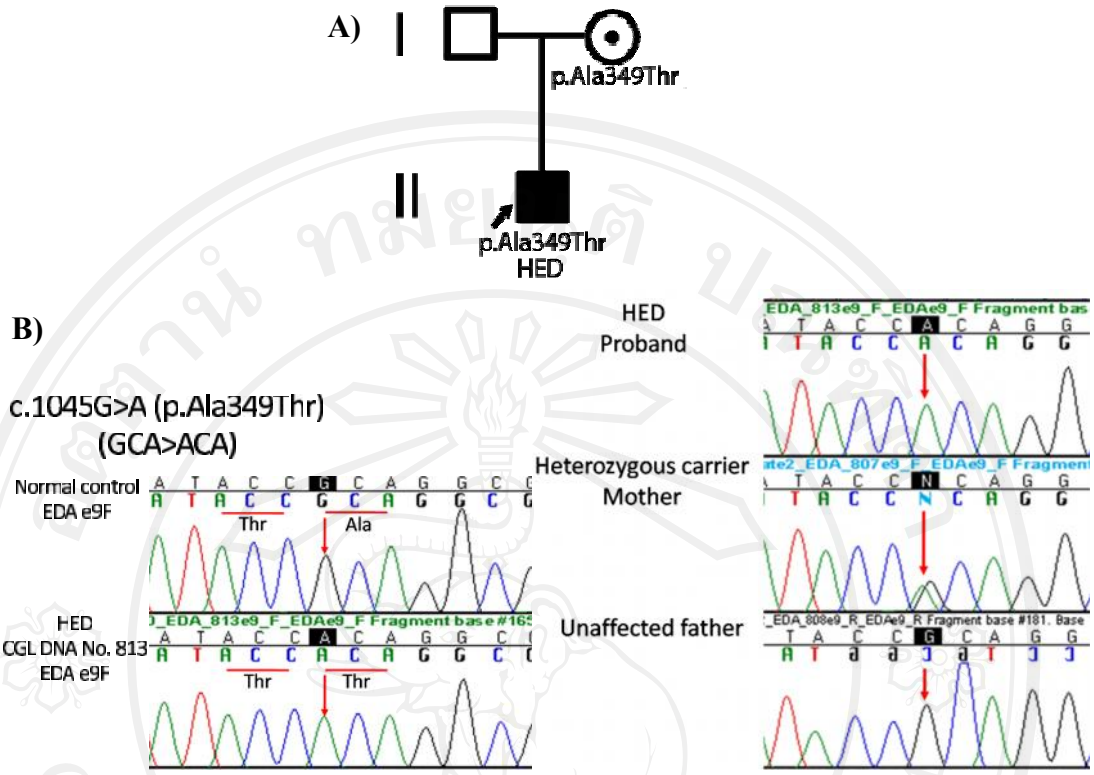

Figure 4.13 Mutation analysis of Family III, affected with p.Arg349Thr. A, pedigree of this family. B, Nucleotide analysis in the affected proband CGL DNA No. 813 and his parents. Chromatograms of some parts of exon 9 shownucleotide sequence a change at position 1045, leading to the p.Ala349Thr missense mutation. The red arrows indicate the position of the single nucleotide transition.

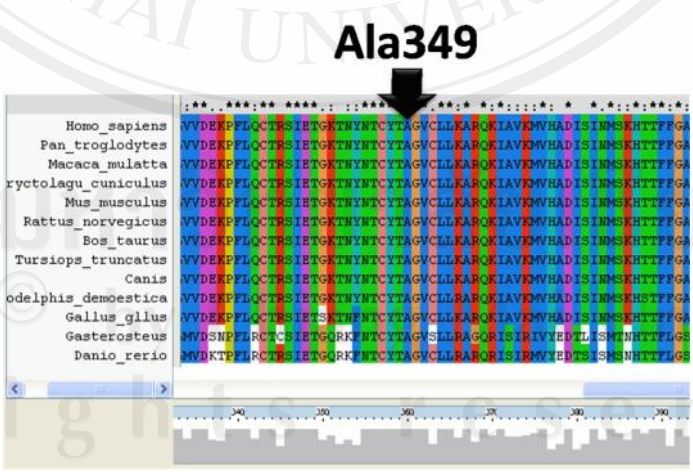

Figure 4.14 Alignment of the human amino acid position 349 with corresponding sequences from other species. The clustalX alignment reveals that the arginine position 349 (the black arrow) was completely conserved among various species.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Family IV

Clinical report

The manifestation of the proband (CGL DNA No.431) in this family was non-syndromic hypodontia (Figure 4.15). Her face, hair, skin and nails were normal. She sweated normally during exercise and work. The proband had hypodontia of both maxillary canines and prolonged retention of tooth 53. Hypodontia was also detected in her father, whose phenotype was lack of both maxillary third molars, but otherwise he had no dental or physical problems. The other members of this family were not affected with hypodontia or any ectodermal defects.

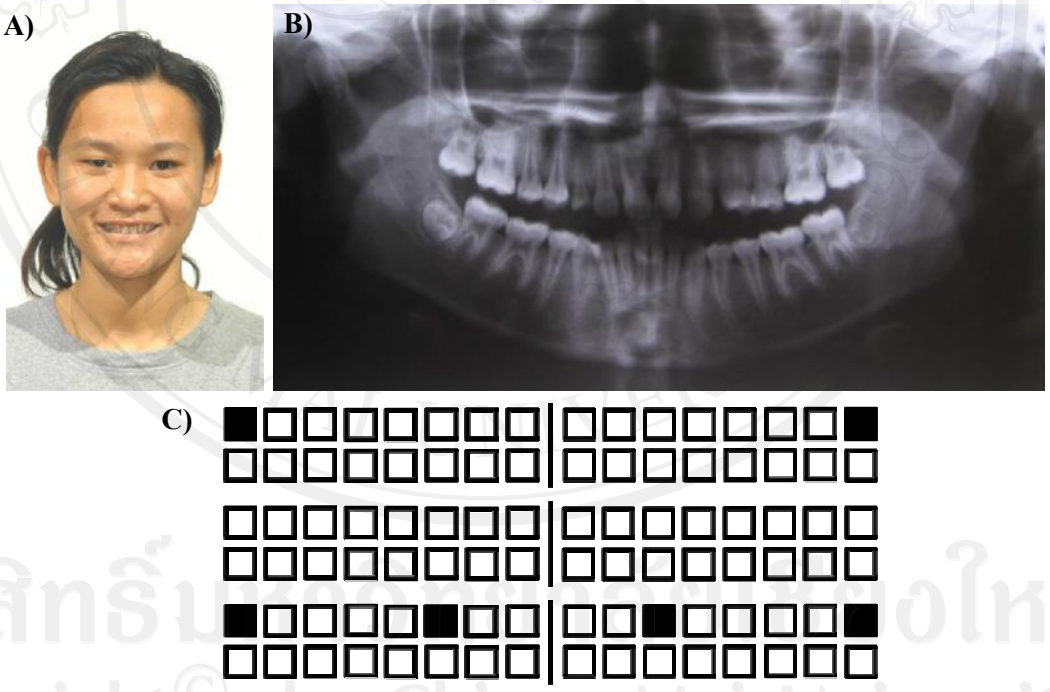

Figure 4.15 Clinical and radiographic examination of proband (CGL DNA No.431). A and B, Upon examination, the proband had only hypodontia of both maxillary canines and third molars and prolonged retention of right maxillary deciduous canine. Otherwise she was healthy. C, The diagram represents the teeth of the father (CGL DNA No.811), mother (CGL DNA No.812) and proband (CGL DNA No.431), respectively, from upper to lower.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Mutation analysis

A pedigree of this family show two members of a family affected with non-syndromic hypodontia. Mutation analysis of the proband showed a nucleotide change (c.491A>C) in the coding sequences of exon 3 in the *EDA* gene (Figure 4.17). The A to C transition results in the substitution of glutamic acid with cysteine at amino acid position 164 (p.Glu164Ala). In accordance with the sequencing, this nucleotide change was identified not only in the proband, but also in her father, suggesting that the particular mutation had vertically transmitted from the father to his offspring. No other nucleotide change in the *EDA* gene was found in this family. The ClustalX alignments of the EDA amino acid demonstrated that the glutamic acid position 164 was highly conserved during evolution (Figure 4.18).

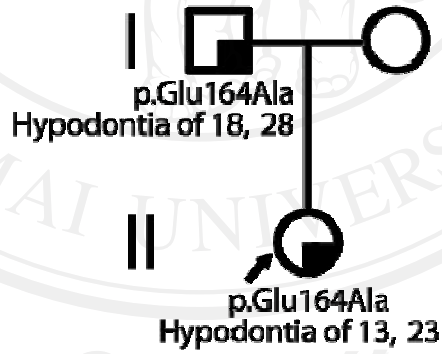

Figure 4.16 Pedigree shows the mutation was transmitted from her father who was hypodontia of teeth 18 and 28. One-quarter blackened objects represent the phenotype of non-syndromic hypodontia.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

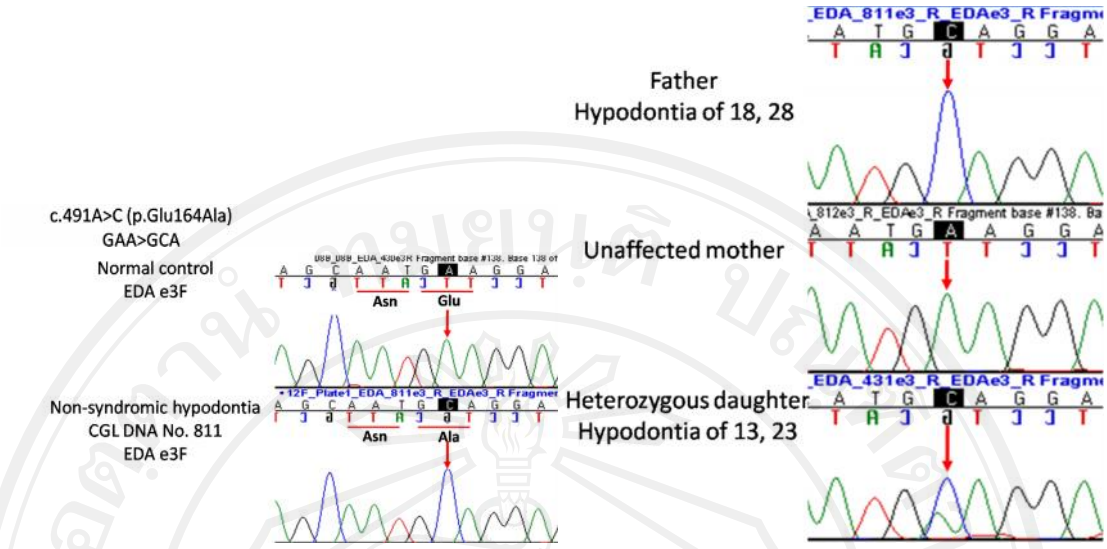

Figure 4.17 Mutation analysis of the *EDA* gene of Family IV, with p.Glu164Ala. Chromatograms of the proband reveal a nucleotide change c.491A>C, resulting in substitution of p.Glu164Ala. The right figure shows that the sequences harboring the particular mutation were found in the hemizygous father and in the heterozygous daughter. The mother was unaffected. The red arrows mark the mutation sequence.

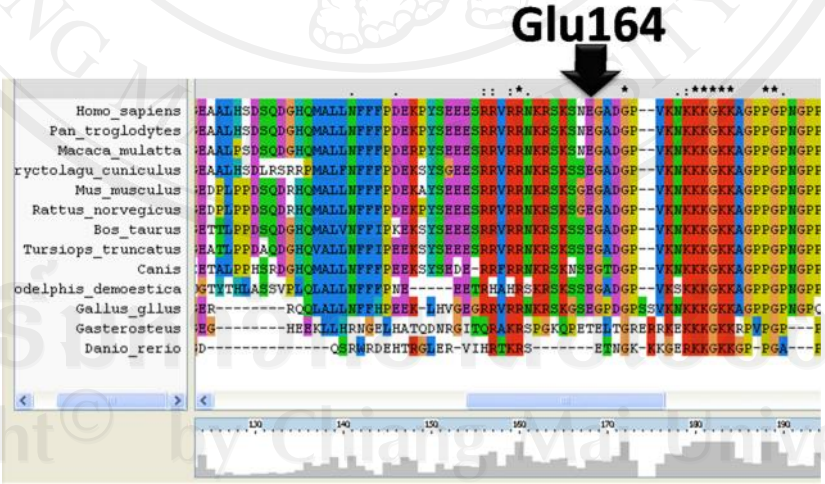

Figure 4.18 Alignment of the human amino acid position 164. The clustalX alignment reveals that the human glutamic acid with its corresponding sequences from other species (the black arrow) was highly conserved.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

Family V

Clinical report

This family comprises four members (Figure 4.19). Of these, three participants had isolated hypodontia. The proband (CGL DNA No.728) was missing six teeth: two maxillary canines and four second premolars (Figure 4.20). Her father (CGL DNA No.810) had hypodontia of the right maxillary lateral incisor, whereas her mother (CGL DNA No.809) had hypodontia of all four third molars. There was no overlapping of the position of hypodontia among these subjects. The relevant family background did not show consanguinity. Clinical examination of three participating subjects demonstrated normal scalp and body hair, skin, nails and sweating, excepting sparse scalp hair in the mother. All of them could tolerate heat and were not affected with any medical problems.

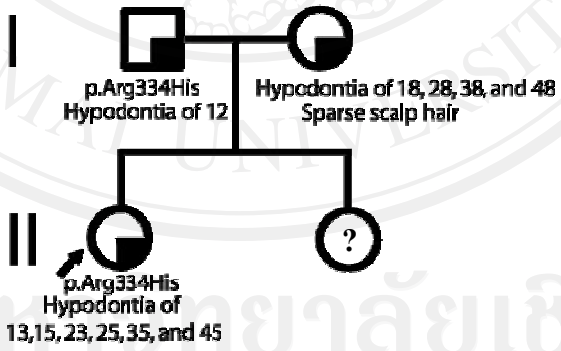

Figure 4.19 Pedigree of the family harboring p.Arg334His. The father had one absent tooth and transmitted this mutation to his elder daughter whose phenotype was hypodontia of six teeth. Sequencing of the mother did not show any *EDA* mutation. One quarter blackened objects represent phenotype of non-syndromic hypodontia.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

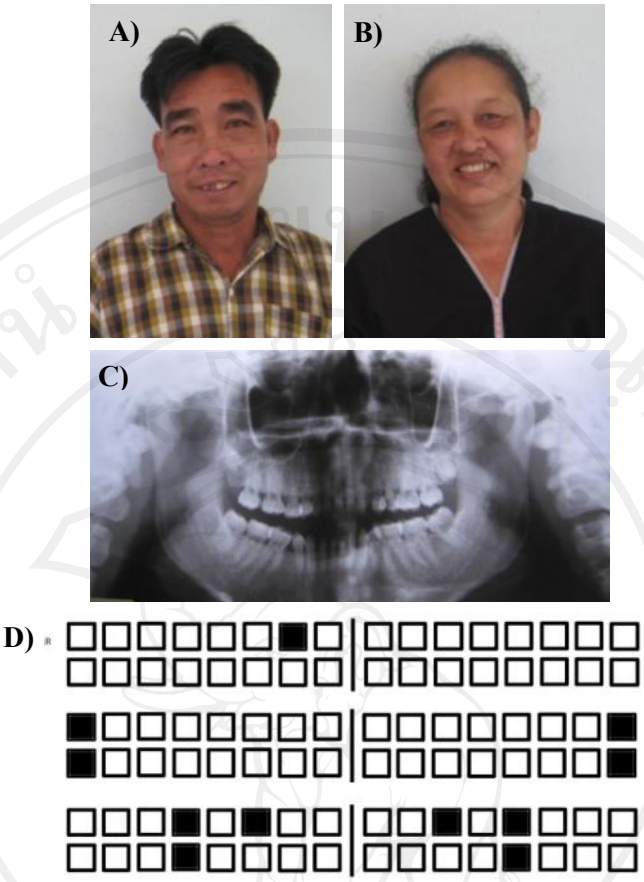

Figure 4.20 Phenotype of members in the Arg334His-affected family. The figure shows clinical features of: A, father (CGL DNA No.810); B, mother (CGL DNA No.809). C, Panoramic radiograph of the proband (CGL DNA No.728) demonstrates missing of second premolars and maxillary canines. D) The diagram represents the teeth of the father, mother and daughter, respectively, from upper to lower. The blackened boxes indicate position of absent teeth, whereas the clear boxes represent present teeth.

Mutation analysis

EDA mutations were screened in this family. Direct sequencing of all coding regions in the *EDA* gene revealed a c.1001G>A transition, which caused substitution of p.Arg334His in the father (Figure 4.21). This variant was also investigated in the heterozygous elder daughter, whereas the mother was normal and had no mutations in *EDA*. Because a sample was not obtained from the younger daughter, it could not be

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved

confirmed whether she really carried this variant. As above described, this variant is nonpathogenic because it was also found in normal controls.

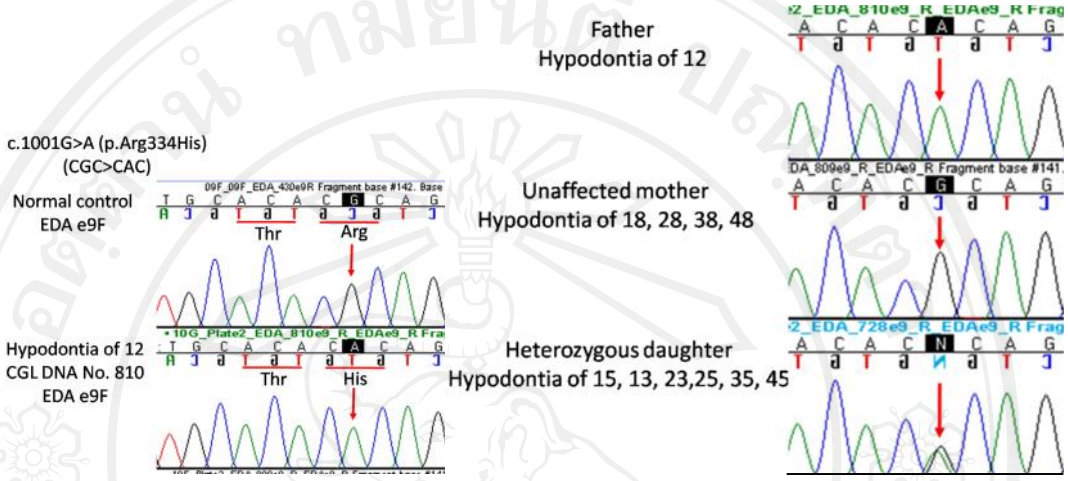

Figure 4.21 Analysis of the p.Arg334His polymorphism in exon 9 of Family V. Using direct sequencing, the finding was c.1001G>A variant, resulting in arginine substitution with histidine at the amino acid position 334. Chromatograms of some parts of exon 9 display a hemizygous pattern in the father and a heterozygous pattern in the elder daughter. The nucleotide changes are identified by red arrows.

ลิขสิทธิ์มหาวิทยาลัยเชียงใหม่
Copyright© by Chiang Mai University
All rights reserved