Enamel protein in smooth hypoplastic amelogenesis imperfecta

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Abstract

Amelogenesis imperfecta (AI) remains a poorly understood group of hereditary enamel defects character-ized by a wide array of clinical presentations. Although numerous reports have described the histological features of AI, knowledge concerning the biochemical composition of the affected enamel remains minimal. The purpose of this investigation was to examine the protein of smooth hypoplastic AI enamel. Exfoliated primary teeth were obtained from an individual with smooth hypoplastic AI together with exfoliated teeth from normal healthy individuals for controls. Enamel was dissected from the AI and control teeth to determine protein content and amino acid profile. The analyses showed that the hypoplastic AI teeth contained 2% protein, compared with 0.3% in normal primary enamel. The protein content of the hypoplastic AI enamel was similar to that reported for the late maturation stage of normal primary enamel. The amino acid profiles of both normal and AI enamel were similar although there appeared to be increased amounts of glycine in the AI enamel. Hypoplastic AI enamel showed an amino acid profile similar to normal mature primary enamel in contrast to hypomaturation AI that exhibits an amelogenin-like character. The amount of retained protein also was different from that reported for hypomaturation AI enamel which contains approximately 5% protein compared with the 2% seen in hypoplastic AI enamel. This study emphasizes the potential usefulness of protein characterization in delineating different AI types and illustrates how this information may lead to an understanding of the developmental defects responsible for producing abnormal enamel. (Pediatr Dent 14:331–37, 1992)

Introduction

Amelogenesis imperfecta (AI) comprises a diverse group of hereditary conditions characterized by enamel defects without evidence of a generalized or systemic disorder. The presentation of diverse clinical manifes-tations is thought to result from the heterogeneous structural and chemical defects. Classification of the AI types considers mode of inheritance and clinical mani-festations. The most widely accepted classification sys-tem recognizes three major groups; i.e., hypoplastic (thin enamel), hypocalcified (primary mineralization defect), hypomaturation (defect in enamel maturation).[1] Delineating specific AI types can be confusing given the phenotypical similarity of many forms and that the most recent classification lists 14 different AI types.[2] Although numerous reports have described the heredi-tary, clinical, radiographic, and histological features of the different AI types, little is known concerning the molecular genetic and biochemical abnormalities re-sponsible for defective enamel development.[3–7]

Analysis of X-linked AI has shown the defective gene for this specific AI type to be closely linked to the locus DXS85 at Xp22.[8] Interestingly, this also has been identified as the general location of the human gene for amelogenin, the principle protein in developing enamel.[9, 10] Recently, one kindred with X-linked hypomaturation AI demonstrated a large deletion in the amelogenin gene.[11] Information from molecular genetic studies will ultimately lead to identification of the genes involved in normal and pathological enamel formation and provide a basis for definitive diagnostic tests. To date, however, only the X-linked AI type has demonstrated linkage allowing identification of the af-fected gene locus. The genetic, biochemical, and devel-opmental mechanisms leading to the distinct enamel aberrations remain unknown for all AI types.

Many investigators feel that the enamel proteins are involved intimately in initiating and controlling crystal growth in normal enamel development; however, the exact mechanisms involved are poorly under-stood.[12–14] While normal enamel proteins have been characterized extensively over the past two decades, little information exists regarding the organic content or character of AI enamel. At present, only one publica-tion appears in the literature characterizing the enamel proteins of AI teeth.[15] Normal mature human enamel is reported to contain between 0.01% to 1.0% protein by weight with some areas such as the cervical enamel and that closest to the dentinoenamel junction having greater than 0.6% protein.[16–20] Analysis of autosomal reces-sive hypomaturation AI showed a protein content of approximately 5.0% by weight in the fully developed enamel.[15] The protein content in these AI teeth was similar to that seen in normal maturation stage enamel indicating that the AI enamel did not progress beyond

this stage of development. Amino acid analysis revealed that the retained AI enamel proteins showed an amino acid composition similar to that reported for normal amelogenins, the most prevalent protein in early maturing enamel.[21, 22] In this AI type, retention of the enamel proteins may be due to: 1) a protein structural abnormality that renders the normal proteolytic processes ineffectual; 2) abnormal proteolytic activity; or 3) defective transport during protein withdrawal. It remains unknown whether qualitative or quantitative alteration of the enamel proteins occurs in other AI types.

The enamel proteins in normal teeth have shown multiple components of varying molecular weight. In secretory enamel these proteins consist predominantly of amelogenins having a molecular weight of around 25,000 daltons.[23, 24] Proteins from mature enamel apparently consist of retained degradation products of amelogenins and higher molecular weight proteins (50,000 – 70,000 daltons) which are mineral bound, although this remains controversial.[20, 25, 26] The protein profile changes during development from one rich in proline, leucine, and histidine (amelogenins) to one that is rich in aspartic acid, glycine, and alanine (nonamelogenins).[20, 27] During development the protein content of enamel declines quantitatively from approximately 20–50% to less than 1% although there is considerable site-to-site protein content variation in normal enamel.[14, 16, 18, 19]

There remains considerable controversy as to the origin and nature of the proteins present in mature enamel and their possible function.[28–30] Proteins with molecular weights ranging from 20,000 daltons to 220,000 daltons, as determined by gel electrophoresis, have been described in mature enamel.[28] Recent investigations have demonstrated that a number of proteins which are not of ameloblast origin also may be present in unerupted mature enamel.[29, 30] None of the proteins in AI enamel have been characterized as to their molecular weight, changes during developmental stages, or cross reactivity with antibodies to normal enamel proteins.

The purpose of this investigation was to quantify and characterize the enamel protein in smooth hypoplastic AI for comparison with normal enamel protein and protein from hypomaturation AI.

Materials and Methods

Five noncarious exfoliated primary teeth were collected from an individual having smooth hypoplastic AI. Two of these teeth were used for the biochemical analysis presented in this report, while the remainder were used to evaluate the histological features and mineral composition of the enamel which have been

presented in greater detail elsewhere.[31] Due to the rarity of this disorder, the thin enamel, and retention of the remaining dentition in the affected individual, additional material was not available for analysis. Given the rarity of this specific AI type and the difficulty of obtaining material from additional cases, we felt that presentation of the findings in this limited sample of human material was important. Exfoliated primary teeth that matched the AI tooth types (i.e., anterior teeth) were gathered from healthy unaffected individuals to serve as controls. Two AI and three normal primary enamel samples were analyzed in this study.

Thin sections of the teeth were obtained using a diamond blade for histological and biochemical analyses. Sections for histological evaluation were examined dry, and imbibed in H_2O and chloronapthalene using transmitted light microscopy. Tooth sections used for biochemical analyses were cut dry and ground to a suitable thickness (approximately 100 µm) without lubrication to prevent the possible loss of organic material. Chemical cleaning of the diamond sectioning blade between specimens reduced the possibility of cross contamination. Due to the very thin enamel in these pathological specimens it was necessary to dissect the full thickness of enamel from the incisal edge to the cervical margin in multiple sections to obtain sufficient material for analysis. Specimens of the AI and normal enamel therefore represent bulk enamel obtained from multiple sections. Careful dissection was carried out under a microscope by gently separating the enamel from the dentin at the dentinoenamel junction using a #11 scalpel. After dissection the enamel was weighed to an accuracy of 0.2 µg and then hydrolyzed in triply distilled 6 M HCl at 105° C in vacuo for 24 hr. Adding phenol crystals to the samples during hydrolysis assisted in the preservation of tyrosine residues. The hydrolysates were dried over P_2O_5 in vacuo to remove the acid and the residues washed three times with triply distilled H_2O. The samples were dried after each wash in a desiccator under vacuum. The residues were taken up in a minimum weighed volume of 0.01 M HCl containing 150 nM/mL norleucine as an internal standard and loaded onto an amino acid analyzer. Amino acid analysis was accomplished using an automated amino acid analyzer with traditional post column ninhydrin derivatization and the quantity of each amino acid residue determined by integration of the standardized absorption profiles.[15, 19, 25]

Amino acid profiles for enamel samples were compared and presented as residues per thousand and graphically illustrated as rose diagrams for visual comparison.[19] Quantitative assessment of the protein content was determined by calculating the residue weight of amino acids recovered for each sample and express-

ing the total protein as percentage yield in relation to the total enamel sample weight.[25] The amino acid composition and protein content of normal primary enamel and hypoplastic AI enamel were compared with published data for normal permanent enamel and hypomaturation AI enamel.[15]

Results

The teeth displayed a yellow coloration with patchy white opaque mottled areas and generalized interdental spacing in both the transitional and full permanent dentitions. Radiographically, the teeth showed normal pulpal morphology and a thin layer of enamel that was only slightly more radiopaque than the dentin. Detailed histological studies presented elsewhere showed the AI enamel to be 40% the thickness of normal enamel.[31] The enamel also showed increased porosity compared with normal enamel (Fig 1) as evidenced by an opaque appearance in the dry sections that greatly diminished after imbibition, with H_2O and chloronapthalene. Some of the opaque areas remained after imbibition with chloronapthalene potentially indicating regions of retained organic material. Evaluation of the family revealed no evidence of other clinically affected individuals. Clinical evaluation of the mother and the proband's sibling revealed normal dental morphology and coloration. The father was not available for examination but was reported to have a normal dentition. Reviewing the family history also gave no indication of enamel defects in the proband's grandparents, aunts, or uncles.

The total protein content of smooth hypoplastic AI and normal enamel were markedly different, with substantially more protein being present in the AI enamel compared with the normal control. Normal healthy primary enamel contained a mean of 0.3% protein in contrast to the AI enamel which yielded 2.0% total protein. Therefore, total recoverable protein from the AI enamel was six and a half times the amount obtained from the normal primary enamel analyzed.

The amino acid profile of the hypoplastic AI enamel was similar in character to that of the control primary enamel (Figs 2 and 3). Both hypoplastic AI and normal primary enamel showed a predominance of glutamic acid, proline, glycine, and alanine (Table, page 334). The hypoplastic AI enamel showed slight elevations in proline, glycine, and alanine while aspartic acid, serine, glutamic acid, phenylalanine, and histidine showed slight reductions compared with the normal primary enamel. With the exception of these minor variations, the remaining amino acids were otherwise very similar between the hypoplastic AI and control primary enamel.

In general the amino acid compositions of the normal primary and hypoplastic AI enamel were similar to that reported previously for normal permanent enamel
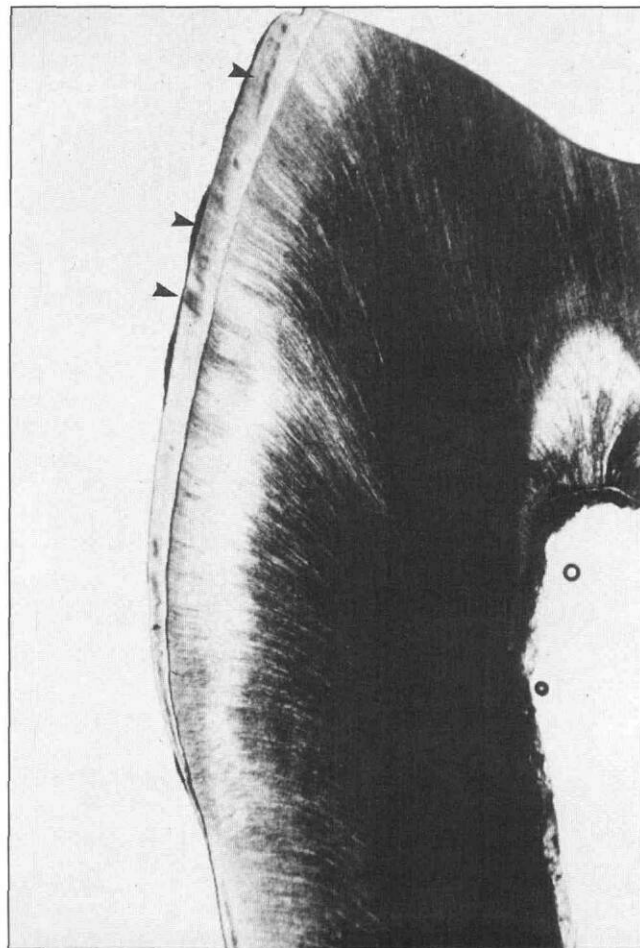

Fig 1. The hypoplastic AI enamel was uniformly thin and showed opaque areas in the enamel (arrows) even after imbibition possibly indicating areas of retained organic material as seen in this thin ground section examined with light microscopy.

(Table). There were, however, minor variations in many of the amino acids, most notable being the elevated glycine content seen in the normal primary (211 residues/1000) and hypoplastic AI enamel (298 residues/1000) compared with the permanent enamel (159 residues/1000). The differences and similarities of amino acid compositions in normal primary and permanent enamel compared with hypoplastic and hypomaturation AI enamel can be seen visually in Figs 2–5 (page 335). The amino acid profile previously reported for hypomaturation AI was strikingly different from the hypoplastic AI in this study concerning its elevated proline (196 vs. 98 residues/1000 respectively) and reductions in glycine (95 vs. 298 residues/1000 respectively) and alanine (21 vs. 106 residues/1000 respectively).[15] Hypomaturation AI enamel also showed higher levels of histidine and tyrosine compared with all other enamel samples.

Discussion

Dramatic differences in the quantity of enamel protein present in AI teeth compared with normal enamel were demonstrated in this investigation. Interestingly, there also appeared to be distinct differences in the amount and amino acid profiles of enamel proteins from different AI types. While the smooth hypoplastic AI enamel evaluated in this study had 2.0% protein, six and a half times the amount present in normal primary enamel, a previous report of hypomaturation AI enamel has shown it to have approximately 5% protein in the fully developed enamel.[15] This investigation indicates, however, that these two clinically and histologically different AI types both may exhibit an increased quantity of protein in the fully developed enamel. The presence of increased protein in hypoplastic AI has not been previously described although it has been alluded to by investigators conducting histological studies that visualized organic material retained in decalcified sections.[32] Ultrastructural studies of hypoplastic AI enamel also have revealed globular and amorphous structures within and around the enamel prisms that most likely represent retained organic material.[31, 33] The finding of increased enamel protein in hypoplastic AI enamel provides further evidence of qualitative alteration as well as a reduction in thickness.

The quantity of protein present in fully developed hypoplastic AI enamel (2%) corresponds quantitatively to amounts reported for the late maturation stage of normal primary teeth in humans.[14] Forming human primary enamel has approximately 20% protein by weight, which is reduced to about 7% in the early maturation stage of development.[34] By late maturation the protein content of human primary enamel is reduced to 2% with this being further reduced to less than 1% in fully mature enamel. In contrast, hypomaturation AI enamel contained protein amounts (5%) similar to transition/early maturation stage normal human enamel (7%).[14, 15, 34] Although the amount of protein present

Table. Amino acid analyses of normal and AI enamel

Amino Acid	Normal Primary*	Normal Permanent†	Hypoplastic AI‡	Hypomaturation AI†
Cystic acid	0	0	0	4.2
OH-proline	0	0	0	0
Aspartic acid	83.2	93.5	64.5	38.6
Threonine	41.0	48.4	28.7	36.2
Serine	83.4	127.2	54.1	79.2
Glutamic acid	152.0	124.1	119.8	122.8
Proline	84.3	138.8	98.1	196.8
Glycine	211.7	159.7	298.8	95.4
Alanine	76.5	61.9	106.5	21.1
Cystine	0	0	0	0
Valine	30.4	44.8	30.8	33.1
Methionine	0	0	0	28.3
Isoleucine	21.3	22.4	12.5	29.5
Leucine	58.4	62.2	39.4	80.3
Tyrosine	26.5	8.6	17.5	83.2
Phenylalanine	27.3	28.3	21.7	32.6
OH-lysine	0	0	0	0
Lysine	27.1	44.0	33.4	29.7
Histidine	42.6	20.8	25.6	51.8
Arginine	33.0	24.3	46.7	36.4

* (N = 3); † (N = 5) (Wright and Butler 1989); ‡ (N = 2).

in normal primary teeth (less than 1%) was similar to amounts previously reported, it could be argued that the increased protein content of hypoplastic AI enamel resulted from posteruption changes and that the porosity of the tissue allowed uptake of proteins from the oral cavity. While this can not be discounted absolutely, previous studies of early enamel carious lesions have not shown significant changes in the quantity or quality of enamel proteins compared to adjacent noncarious enamel from the same tooth.[35] Studies of proteins in carious enamel have shown that initial lesions did not show significant reduction or ingress of protein into the affected enamel. While the nature of early carious lesions may be quite different from the enamel seen in hypoplastic AI, it would appear that the quantity and quality of enamel proteins do not change readily in the oral environment. Therefore, we feel that the increased amount of enamel protein seen in hypoplastic AI enamel is most likely related to a developmental abnormality and does not reflect posteruption change.

The amino acid profile of hypoplastic AI enamel was largely similar to that seen in fully developed primary enamel and in many respects was similar to enamel from normal unerupted permanent teeth.[15] The glycine
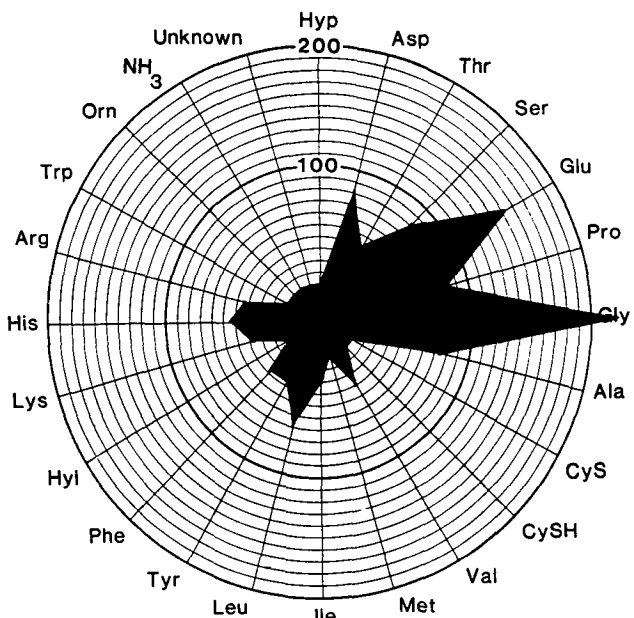
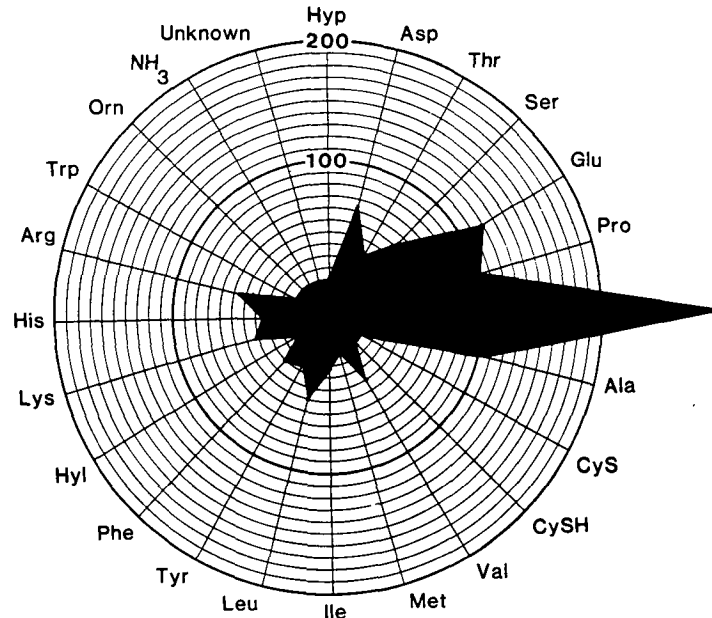**Fig 2.** Rose diagram of normal primary enamel protein amino acid profile.

Fig 3. Rose diagram of smooth hypoplastic amelogenesis imperfecta enamel protein amino acid profile.
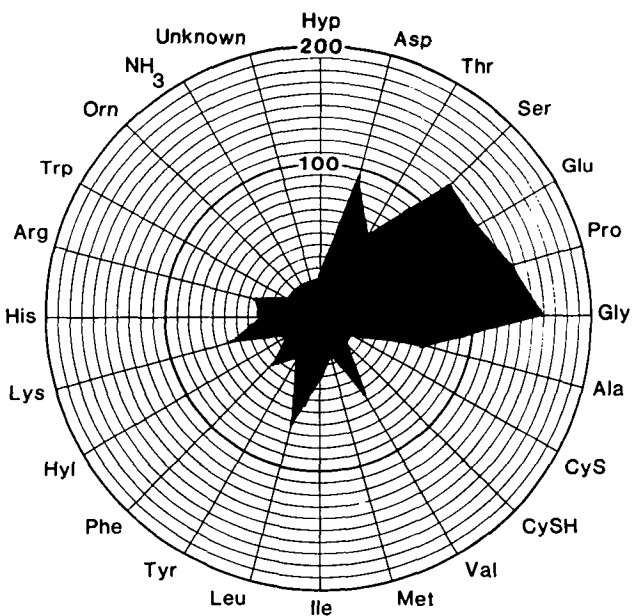

Fig 4. Rose diagram of normal permanent enamel protein amino acid profile.
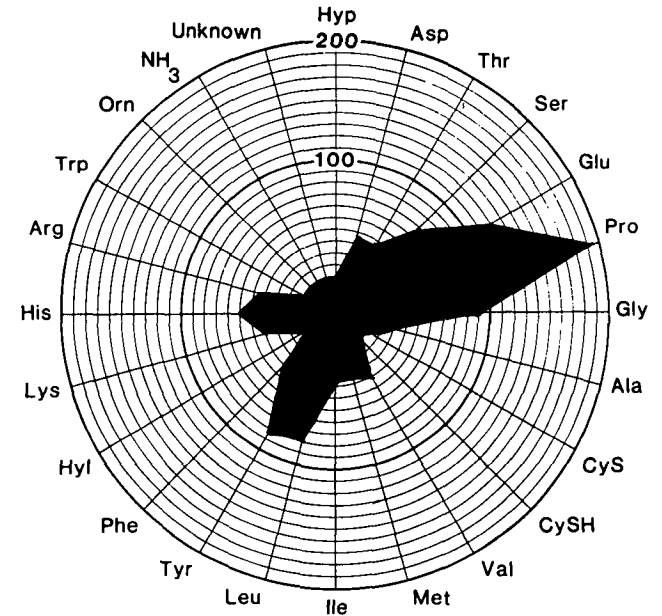

Fig 5. Rose diagram of hypomaturation amelogenesis imperfecta enamel protein amino acid profile.

content did vary markedly between samples (ranging from 135 to 277 residues/1000 in normal primary enamel) and was especially high in the primary teeth compared with the permanent enamel. Whether this represents a true compositional change in the protein or is the result of tissue sampling and/or a small sample size can not be definitively determined from this investigation. The amino acid profile of hypoplastic AI enamel did not, however, have the high proline content charac-

teristic of developing primary enamel that results from a high amelogenin content.[34] This is in stark contrast to hypomaturation AI enamel which displays an amino acid content rich in proline and amelogenin-like character.[15] The mature enamel profile of hypoplastic AI enamel indicates that the developmental abnormality involved may be quite different from that seen in hypomaturation AI. In hypoplastic AI the transition from the proline rich amelogenin appears to have taken

place, resulting in the expected nonamelogenin amino acid profile characteristic of fully mature enamel. This could indicate that proteases thought to be responsible for degradation of the enamel proteins are functional in hypoplastic AI and not in hypomaturation AI.[15, 36, 37] Despite this transition in protein character, the hypoplastic AI teeth retained an excessive amount of protein in the fully developed enamel. This may be, at least in part, due to formation of only the protein rich enamel adjacent to the dentinoenamel junction while the remainder of enamel, which has less protein, is absent.[38] The developmental mechanism leading to enamel with a reduced thickness and exhibiting a marked increase in the final protein content appears to be complex. Alteration of the amount and/or structure of the enamel protein could lead to abnormal enamel formation characterized by protein retention and deficient enamel thickness through, as yet, undefined feedback or regulatory mechanisms. While investigators are gaining an understanding of enamel matrix deposition and mineralization, little is known concerning what determines the life cycle of an ameloblast or this cell's ability to form enamel of a specific thickness.

Initial characterization of the enamel in hypoplastic AI showed retention of an increased amount of protein which has an amino acid profile similar to normal enamel protein in the fully developed AI enamel. This investigation demonstrated that hypoplastic AI enamel was altered not only in thickness, but also exhibited a distinct change in the amount of retained enamel protein. Although the mechanism for this developmental abnormality remains elusive, the diagnostic potential of this information should be considered. The protein content of fully developed enamel from different AI types appears quantitatively and qualitatively different. This should allow discrimination of AI types based on enamel composition along with the clinical, hereditary, and histological features. Furthermore, the distinct difference in amino acid profiles of the enamel protein seen in different AI types provides an objective biological marker that appears useful for delineating the different AI types.

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