AMELOGENESIS IMPERFECTA
An Epidemiologic, Genetic, Morphologic and Clinical Study

BIRGITTA BÄCKMAN
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Amelogenesis imperfecta (AI) is a genetically determined enamel defect characterized by genetic and clinical heterogeneity.

The prevalence and incidence of AI were established in the county of Västerbotten, northern Sweden, in 3-19-yr-olds born 1963-79, as were the mode of inheritance and clinical manifestation of AI. The distribution of the inorganic component in the enamel of AI teeth was studied as well as the surface morphology and other morphological details, and the findings were correlated to genetic and clinical data.

AI was diagnosed in 79 children and adolescents (index cases). The prevalence in the study population was 1.4:1 000. The mean yearly incidence 1963-79 was 1.3:1 000.

The inheritance patterns for AI were established in 78 index cases from 51 families. Pedigree and segregation analyses suggested autosomal dominant (AD) inheritance in 33 families, autosomal recessive (AR) in six families, and X-linked recessive in two families; in ten families only sporadic cases were found. In one of the families with an AD inheritance pattern, X-linked dominant was a possible alternative.

Examination of the families of the 78 index cases revealed 107 new cases of AI. The hypoplastic form was seen in 72% of all diagnosed cases and the hypomineralization form in 28% of the cases.

A further classification of the clinical manifestations led to the identification of eight clinical variants. In 33 of the 51 families the same clinical variant was found in all affected members. In eight families affected members were assigned to different clinical variants. In three families with an X-linked inheritance pattern for AI, the clinical manifestation differed between women and men due to lyonization. Among the remaining five families, with an AD inheritance pattern for AI, variants clinically characterized by hypoplasia as well as variants characterized by hypomineralization were found in three families; in the other two families the clinical manifestation varied within the same main form of AI, i.e. hypoplasia or hypomineralization.

Hypoplasia as well as hypomineralization were observed microradiographically in the enamel of most of the examined teeth. These findings were supported by scanning electron microscopy (SEM).

Both clinically and microradiographically as well as by SEM, similar variants of AI were found as AD and AR traits and/or among the sporadic cases. In the families with AI as an X-linked trait the genetic hypothesis was confirmed by the clinical, microradiographic and scanning electron microscopic findings.

Key words: amelogenesis imperfecta, enamel defect, epidemiology, genetics, microradiography, scanning electron microscopy.

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Umeå 1989
<table>
<thead>
<tr>
<th>CONTENTS</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>7</td>
</tr>
<tr>
<td>PREFACE</td>
<td>9</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>11</td>
</tr>
<tr>
<td>AIMS</td>
<td>17</td>
</tr>
<tr>
<td>MATERIAL AND METHODS</td>
<td>18</td>
</tr>
<tr>
<td>RESULTS</td>
<td>22</td>
</tr>
<tr>
<td>Prevalence and incidence (I)</td>
<td>23</td>
</tr>
<tr>
<td>Inheritance patterns (II)</td>
<td>24</td>
</tr>
<tr>
<td>Clinical manifestations (III)</td>
<td>25</td>
</tr>
<tr>
<td>Microradiography (IV)</td>
<td>27</td>
</tr>
<tr>
<td>Scanning electron microscopy and microradiography (V)</td>
<td>28</td>
</tr>
<tr>
<td>DISCUSSION</td>
<td>31</td>
</tr>
<tr>
<td>SUMMARY AND CONCLUSIONS</td>
<td>38</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>40</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>41</td>
</tr>
<tr>
<td>APPENDIX: Papers I-V</td>
<td>47</td>
</tr>
</tbody>
</table>
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This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


Amelogenesis imperfecta (AI) is a genetically determined enamel defect. In one of the first published reports AI was referred to as "hereditary brown teeth" (Spokes 1890). Later, Finn (1938) named the condition "brown hypoplasia of the enamel" and was the first to make a clear distinction between hereditary defects confined to enamel, i.e. AI, and those confined to dentin. The present designation, AI, was coined by Weinmann et al (1945).

AI may occur either as a trait confined to the enamel or as a symptom in generalized diseases and syndromes (Pindborg 1970, 1982; Witkop 1989).

By definition, either all primary and permanent teeth are affected in AI or only groups of teeth in either dentition. Witkop (1958) described a form which could affect only the primary dentition, and cases have been reported where the primary teeth were affected either not at all or less than the permanent (Toller 1959, Laird 1968).

Even when confined to enamel, a few cases have been reported of AI in combination with other ectodermal disturbances (Tebo 1950, Kerebel 1960, Bergman et al 1964). AI has also been associated with delayed eruption and/or impaction of teeth, often undergoing resorption (Weinmann et al 1945, Chaudhry et al 1959, Toller 1959, Frank & Bolender 1962, Laird 1968, Lehmann 1979, Fritz 1981, Nakata et al 1985, Sewerin & Saitetz 1987, Ooya et al 1988). This finding has been attributed to premature degeneration of the ameloblast and the attendant inability to promote normal eruption (Weinmann et al 1945). Histologic examinations (Nakata et al 1985, Ooya et al 1988) of gingival tissue excised in areas of unerupted teeth have shown bodies of calcification similar to the "enameloid conglomerates" described by Weinmann et al (1945) and associated with disturbances in tooth development (Witkop & Sauk 1976).

Even if AI is an ectodermal disorder, cases including mesodermally derived defects have been described. Pulpal calcifications have been observed in connection with AI (Frank & Bolender 1962, Prince & Lilly 1968, Rosenberg Gertzman et al 1979, Sundell 1986, Sewerin & Saietz 1987, Ooya et al 1988) and have been interpreted as a defence reaction of the pulp to the defective enamel (Rosenberg Gertzman et al 1979, Sundell 1986). As they have been found both in impacted and in erupted teeth, Ooya et al (1988) related them to pulpal injury caused by disturbances in blood supply. Dentinal defects have also been observed (Cameron & Bradford 1957, Winter et al 1969). Winter et al (1969) hypothesized that they might result from interactions between developing ectoderm and mesenchyme. Since the dentinal defects reported were minor, they may have been coincidental (Aldred & Crawford 1988).

AI has been associated both with a low prevalence of caries (Gustafson et al 1947, Shear 1954, Toller 1959, Laird 1968, Witkop et al 1973, Sundell 1986), and with a high (Weinmann et al 1945, Winter et al 1969, Lehmann 1979). Suggested explanations for the low prevalence are flattened fissures due to rapid attrition and lack of approximal contacts (Winter & Brook 1975), while no explanation has been offered for the high caries prevalence. Cases have been reported with heavy calculus formation (Shear 1954, Witkop et al 1973, Giansanti 1973, Alexander 1984) as well as with gingivitis (Malone & Bazola 1966, Prince & Lilly 1968, Rosenberg Gertzman et al 1979, Alexander 1984, Sundell 1986, Sewerin & Saietz 1987, Walls 1987). Both findings seem to be associated with the more severe forms of AI and have therefore been attributed to local etiologic factors (Shear 1954, Giansanti 1973, Sundell 1986, Sewerin & Saietz 1987). Cases with moderate gingival hyperplasia have been reported by Storie & Cheatham (1970) and Ooya et al (1988).

Previous studies have shown that AI is rare, with prevalences of 1:14 000-16 000, i.e. 0.06:1 000, in a study of 4-12-yr-olds in the state of Michigan, USA (Witkop 1958, 1965) and 1:8 000, i.e. 0.1:1 000, in children aged 6-18 yrs in Tel Aviv and Jerusalem, Israel (Chosack et al 1979). In the western part of Sweden the prevalence was about 1:4 000, i.e. 0.2:1 000, among 3-19-yr-olds and thus higher than in the studies mentioned above (Sundell & Koch 1985). In the county of Västerbotten, northern Sweden, the relatively large number of AI cases seen clinically indicates a high prevalence of AI in the region.

Reports on AI have described a condition characterized by the variety of clinical manifestations and by genetic heterogeneity. The first attempt to classify the clinical manifestations was made by Weinmann et al (1945), who defined two main groups of AI, the hypoplastic type and the hypocalcification type. In the former, the enamel of all teeth, primary as well as permanent, presented a quantitative defect; the enamel was fairly well matured and therefore
hard and glossy. In the hypocalcification type, on the other hand, the enamel defect was qualitative; the enamel was soft, soon lost upon mechanical stress, and showed a reduced contrast to the dentin in radiographs. This subdivision was based on genetic, clinical, radiographic and histologic data and supported the current theory of amelogenesis, which regarded the formation of an organic matrix and its subsequent mineralization as distinctly separate stages (Diamond & Weinmann 1940). Later studies have shown amelogenesis to be a continuous process, in which the two stages overlap (Angmar-Månsson 1971). Although based on false assumptions, the subdivision suggested by Weinmann et al (1945) is still used, presumably for its clinical convenience and because none of the subsequent classifications correctly reflects all aspects of AI. A third main type, the hypomaturation form of AI, characterized by enamel of normal thickness but soft and with approximately the same radiodensity as dentin, was defined by Witkop (1957). The three main types have been differentiated in turn and up to 13 subgroups have been described, based upon differences in genetic pattern, and on differences in clinical and histologic appearance.

Of the various classification systems, those by Darling (1956) and Sundell & Koch (1985) were based on the clinical manifestations of AI found in Great Britain and western Sweden, respectively. Shields (1983) used a statistical model to analyse and classify AI. Schulze (1956) examined nine large families from a district between Göttingen and Hannover in Germany and connected clinical and histologic manifestations to genetic patterns. This was also done in the classification initiated by Witkop (1957) and later updated and modified (Witkop & Rao 1971, Winter & Brook 1975, Witkop & Sauk 1976, Chosack et al 1979, Melnick et al 1982).

The genetically determined nature of AI was established by Spokes (1890), who presented a genealogical tree and included "hereditary" in his designation of AI. In the earliest
reports the inheritance pattern was considered to be autosomal dominant. Haldane (1937), using pedigree analysis, was the first to suggest an X-linked inheritance pattern. This was established in six of the nine families examined by Schulze (1956), who also observed a sex difference in clinical manifestation, men being more seriously affected. This could be explained by the Lyon hypothesis (Lyon 1961) that women are mosaics with regard to genes on the X-chromosome. Early in embryonic life one of the two X-chromosomes in each somatic cell of the woman is inactivated and appears as a sex chromatin body. Either of the X-chromosomes can be inactivated, but the outcome applies to all subsequent descendants of that cell. Thus, in women with X-linked AI, some of the ameloblasts are controlled by the X-chromosome carrying the abnormal gene, others by the chromosome with the normal gene. Clinically, this is reflected in women as alternating areas of normal and defective enamel; in men with this inheritance pattern for AI, only defective enamel is formed. In the light of findings from a few more extensive studies (Schulze 1956, Witkop 1957, Chosack et al 1979, Sundell & Valentin 1986) and many case reports (for review see Witkop & Sauk 1976), the inheritance patterns autosomal dominant (AD), autosomal recessive (AR), X-linked dominant (XD) and X-linked recessive (XR) have been suggested for different forms of AI.

Histologic studies of AI are relatively few. Weinmann et al (1945) examined ground and decalcified sections of primary and permanent teeth and proposed a clear distinction between hereditary enamel hypoplasia and hereditary enamel hypocalcification, even though the results indicated that the enamel matrix was not fully mature also in teeth characterized as hypoplastic. In later studies, hypoplastic and hypomineralization defects have been found in the same tooth, regardless of the predominant clinical manifestation (Darling 1956, Hals 1958, Bergman et al 1964, Erpenstein & Wannenmacher 1968, Sauk et al 1972a, Sauk et al 1972b, Kerebel & Daculsi 1977, Kerebel & Dubois 1982, Wright 1985, Ooya et al 1988).
Diagnostic accuracy could be improved with a mode of examination that yields more detailed knowledge of the surface morphology. The replica technique for the examination of tooth surfaces under the scanning electron microscope provides such detailed information (Lambrechts et al 1981).

Many of the subgroups identified in AI are based on observations of single cases from families with relatively few examined members and with anamnestic data used as the basis for deducing heredity. This might explain the ever-increasing variety of reported forms of AI. Few studies have covered a large number of families, with clinical data on many members and teeth available for histologic examination. Such a family study would provide more accurate information on the genetic pattern and clinical manifestation of AI and help to verify or reject the earlier picture of genetic and clinical heterogeneity.
The aims of this study were:

. to establish the prevalence and incidence of AI in the county of Västerbotten, northern Sweden
. to study the inheritance patterns for AI
. to describe the clinical manifestations of AI
. to study the distribution of the inorganic component in the enamel of AI teeth
. to study the surface morphology and other morphological details of the enamel in AI teeth
. to study the correlation between mode of inheritance and manifestation of AI.
The study population in paper I consisted of all 3-19-yr-olds (n=56,663) born 1963-79, receiving annual dental care in the Public Dental Health Service in the county of Västerbotten, northern Sweden. Virtually all 3-19-yr-olds in the county were included. The study was performed from September 1, 1982 to June 30, 1983. All patients with the tentative diagnosis of AI, identified at the annual examination, were reported to the author, as were the children born in the county of Västerbotten and registered as AI cases at specialist clinics in the rest of Sweden. In Sweden the county of birth is evident from the personal identity number.

AI was defined as a clinically detected, general defect of the enamel, not attributable to disease or toxic influence in a particular period. All primary and permanent teeth should be affected or only groups of teeth in either dentition (Witkop & Sauk 1976).

The AI cases were divided into hypoplastic, hypomaturational and hypomineralization forms on the basis of the criteria of Witkop & Sauk (1976), the term "hypomineralization" being used instead of "hypocalcification".

The examinations were performed under optimal clinical conditions and included a thorough registration of enamel defects on all teeth, posterior bitewing radiographs, colour photographs and registration of ectodermal disturbances of hair, nails and skin.

A history was taken of the mother's health during pregnancy and delivery, including medication and complications, as well as of the child's health in the neonatal and preschool periods. Duration of breastfeeding, weaning and associated problems were registered, as well as consumption of fluoride tablets in the preschool period. The history included questions about trauma to primary teeth.
Data on the fluoride content of the drinking water during neonatal and preschool life were available, together with earlier dental records, from the Public Dental Health Service. Medical records from the maternity ward and pediatric clinics were available for children with the AI diagnosis.

In paper II the study material comprised the 79 children and adolescents with AI diagnosed in the prevalence study, three of whom declined to participate. Another two children with AI, not reported in the prevalence study, were included, giving a basic material of 78 children and adolescents (index cases) from 51 families. First- and second-degree relatives of the index cases and first cousins and their children were personally invited to participate in the study. More distant relatives were also included if they were reported to have enamel defects resembling AI. Questions were asked about the dental status of deceased relatives, and information considered to be reliable was registered. The total number of individuals in the examined families was 1127 (II, Table II). In all, 917 individuals (432 women, 485 men) were clinically examined, 686 by the author and 231, living in other parts of Sweden, by their own dentists. Anamnestic data were used in 32 individuals (19 women, 13 men), of whom 21 had full dentures. Due to low age, full dentures or extensive prosthetic reconstructions, 143 individuals (81 women, 62 men) were not examined and another 35 (17 women, 18 men) declined to participate in the study. Dental records, radiographs and, in a few cases, colour slides were available for the patients examined in other parts of Sweden.

Hypomaturation and hypomineralization were treated as one form in this study, since clinical examination had revealed that both severe and less severe hypomineralization could occur in the same family.

Pedigrees were constructed, including first-degree relatives and grandparents. Other relatives were included only when this clarified the mode of inheritance.
Paper III describes the clinical variants of AI in 165 of the individuals from 51 families previously diagnosed (I, II). Detailed clinical information was not available for 20 individuals, who were therefore omitted. The morphologic criteria suggested by Witkop & Sauk (1976) were used as a basis for the clinical classification (Table 1). An additional group, "vertically ridged teeth", was used for the manifestation in women in families with an XR inheritance pattern.

Table 1. Classification of amelogenesis imperfecta according to Witkop & Sauk (1976).

<table>
<thead>
<tr>
<th>HYPOPLASTIC AI</th>
</tr>
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<tbody>
<tr>
<td>1. Autosomal dominant pitted</td>
</tr>
<tr>
<td>2. Autosomal dominant local</td>
</tr>
<tr>
<td>3. Autosomal dominant smooth</td>
</tr>
<tr>
<td>4. Autosomal dominant rough</td>
</tr>
<tr>
<td>5. Autosomal recessive rough</td>
</tr>
<tr>
<td>6. X-linked (dominant) smooth</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HYPOCALCIFIED AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Autosomal dominant</td>
</tr>
<tr>
<td>2. Autosomal recessive</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HYPOMATURATION AI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Autosomal dominant hypomutation - hypoplastic with taurodontism</td>
</tr>
<tr>
<td>2. X-linked (recessive)</td>
</tr>
<tr>
<td>3. Autosomal recessive pigmented</td>
</tr>
<tr>
<td>4. Snow-capped teeth</td>
</tr>
</tbody>
</table>

In paper IV, 22 exfoliated primary and four extracted permanent teeth were examined by microradiography. The teeth came from 22 children (original cases) from 19 families, and represented seven of the eight clinical variants of AI described in paper III. Teeth from two control groups were also examined. One group consisted of 14 primary teeth from non-affected relatives (sisters, brothers or cousins) of the original cases, the other of 15 primary and two permanent
The teeth (IV, V) were embedded in methylmetacrylate and sagittal ground sections, 60-80 μm, were prepared, using Leitz's saw microtome No. 1600 (Norén 1983). Contact micro-radiographs were obtained using Kodak High Resolution Plates. The X-ray source was a Siemens AGW 3 unit, fitted with a copper anode with an insert of tungsten and a beryllium window. The X-rays, emitted at 20 kV and 20 mA, were filtered through a 20 μm Ni filter. The distance between the photographic plate and the focus was 23 cm.

In each microradiograph (IV) the thickness of the enamel was measured using an ocular scale. Duplicate measurements after one week showed a reproducibility of 85%.

In paper V, 12 of the primary teeth with AI and five non-affected control teeth examined microradiographically (IV) were also examined by scanning electron microscopy (SEM). The teeth represented five of the eight clinical variants described in paper III. The buccal surfaces of the teeth were treated with a 5% sodium hypochlorite (NAOCL) solution, followed by spraying with water and drying with compressed air. Impressions and replicas were prepared according to a method described by van Dijken & Hörstedt (1987). Two teeth were divided bucco-lingually with a scalpel and hammer. The positive castings and the two tooth halves were mounted on metal stubs and covered with gold by standard evaporation technique. They were then studied in a Cambridge Stereoscan S4 electron microscope. Evaluation of the morphology of the surfaces was based on photomicrographs at 24- to 2400-fold magnifications.

Statistical methods

To confirm the inheritance patterns (II) a statistical segregation analysis was carried out, using the a priori method and assuming complete ascertainment (Emery 1976). Chi-square test was used to assess the difference between the observed
and the expected number of affected individuals, as well as the differences in the distribution of affected and non-affected women and men within and between different inheritance patterns. In paper IV the Wilcoxon two-sample rank-sum test was used to assess differences in enamel thickness between groups with at least three teeth. In the analyses \( p < 0.05 \) was considered a statistically significant difference.
RESULTS

Prevalence and incidence (I)

Out of a total of 298 children with suspected AI, reported from 28 of the 32 dental clinics in the county, 296 were clinically examined. AI was diagnosed in 79 children (41 girls and 38 boys). The prevalence in the study population was 1:717, i.e. 1.4:1 000. Of the 79 children, 70 were born in the county. One child with AI, born in the county of Västerbotten, was reported from a specialist clinic in another county. The total number of 3-19-yr-olds born in the county 1963-79 was 55 626, and the mean yearly incidence of AI in the period was 1:783, i.e. 1.3:1 000. The year-by-year variation in incidence was considerable, ranging from 0.6 to 2.6 per 1 000, with the highest figure in 1969. When the three children with AI, born 1963-79 and diagnosed in the genetic study (II), were also included in the calculations, neither the prevalence nor the mean yearly incidence changed.

Of the children with AI, 58 (1:1 000) had the hypoplastic form, 16 (0.3:1 000) the hypomaturation form and 5 (0.1:1 000) the hypomineralization form.

Fourteen children had atopic disorders, and one child had no nails on the index fingers of both hands. One child had suffered from complications due to preterm delivery.

A total of 217 children did not meet the criteria for AI. Local enamel defects that could be explained by trauma or by periradicular osteitic processes of the primary predecessors were found in 33 cases. In 21 cases the cause of the local defect was unknown. Generalized defects due to a high intake of fluorides were found in 33 children, and in 35 children the defects consisted of minor opacities of unknown etiology. In the remaining 95 children the enamel defects were restricted to groups of teeth and could be related to a specific period of enamel formation.
Inheritance patterns (II)

The results of the genetic study are shown in pedigrees 1-51 (II, Figs 1-11). In families 1-33 the inheritance pattern was compatible with AD inheritance, though this assumes a reduced penetrance of AI in families 2, 23 and 31 (II, Figs 2, 7, 9). Moreover, in families 32 and 33 (II, Fig 9) the AD inheritance pattern was only highly probable, since a definite diagnosis could not be made in the parental generation on account of individuals with full dentures and no knowledge of the status of their natural teeth. XD inheritance was a possible alternative in family 22 (II, Fig 7). The inheritance pattern in families 34-39 was compatible with AR, and in families 40 and 41 with XR inheritance. The ten probands (5 girls, 5 boys) in families 42-51 were sporadic cases, i.e. only one case was found in each family.

Examinations in the families of the 78 index cases (39 girls, 39 boys) revealed 107 new cases of AI: 89 (50 women, 39 men) in the families with AD inheritance, 3 (1 woman, 2 men) in those with AR inheritance and 15 (11 women, 4 men) in the families with XR inheritance. One of the new cases in the families with AD inheritance was born in the period 1963-79. The hypoplastic form of AI was observed in 133 (72%) cases and the hypomineralization form in 52 (28%). AD inheritance was seen in 142 (77%) cases, AR inheritance in 13 (7%) and XR inheritance in 20 (11%) cases. Ten (5%) sporadic cases were found.

Statistical segregation analysis of families 1-33 did not indicate a statistically significant (p >0.05) deviation from the expected number of normal and affected offspring, assuming AD inheritance. In families 34-39 close agreement was found between the observed (n=13) and the expected (n=12.3) number of sibs assuming AR inheritance. In families 1-33 with an AD inheritance pattern there was a statistically significant (p <0.05) excess of women among the affected individuals compared with the non-affected. No such difference was observed in families 34-41. Between cases with AD and AR inheritance there was a statistically significant
female excess in the dominant group. Among the patients with AR and XR inheritance there was a statistically significant (p < 0.05) excess of men in the AR families and of women in the XR.

Clinical manifestations (III)

Eight clinical variants of AI were found (Table 2). The predominant clinical manifestation was "pitted hypoplastic AI". In 33 families the same clinical variant was seen in all affected members, in eight families the affected members were assigned to different clinical variants (Table 3), and in the remaining ten families only one individual was affected.

Table 2. Individuals with AI subgrouped according to clinical manifestation and related to inheritance pattern. AD=autosomal dominant, AR=autosomal recessive, XR=X-linked recessive.

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>No. of individuals</th>
<th>Inheritance pattern</th>
<th>Sporadic cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pitted hypoplastic</td>
<td>71</td>
<td>68</td>
<td>2</td>
</tr>
<tr>
<td>Local hypoplastic</td>
<td>28</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Smooth hypoplastic</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Rough hypoplastic</td>
<td>15</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Snow-capped teeth</td>
<td>8</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>Hypommaturation</td>
<td>22</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Hypomineralization</td>
<td>9</td>
<td>7</td>
<td>-</td>
</tr>
<tr>
<td>Vertically ridged teeth</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>165</td>
<td>125</td>
<td>13</td>
</tr>
<tr>
<td>Inheritance pattern</td>
<td>Family no.</td>
<td>AD</td>
<td>AD</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>Local hypopli phenotypic teeth</td>
<td>2</td>
<td>4</td>
<td>20</td>
</tr>
<tr>
<td>Rough hypopli teeth</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Smooth hypopli teeth</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Local hypopli phenotypic hypopli teeth</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Smooth hypopli teeth</td>
<td>1</td>
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<tr>
<td>Vertically ridged teeth</td>
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Table 3. Families with different clinical variants of Al related to inheritance pattern.
Of the eight families with different clinical variants of AI, both hypoplastic and hypomineralization forms were seen in families 2, 20 and 28 (Table 3). In families 16, 22 and 30 all affected members could be fitted into the same main form of AI, i.e. hypoplastic in families 16 and 22 and hypomineralization in family 30 (Table 3). In family 22 there was generalized and severe hypoplasia of the enamel in the one boy with "smooth hypoplastic AI". The five affected women in this family presented a variety of manifestations, with zones of seemingly normal enamel interspersed with the hypoplastic areas. "Pitted hypoplastic AI" was seen in three and "rough hypoplastic AI" in two women. A vertical arrangement of the defects could be discerned. In families 40 and 41 there was also a difference in manifestation between women and men (Table 3). In the women with "vertically ridged teeth" the enamel showed alternating bands of normal and defective enamel; in the men the enamel was generally affected, with "smooth hypoplastic AI" in family 40 and "hypomaturation AI" in family 41.

Primary and permanent teeth were similarly affected, except in "pitted hypoplastic AI", "local hypoplastic AI", and "snow-capped teeth", where the primary teeth were less severely affected. In one of the cases with "snow-capped teeth" only the permanent teeth were affected.

Microradiography (IV)
The microradiographic and the clinical observations were in good agreement in almost all the 26 examined AI teeth. Of the three teeth with "local hypoplastic AI", however, only one showed hypoplastic defects, and in the tooth from the clinical group "snow-capped teeth" only small subsurface areas of hypomineralization were found in the enamel.

The findings in the one tooth with "smooth hypoplastic AI" from family 40 (Table 3) did not correspond with the clinical appearance. In this tooth, the microradiographs showed demineralized channels of varying size, extending in a direction perpendicular to and in some cases penetrating the
enamel surface. The inner zone of the enamel exhibited hypomineralization, involving about 2/5 of the total thickness but with a seemingly normal mineral content in the surface area and in a thin zone adjacent to the dentinoenamel junction. The extent of the zone of hypomineralization corresponded to that seen in teeth from the clinical groups "hypomaturation AI" and "hypomineralization AI". Enamel hypoplasia was found in one cusp, and the enamel was thinner than in the control teeth.

Apart from the clinical variants "local hypoplastic AI" and "snow-capped teeth", the microradiographs of most teeth from the other five clinical variants showed both hypoplasia and hypomineralization of the enamel, regardless of the predominant clinical manifestation.

The teeth from the clinical variants "hypomaturation AI" and "hypomineralization AI" did not differ in their microradiographic appearance.

The enamel was thinner in teeth from the clinical variants "local hypoplastic AI" (p <0.05) and "rough hypoplastic AI" (p <0.01) than in the control teeth. The enamel in both primary and permanent teeth from one girl with the clinical variant "hypomineralization AI" was considerably thicker than in the control teeth.

A limited subsurface area of hypomineralization was found in one control tooth. The remaining control teeth showed no hypoplasias or areas of hypomineralization.

Scanning electron microscopy and microradiography (V)

In SEM, the enamel hypoplasias observed clinically and microradiographically were manifested as smoothly delineated depressions, varying in extent and frequency between the clinical variants. In "rough hypoplastic AI" the enamel was generally affected with irregular hypoplasias, overhung by relatively smooth enamel (V, Fig 2). Some of the hypoplasias were similar to those seen in "pitted hypoplastic AI". In
this variant rounded depressions with a diameter of 100-200 μm were distributed over the surface, interspersed with areas of seemingly normal enamel (V, Fig 1). In "hypominenralization AI" corresponding depressions were seen but with a sporadic occurrence (V, Fig 4). From the clinical variant "rough hypoplastic AI" a tooth-half was also examined and showed an organized crystalline pattern, except in limited areas in the inner part of the enamel, corresponding to hypomineralized areas in the microradiographs.

SEM showed that the enamel defects in the incisal area of teeth from the clinical groups "hypomaturation AI" and "hypomineralization AI" were sharply delineated (V, Fig 4). A honeycomb pattern was seen in the bottom of most defects. Microradiographically, in teeth from both variants areas of hypomineralization were found connected with the defects.

In SEM, the teeth from the clinical variant "smooth hypoplastic AI" with an XR inheritance pattern exhibited an enamel surface with rounded openings, 5-20 μm in diameter (V, Fig 3). Orifices of empty channels with the same diameter were seen near the surface of the enamel in the examined tooth-half. The manifestation corresponded with the demineralized channels seen microradiographically.

Correlations between inheritance pattern and clinical, microradiographic and SEM manifestations of AI (II-V)

AD inheritance was seen in 89% of all cases with the hypoplastic form and in 44% of all cases with the hypomineralization form of AI (II). If the sporadic cases were considered to be either new mutations with AD inheritance or members of families with reduced penetrance of the gene for AI, the corresponding figures were 95% and 50%, respectively.

Of the eight clinical variants of AI described, "vertically ridged teeth" was seen only in women in families with an X-linked inheritance pattern and "local hypoplastic AI" only as an AD trait (III). "Smooth hypoplastic AI" was seen in
one boy whose inheritance pattern was either AD or XD and in one boy with AI as an XR trait (Table 3).

The other five clinical variants were found in the AD as well as in the AR group and/or among the sporadic cases, and none of them was found exclusively in families with AI as an AR trait (III).

Both microradiography and SEM gave findings that were similar in teeth from children with the same clinical variant but different inheritance patterns, the sporadic cases included (IV, V). The findings in the one tooth with an XR inheritance pattern were not seen in any other tooth in the present study. The only control tooth with areas of hypomineralization came from a sister of one of the sporadic cases.
DISCUSSION

The prevalence figure for AI in the county of Västerbotten is the highest reported to date (I). It is based on the number of 3-19-yr-olds diagnosed from September 1, 1982 to June 30, 1983. Three additional AI cases were diagnosed later than June 30, 1983 (II). In view of the wide variation in the manifestations of AI, it is conceivable that additional, very discrete clinical variants may have been overlooked. On the other hand, as many as 298 tentative cases were reported, indicating that the dentists were actively involved in the selection of suspected cases of AI. Even so, the prevalence figure should be regarded as a minimum.

Due to the limited ability of the ameloblast to react specifically to noxious influences, enamel defects can be found that are similar to those in AI but have a different etiology. Medical records were therefore used to support the historical data. In the one child who had suffered from complications due to preterm delivery, the AI diagnosis was supported by the fact that his father and three brothers had AI (I).

Epidemiologic studies have shown a higher prevalence for other genetically determined diseases in the county of Västerbotten than in the rest of Sweden (Beckman & Bergen-holtz 1975, Gustavson et al 1977, Jagell et al 1981, Forsgren et al 1988, Holmgren et al 1988). One suggested explanation is the founder effect in conjunction with unbroken isolates in the county, where migration and population mixing only began in recent decades (Beckman & Cedergren 1971). In time, increased migration will therefore probably tend to decrease the prevalence of AI in the county. A plot of the mean yearly incidence in 1963-79 showed a downward trend since 1969, but the study period is too short for definite conclusions. The incidence showed a large year-by-year variation, as expected when the annual number of diagnosed cases is low.
The inheritance patterns AD, AR, XD and XR were found for AI in the present study (II), with AD as the predominant mode of inheritance. The decision to allocate families 2, 23 and 31 (II, Figs 2, 7, 9) to the AD group was based on the assumption of reduced penetrance of the AI gene in the parental generation, a view supported by the close relationship between the affected individuals in families 23 and 31. In the six families where AR inheritance was considered to be probable (II), AD inheritance with reduced penetrance of the gene might also be conceivable. The possibility that reduced penetrance obscures the AD inheritance pattern has been discussed earlier (Schulze 1956, Cameron & Bradford 1957, Toller 1959, Alexander 1984) and could also explain the ten sporadic cases of AI. Thus, the only control tooth with areas of hypomineralization as seen by microradiography came from a clinically unaffected sister of a sporadic case (IV). Apart from the possibility of being a normal variation of the subsurface mineral content (Norén 1982), this could represent a subclinical manifestation of AI, i.e. a very mild expression of the gene. A genetic trait might be defined as recessively inherited, if the analytic method is not sufficiently sensitive to diagnose the defect. In the sporadic cases, mutations or illegitimacy are alternative explanations.

To test the hypothesis that AI is solely an AD or an X-linked trait, the correlation between mode of inheritance and clinical variant was studied. Apart from the clinical manifestations found in the families with AI as an X-linked trait and "local hypoplastic AI", which was seen only as an AD trait, similar clinical variants were found in families with AI as an AD and an AR trait and/or among the sporadic cases (III). Thus, contrary to previous reports (Witkop & Sauk 1976), "pitted hypoplastic AI" and "hypomaturation AI" were found both as AD and as AR traits (Table 2). The clinical variant "snow capped teeth" was also seen both as an AD and as an AR trait, as previously stated (Winter & Brook 1975, Witkop & Sauk 1976), but not connected with X-linked inheritance, as reported by Escobar et al (1981). When
similar clinical variants were found in connection with both AD and AR inheritance, no specific difference was observed in their manifestation, i.e. severity (III). The microradiographic findings supported this observation (IV). The clinical variant "rough hypoplastic AI" as an AR trait did not correspond with the serious variant - "enamel agenesis" - described by Witkop & Sauk (1976), but similar cases were found in the AD group. Thus, there is support for the hypothesis that reduced penetrance obscures the inheritance pattern in families with AI as an AR trait.

The clinical variants found in the examined families were mainly the same as those reported earlier (Witkop & Sauk 1976, Chosack et al 1979, Sundell & Koch 1985). In agreement with Chosack et al (1979) and Sundell & Koch (1985), the hypoplastic form of AI was the most common manifestation (I, II).

The excess of women found in the affected families with an AD inheritance pattern for AI is not easily explained. It still applied when family 22, with a possible XD inheritance pattern, was excluded. A similar difference in the sex ratio was observed in families reported by Tebo (1950), and Simm & Goose (1976).

In all but eight of the families the clinical variants were similar in all affected members (III). The variation in clinical manifestation between families with the same inheritance pattern for AI could be explained by the influence of modifying genes or environmental factors, which should differ more between than within families. The primary action of a gene is often many developmental steps away from the observed effect, and the expression of the gene at that locus is modified by the "background" genes of the organism.

The eight families with affected members assigned to different clinical variants (Table 3) illustrate the ability of the same genetic defect to modify its expression. In each of the families 2, 20 and 28 (Table 3) different clinical
variants were found of both the hypoplastic and the hypomineralization forms of AI. Clinically (II,III) and, in family 2, microradiographically (IV), hypoplasias as well as areas of hypomineralization were found in the enamel of most of the affected individuals.

In family 16 (Table 3) "pitted hypoplastic AI" and "rough hypoplastic AI" were seen; in the latter variant all ameloblasts seemed to have been affected, in the former case only a limited number. In the microradiographs, areas of hypomineralization as well as hypoplasias were found in both variants (IV). SEM confirmed that these variants seemed to differ mainly in the extent of the disturbances and that single defects showed a similar morphology (V). Shields (1983) stressed the similarity in clinical appearance between "pitted hypoplastic AI" as an AD trait and the female manifestation of AI as an X-linked trait. In AI as an X-linked trait the programmed cell death could be explained by lyonization (Lyon 1961), while in AI as an AD trait allelic exclusion was suggested. In allelic exclusion, which is a rather unusual phenomenon, when one of the alleles at a locus is active, the production of the other is suppressed (Melnick et al 1982).

In family 30 (Table 3) the clinical variant "hypomineralization AI" was found in two children with primary teeth and "hypomaturation AI" in the permanent teeth of their mother (III). The enamel of primary teeth is normally thinner and has a somewhat lower degree of mineralization than the enamel of permanent teeth (Wilson & Beynon 1989), which probably explains why the degree of hypomineralization varied within this family. Light microscopy has revealed mineralization defects in the intraprismatic zone in "hypomineralization AI" and in the interprismatic zone or in the rod sheath in "hypomaturation AI" (Witkop & Sauk 1976). The mechanisms responsible for maturation of human enamel are poorly understood and a subdivision of AI into hypomineralization and hypomaturation forms has been called "functionally erroneous" (Shields 1983). In cases with AI as an AD and
an AR trait and in the sporadic cases, the microradiographic and SEM findings did not distinguish between these two variants (IV, V).

In the families with an X-linked inheritance pattern for AI, the clinical manifestations differed between women and men (Table 3). In family 22 (III, Fig 3) the XD inheritance pattern was only highly probable, since only two affected men were diagnosed and one of them had had dentures for many years. Only a few genetic disorders exhibit an XD inheritance pattern. According to Witkop (1971), hypoplastic AI was the first XD trait to be described in man. In XD diseases men are affected uniformly and severely, whereas women have varied manifestations due to lyonization (Lyon 1961). In family 22 both pedigree analysis and clinical manifestations supported an XD inheritance pattern (III, Fig 3). Similar families have been described earlier (Schulze 1956, Rushton 1964, Shokeir 1971, Berkman & Singer 1971). For the two families with an XR inheritance pattern (II, Fig 11), the clinical manifestation in affected men was "hypomature AI" in family 41 and "smooth hypoplastic AI" in family 40. Microradiographic and scanning electron microscopic examination of teeth from the boy in the latter family showed a morphology not seen in any other tooth in the present study (IV,V). Similar findings have been reported in teeth with "hypomature AI" and an XR inheritance pattern (Sauk et al 1972c, Mc Larty et al 1973, Witkop & Sauk 1976). The manifestation in this boy should therefore have been assigned to that group. In the affected women the vertical distribution of normal and defective enamel constituted a pattern attributable to the amelogenesis (III). An XR trait usually defies clinical diagnosis in female carriers, but defective enamel formed by the genetically mutant ameloblast is an exception. This could explain why an excess of women was found among the affected members of these families (II), in contrast to the usual sex ratio in this mode of inheritance. Accordingly, two variants of AI connected with X-linked inheritance were found; "smooth hypoplastic AI" as an XD trait and "hypomature AI" as an XR trait.
The teeth available for microradiographic examination were mainly primary teeth (IV). In previous reports the manifestation of AI has been found to be either absent in primary teeth or less prominent than in permanent teeth (Witkop 1958, Toller 1959, Laird 1968). Clinically, the manifestations found in the primary teeth with "pitted hypoplastic AI", "local hypoplastic AI" and "snow-capped teeth" were less pronounced than in the corresponding permanent teeth (III). The microradiographic findings were also mild in the teeth examined from the latter two groups, while no appreciable difference was found between the primary and the permanent teeth with "pitted hypoplastic AI" (IV). The genetic control of amelogenesis should be the same in primary and permanent teeth. The observed differences in the severity of manifestations could be interpreted as exponents of the wide variation in AI manifestations.

Both hypoplasias and areas of hypomineralization were microradiographically found in the enamel of most of the examined teeth (IV). Similar observations have been reported earlier (Darling 1956, Hals 1958, Bergman et al 1964, Erpenstein & Wannenmacher 1968, Sauk et al 1972a and b, Kerebel & Daculsi 1977, Wright 1985, Ooya et al 1988). This finding indicates a generalized disturbance of the amelogenesis in AI. It supports the theory that the variation in clinical manifestation is due to the modifying effect of "background" genes or environmental factors on the genetically mutant ameloblast.

The issue of whether AI is solely an AD or an X-linked trait cannot be settled for certain without DNA-based diagnostics. This technique could also show, whether the six clinical variants connected with autosomal inheritance and the two found in the X-linked families represent genes at different loci, alleles at the same locus or a varying expressivity of the same genetic defect.
Little is known about the genetic control of the amelogenesis. The developing enamel matrix is composed almost entirely of two classes of enamel proteins: amelogenin, which predominates and has been suggested to play an important role in the control of biomineralization, and enamelin (Termine et al 1980). In two of the X-linked families in the present study (II, families 22, 41), analysis of restriction fragment length polymorphisms (Southern 1975), has been made. Preliminary results locate the locus for AI to the end of the short arm of the X-chromosome (Lagerström et al 1989). The final analyses of whether the two families show the same location remain to be done. The chromosomal assignment corresponds to that recently found for amelogenin (Lau et al 1989). Continued research, applying this new knowledge, might clarify the nature of the genetic defect in AI as an X-linked trait. According to Lau et al (1989), defective encoding of enamelin or the proteases associated with the mineral phase of enamel maturation might ultimately explain the autosomal types of AI.
SUMMARY AND CONCLUSIONS

The prevalence of AI in 3-19-yr-olds born 1963-79 in the county of Västerbotten, northern Sweden, was 1.4:1 000 and thus considerably higher than reported elsewhere. The mean yearly incidence was 1.3:1 000.

Pedigree and segregation analyses suggested AD, AR, XD and XR inheritance for AI, with AD as the predominant genetic pattern. Sporadic cases were found in ten families. It is conceivable that AI is solely due to AD and X-linked genes. In families considered as AR and in families with sporadic cases, a reduced penetrance of the gene or new mutations might explain the presence of AI.

Eight clinical variants of AI were identified. In most families the clinical variant was similar in all affected members.

The hypoplastic form of AI was the most common and connected in 89% with an AD inheritance pattern. If the sporadic cases with hypoplasia were considered to be either new mutations with AD inheritance or members of families with reduced penetrance of the gene for AI, the figure for AD inheritance is 95%.

In the families with AI as an X-linked trait the genetic hypothesis was confirmed by the clinical, microradiographic and scanning electron microscopic findings. The manifestation of AI in men differed from that in women due to lyonization.

Both clinically and microradiographically as well as by scanning electron microscopy, similar variants of AI were found in families with AD and AR inheritance and/or among the sporadic cases.

The microradiographs showed hypoplasia as well as hypomineralization in the enamel of most teeth, independent of
predominant clinical manifestation, indicating a generalized disturbance of the amelogenesis in AI.

The variations in clinical and histologic characteristics of AI connected with the same inheritance pattern suggest that the genetic defect, in conjunction with the influence of modifying genes or environmental factors, could explain the multiplicity of clinical expression that characterizes AI.
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REFERENCES


44


