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ORIGINAL ARTICLE

Enamel defects and salivary methylmalonate in methylmalonic acidemia

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INTRODUCTION AND OBJECTIVE: To characterize enamel defects in patients with methylmalonic acidemia (MMA) and cobalamin (cbl) metabolic disorders and to examine salivary methylmalonate levels in MMA.

SUBJECTS AND METHODS: Teeth from patients (n = 32) were evaluated for enamel defects and compared with age- and gender-matched controls (n = 55). Complementation class (*mut*, *cblA*, *cblB* and *cblC*) and serum methylmalonate levels were examined. Primary teeth from two patients were examined by light and scanning electron microscopy and salivary methylmalonate levels from two patients were analyzed.

RESULTS: Enamel defects were significantly more prevalent per tooth in the affected group than the control group, across complementation types (P < 0.0001). The *mut* MMA subgroup had a significantly higher prevalence per individual of severe enamel defects than controls (P = 0.021), and those with enamel defects exhibited higher serum methylmalonate levels than those without (P = 0.017). Salivary methylmalonate levels were extremely elevated and were significantly higher than controls (P = 0.002). Primary teeth were free of enamel defects except for two *cblC* patients who exhibited severe enamel hypoplasia. One primary tooth from a *cblC* patient manifested markedly altered crystal microstructure.

CONCLUSION: Enamel anomalies represent a phenotypic manifestation of MMA and cbl metabolic disorders. These findings suggest an association between enamel developmental pathology and disordered metabolism. Oral Diseases (2009) 15, 196–205

Keywords: enamel; methylmalonic acidemia; enamel hypoplasia; enamel microstructure; salivary biomarker

Introduction

The hereditary methylmalonic acidemias (MMAs) comprise a group of autosomal recessive enzymopathies characterized by massive accumulation of methylmalonate and propionate-derived metabolites in the bodily fluids (Fenton et al, 2001). The collective incidence of isolated MMA in the population is unknown but likely lies between 1 in 50 000-100 000 births (Chase et al, 2001). Deficient activity of the methylmalonyl-coenzyme A mutase (MUT) enzyme accounts for approximately half of all cases (Horster et al, 2007) and over one hundred disease causing mutations have been identified at the MUT locus (Acquaviva et al, 2005; Worgan et al, 2006). Impaired intracellular metabolism of cobalamin (cbl) define another group of hereditary defects designated by the cellular complementation status (cblA-F)(Fenton and Rosenblatt, 2001). These disorders can feature isolated MMA (cblA, cblB, cblD variant 2) caused by a functional impairment of the methylmalonyl-CoA mutase reaction, combined MMA/aciduriahyperhomocysteinemia/homocystinuria (cblC, cblD and *cblF*) or isolated hyperhomocystinemia (*cblD variant 1*, *cblE* and *cblG*) (Venditti, 2005; Adams and Venditti, 2008). Figure 1 depicts the general steps of cbl metabolism and the location of the main enzyme defects in this pathway (Chandler and Venditti, 2005).

The elevated metabolites universally present in individuals with isolated or combined MMA derive from endogenous catabolism of certain essential amino acids (isoleucine, methionine, threonine and valine), odd chain fatty acids, and cholesterol, as well as gut bacterial derived propionate (Leonard, 1997). These compounds are metabolized to methylmalonyl-CoA, and then into succinyl-CoA, a Krebs cycle intermediate, via the methylmalonyl-CoA mutase reaction. When this reaction is impaired, methylmalonic acid as well as propionyl-CoA derived metabolites, such as 2- methylcitrate and 3-hydroxypropionate, begin to accumulate in the body with detrimental effects (Ando *et al*, 1972a,b; Cheema-Dhadli *et al*, 1975).

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Received 16 July 2008; revised 19 November 2008; accepted 19 November 2008



Figure 1 Pathway of cellular processing of cobalamin (OH-cbl) and location of main enzyme defects in the pathway leading to methylmalonic acidemias and cobalamin metabolic disorders

Collectively, patients affected with these conditions display clinical and biochemical heterogeneity. Those with MUT deficiency can exhibit failure-to-thrive, strokes, kidney disease, pancreatitis, and early mortality (Matsui et al, 1983; vanderMeer et al, 1994; Baumgater and Viardot, 1995; Nicolaides et al, 1998; deBaulny et al, 2005; Horster et al, 2007). Extreme elevations of blood and urinary methylmalonic acid are typical and can rise to the millimolar range (compared with normal nanomolar range) in the setting of crisis or renal insufficiency (Walter et al, 1989). The mainstay of treatment includes protein restriction to reduce the metabolite load as well as cofactor and carnitine supplementation (deBaulny et al, 2005). Despite conventional medical management, mortality has remained substantial and some severely affected patients have been treated using liver and/or combined liver-kidney transplantation (Leonard et al, 2004). Disordered intracellular metabolism of cbl produces another group of disorders named after the corresponding cellular complementation class - cobalamin C (*cblC*), D and F. These conditions are also heterogenous (Rosenblatt et al, 1997; Fenton and Rosenblatt, 2001), clinically and biochemically, and present a spectrum of clinical phenotypes, ranging from intrauterine growth retardation (Andersson et al, 1999) to adult onset neurodegeneration (Powers et al, 2001). The dental phenotypes in all these disorders have remained uninvestigated, even though bone demineralization, osteopenia, or osteoporosis, is often observed (Baumgater and Viardot, 1995). The abnormalities of hard tissue mineralization led us to begin observing the dental manifestations of these diseases.

Enamel is the most mineralized tissue in the body, with approximately 97% by weight mineral content, and is made up of small calcium phosphate crystallites oriented in a specific pattern to form enamel prisms (Robinson et al, 2003). Enamel formation is a complex and highly regulated process involving the secretion and processing of a protein extracellular matrix and the regulation of ion flow and crystal growth (Simmer and Fincham, 1995; Fincham et al, 1999). Initial calcification of a primary human anterior tooth begins at 14-17 weeks in utero and enamel formation is completed at 1-9 months after birth. Permanent human anterior teeth begin calcification at 3–12 months and complete enamel formation at 4–7 years of age (Ash and Nelson, 2003). Once developed, enamel remains fairly inert, biologically non-responsive to internal stimuli, and functions without further remodeling (Hu et al, 2007). The presence of enamel defects, therefore, may be highly dependent on conditions occurring during the critical time of enamel formation and mineralization.

Enamel defects have been associated with pediatric systemic illnesses during the critical period of enamel formation (Pindborg, 1982; Bhat and Nelson, 1989; Russell *et al*, 1996; Lucas and Roberts, 2005; Kusiak *et al*, 2007). Enamel anomalies have also been described in patients with specific metabolic disorders, such as phenylketonuria, an amino acid disorder (Myers *et al*, 1968; Lucas *et al*, 2001), and in those with mucopoly-saccharidoses, a group of lysosomal storage diseases (Rølling *et al*, 1999; Barker and Welbury, 2000; Guven *et al*, 2008). However, the consequences of the effects of inborn errors of organic acid metabolism on enamel development have not been examined.

The objective of this study was to characterize enamel defects in a group of patients with MMA and cbl metabolic disorders. These findings were then investigated for association with complementation status and serum methylmalonate concentrations. Microstructural analysis of two deciduous teeth and salivary methylmalonate level analysis from two *mut* deficient patients was also performed.

Materials and methods

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Thirty-two individuals with MMA or cbl metabolic disorders, designated the affected group, were evaluated as part of the National Institutes of Health study 04-HG-0127 'Clinical and Basic Investigations of Methylmalonic Acidemia and Related Disorders' after informed consent was obtained. Patient studies were conducted in compliance with the Helsinki Declaration and were approved by the Institutional Review Boards of the National Human Genome Research Institute and the National Institute of Dental and Craniofacial Research. Fifty-five age- and gender-matched individuals enrolled in other NIH protocols, but not diagnosed with an inborn error of metabolism and transport, received dental evaluation and comprised the control group. Exclusion criteria for the control group included hereditary diseases that have enamel or dentin defects associated with its phenotype, such as osteogenesis imperfecta, amelogenesis imperfecta, Turner syndrome, cystic fibrosis, hypoparathyroidism and rickets.

All patients with MMA and cbl metabolic defects had diagnoses confirmed by cellular biochemical studies and/or mutation analysis (Lerner-Ellis et al, 2005; Worgan et al, 2006; Chandler et al, 2007). Affected individuals were divided into two groups based on whether they possessed permanent (n = 22 subjects) or primary (n = 10 subjects) maxillary anterior incisors. Table 1 shows the affected groups and the control group characteristics. Serum or salivary methymalonic acid was determined for the affected group by either electrospray tandem mass spectrometry (Magera et al, 2000) or by gas chromatography-mass spectrometry with stable isotopic internal calibration (Allen et al. 1993). The serum methylmalonate levels were determined near the time of dental evaluation. Ten study subjects had received combined liver-kidney or kidney transplants.

All subjects (affected and control groups) received a comprehensive dental evaluation, including an exami-

nation for developmental anomalies of tooth number and dental structural defects. Radiographic evaluation included a panograph and intraoral radiographs. Intraoral photographs of the anterior teeth were taken on all subjects. Facial aspects of index maxillary anterior index teeth (no. 7, 8, 9, 10) were reviewed by the same dentist and assigned enamel defect scores using the Modified Developmental Defects of Enamel (DDE) Index (Clarkson, 1989). In this Index, both a coding score (0-9) indicating type of enamel defect (with a higher number indicating a more severe type of defect) and an extent (0-3) of enamel defect (indicating the area of tooth surface affected) is determined. A score of 0 indicates normal enamel, a score of 1-2 indicates demarcated opacities, a score of 3-6 indicates diffuse opacities or lining, and a score of 7-9 indicates hypoplasia in the form of pitting or missing enamel. A second dentist, blinded to the study subject's diagnosis, reviewed the photographs and also assigned DDE scores to the index teeth. The average of the two scorers was used for analysis.

The prevalence of the types of enamel defect between groups was then compared to determine differences of DDE types for index teeth. The prevalence of an individual having at least one index tooth with severe enamel defects was then compared across groups, with a severe enamel defect defined as a DDE score of ≥ 3 . The difference in extents between groups was also compared.

Microstructural analysis was performed on exfoliated deciduous teeth from two affected patients, a 10-yearold Caucasian female with *mut* MMA (tooth L) and a 9-year-old Caucasian female diagnosed with *cblC* (tooth N). Each tooth was sectioned and analyzed with light microscopy (LM) and with scanning electron microscopy (SEM). Thin sections approximately 100 microns thick were cut with a diamond disc for LM examination. Using SEM, fractured enamel samples were examined after removal of organic material using NaOCl as described previously (Wright *et al*, 1993).

Table 1	Summary	statistics	comparing	affected	group	with	control	group
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	Number of subjects	Age range (years old)	$Mean age \pm s.d.$ (years old)	$\begin{array}{l} \mbox{Mean age of} \\ \mbox{diagnosis } \pm \ s.d. \\ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ $	Number of subjects with kidney transplant	Males	Females
Permanent In	cisors Teeth						
MMA	22	8-34	17.50 ± 7.88	6.11 ± 8.92	8	10	12
cblA	2	32-34	33.00 ± 1.41	24.75 ± 16.62	1	1	1
cblB	1	-	9	11	0	0	1
$cblC^{a}$	4	9-21	14.75 ± 5.31	3.60 ± 1.74	0	2	2
mut	15	8-25	16.73 ± 6.69	3.82 ± 6.14	7	7	8
Controls	45	8-29	18.29 ± 6.16	NA	NA	23	22
Primary Incise	ors Teeth						
MMA	10	2-6	4.30 ± 1.42	0.65 ± 0.93	2	6	4
cblA	1	6	-	0.10	0	1	0
cblB	1	4	-	0.17	0	1	0
$cblC^{a}$	3	2–4	3.33 ± 1.15	1.60 ± 1.35	0	2	1
mut	5	3-6	4.60 ± 1.52	0.29 ± 0.25	2	2	3
Controls	10	4–7	$5.40~\pm~1.17$	NA	NA	4	6

^aCombined MMA and Homocystinuria.

MMA, methylmalonic acidemias.

Further, two of the patients with *mut* MMA provided unstimulated, whole saliva samples that were collected concurrently with blood samples for determination of methylmalonic acid concentrations. The levels were compared to two healthy control saliva and blood samples collected in an identical fashion.

Statistical analyses were performed using the R statistical analysis package (R Development Core Team, 2006). Normally distributed continuous variables were expressed as means \pm s.d. Student's *t*-tests were used to analyze continuous differences in groups. A Chi-Squared test with Fisher's exact test for significance was used to examine frequency differences between groups. A multivariant regression analysis was used to determine the associations between groups while accounting for the influence of confounders. Two-tailed *P* values < 0.05 were considered statistically significant.

Results

No significant difference was found between the first and second scorers for DDE score or DDE extents for each tooth, indicating intra-scorer reliability index (P > 0.75). For affected subjects with permanent maxillary central incisors, no significant differences in age (P = 0.664) or gender (P = 0.803) were found between the affected (n = 22) and control (n = 45) groups (Table 1). The prevalence of severe enamel defects in the teeth of affected subjects was significantly higher when compared with controls, for both diffuse opacities (P < 0.0001) and for hypoplasia (P < 0.0001). The prevalence of the number of subjects who had at least one tooth with a severe enamel defect, of at least a diffuse opacity, was significantly higher in the affected group than controls (P = 0.010). The extents of the defects were also significantly greater in the affected group when compared with the control group (P < 0.0001, Table 2).

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An analysis of the enamel defects in teeth between various complementation types for affected patients with permanent index teeth (n = 22) showed no significant association between the specific metabolic defect and type of enamel defect. All the metabolic subtypes examined demonstrated severe enamel defects (diffuse opacities or hypoplasia) on teeth with a significantly higher prevalence when compared to controls (P < 0.0001). However, the prevalence of the number of subjects who had at least one tooth with a severe enamel defect, of at least a diffuse opacity, was significantly higher only in the *mut* MMA subgroup when compared with the control group (P = 0.021).

Figure 2 shows a clinical photo of the teeth of a control subject with normal enamel and several affected subjects with a spectrum of severe enamel defects, demonstrating diffuse opacities and enamel pitting.

For subjects with permanent index teeth (n = 22). those who demonstrated severe enamel defects (diffuse opacities or enamel hypoplasia, n = 12) had significantly higher serum methylmalonic acid levels than those without (n = 10) (1467.33 ± 1662.78 vs $150.42 \pm 209.28 \ \mu \text{mol} \ l^{-1}, P = 0.022$). Within the *mut* MMA subgroup (n = 15), where serum methylmalonate levels can be expected to be elevated, the subjects who had severe enamel defects (n = 8, five transplant)subjects (62%), 2050.50 \pm 620.48 µmol 1⁻¹) had significantly higher serum levels of methylmalonic acid than those without (n = 7, two transplant subjects (29%), $209.57 \pm 86.24 \ \mu \text{mol} \ l^{-1}, \ P = 0.017)$ (Figure 3). This association of DDE scores with serum levels of methymalonic acid remains significant, even when adjusted for transplant status (P = 0.040). No trend towards congenitally missing or supernumerary teeth was found in the affected group.

 Table 2 Enamel defect prevalence between affected and control groups

	Number of subjects	Number of teeth	Number of teeth (percentage) DDE < 3	Number of teeth (percentage) diffuse opacity (DDE 3–5)	Number of teeth (percentage) hypoplasia (DDE ≥ 6)	Enamel defect extent	Number of subjects (percentage) with a tooth $DDE \ge 3$
Permanent i	ncisors teeth						
MMA	22	86	40 (47)	32 (37)**	14 (16)**	$1.85 \pm 1.35^{**}$	12 (55)*
cblA	2	8	4 (50)	1 (12)	3 (38)**	$2.75 \pm 0.46^{**}$	1 (50)
cblB	1	4	2 (50)	0 (0)	2 (50)*	$2.50 \pm 0.58^{**}$	1 (100)
cblC ^a	4	16	8 (50)	8 (50)**	0 (0)	$1.50 \pm 1.55^{**}$	2 (50)
mut	15	58	26 (45)	23 (40)**	9 (15)**	$1.75 \pm 1.35^{**}$	8 (53)*
Controls	45	179	162 (90)	16 (9)	1 (1)	$0.57~\pm~0.05$	9 (20)
Primary inci	sors teeth						
MMÅ	10	38	30 (79)	0 (0)	8 (21)*	$0.72 \pm 1.21*$	2 (20)
cblA	1	4	4 (100)	0 (0)	0 (0)	0 ± 0	0 (0)
cblB	1	4	4 (100)	0 (0)	0 (0)	0 ± 0	0 (0)
$cblC^{a}$	3	12	4 (33)	0 (0)	8 (67)**	$3.00 \pm 0^{**}$	2 (67)*
mut	5	18	18 (100)	0 (0)	0 (0)	0.14 ± 0.35	0 (0)
Controls	10	40	40 (100)	0 (0)	0 (0)	$0.15~\pm~0.37$	0 (0)

^aCombined MMA and Homocystinuria.

*Significantly higher prevalence in affected group than controls, P < 0.050.

**Significantly higher prevalence in affected group than controls, P < 0.001. DDE, Developmental Defects of Enamel; MMA, methylmalonic acidemias.

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Figure 2 Control patient (row a) with normal enamel. Affected patients (row b-row e) showing Developmental Defects of Enamel (right panel shows detail of the enamel defects of left panel with arrows pointing to specific diffuse opacities and arrowheads pointing to hypoplasia)



*graph bars are mean ± 2 times the standard deviation

Figure 3 Affected patients with *mut* type methylmalonic acidemia and severe enamel defects, \blacksquare have significantly* higher serum levels of methylmalonic acid than those with normal enamel, \blacksquare

For subjects with primary maxillary central incisors, no significant differences in age (P = 0.075) or gender (P = 0.656) were found between the affected (n = 10) and control (n = 10) groups (Table 1). The prevalence of severe enamel defects in the teeth of affected subjects were significantly higher when compared with controls (P = 0.044), wholly due to two *cblC* subjects who exhibited very severe enamel defects of generalized missing enamel (Table 2). For this *cblC* subgroup, the prevalence of the number of subjects who had at least one tooth with a severe enamel defect, here severe enamel hypoplasia, was significantly higher than the control group (P = 0.038). Figure 4 shows a control subject with primary teeth covered with normal enamel and the two *cblC* affected subjects with cone-shaped, severely dysmorphic primary teeth and radiographic evidence of enamel hypoplasia.

Both primary teeth provided by the affected subjects for microstructural analysis were grossly normal morphologically (Figure 5a,b). Microstructurally, the tooth sample from the *mut* patient (molar) was found to have normal enamel thickness and regular crystalline structure. In contrast, the tooth sample from the *cblC* patient (incisor) was found to have normal enamel thickness but markedly altered microstructure. As seen by LM of thin sections, the incisor sample had altered enamel opacity indicative of increased porosity as compared with the molar sample (Figure 5c,d). Also, when viewed with SEM, the incisor enamel showed flat, plate-like crystallites that were intermixed with the more classically normal appearing hexagonal enamel crystal that were seen throughout in the normal appearing molar sample (Figure 5e.f).

Salivary levels of methylmalonic acid were massively elevated in the two *mut* patients whose saliva was tested (21 904 \pm 1438 nmol l⁻¹) when compared to two control salivary concentrations (427 \pm 142 nmol l⁻¹, P = 0.002). This finding parallels the difference in the serum levels of MMA in the patients (191 887 \pm 103 538 nmol l⁻¹) and controls (121 \pm 18 nmol l⁻¹, P = 0.120).

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Figure 4 Primary teeth from control subject and radiograph (row **a**). Severely dysmorphic, cone shaped primary teeth and radiographic evidence of enamel hypoplasia in two affected patient with *cblC* disorders (row **b** and **c**)

Discussion

This study demonstrated that enamel defects were significantly more prevalent and more extensive in the permanent teeth of the affected group, comprised of isolated and combined forms of MMA, when compared to the control group (P < 0.0001, Table 2). The DDE scores found in this study can be compared to the reported normal prevalence of enamel defects of the facial surfaces of anterior teeth using the DDE Index: up to 5% of teeth with diffuse opacities and 1% of teeth with hypoplasia (Clarkson and O'Mullane, 1989). Compared with this reported prevalence, the affected group is found to have a significantly higher prevalence of severe enamel defects than the normal population (P < 0.0001). The presentation of the severe forms of enamel defects, seen as diffuse opacities of enamel or as enamel hypoplasia (Figure 2), at a highly significant greater prevalence in the study group compared to the controls suggests that these types of enamel defects are a clinical expression of MMA. Although only index teeth were analysed for DDE, the finding of severe enamel defects in the maxillary anterior incisor teeth for affected subjects can be generalized for all smooth surfaces of all teeth, in contrast to the isolated enamel defects found on one or two teeth of the control group.

Affected subjects with *mut* MMA and adult index teeth had a significantly higher prevalence of having at least one index tooth with a severe enamel defect (P = 0.021). Affected subjects in this sub-group with a severe enamel defect also had higher serum methylmalonic acid levels compared to those without enamel defects (P = 0.017, Figure 3). Assuming that a current elevated MMA level can be associated with a past

history of difficult metabolic control, this difference suggests that dysregulated metabolism during enamel development may lead to developmental defects of enamel which are then permanent. Though kidney transplant may influence serum methylmalonic acid levels, the association between enamel defect score and serum methylmalonic acid remained when adjusted for transplant status (P = 0.040).

Markedly elevated levels of methylmalonate can also be indicative of renal involvement (Walter et al, 1989). In pediatric populations, systemic disturbances that occur during enamel development have been associated with enamel anomalies (Pindborg, 1982; Bhat and Nelson, 1989), and renal dysfunction in particular has been linked with enamel hypoplasia (Lucas and Roberts, 2005). Our results suggest that the current enamel defects may retrospectively reflect pediatric disease, possibly renal insufficiency or a local influence of MMA related biochemical malfunction at the site of tooth agenesis, which interfered with normal enamel development. Enamel defects seen in permanent teeth of our study subjects would then represent the permanent sequela of disturbed enamel formation during the first 7 years of life. The possible influence of renal disease is supported by our finding of severe defects in the mut type subgroup (where renal disease is a significant complication) and the association of severe defects with elevated serum methylmalonate levels.

Our analysis of affected subjects with primary index teeth suggests that primary teeth are generally spared developmental defects of enamel in these disorders. However, the finding of two *cblC* type MMA patients with severely dysmorphic primary teeth and extreme hypoplastic enamel development (Figure 4), suggests 201

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that this subgroup may have dental developmental problems in-utero, when these teeth begin calcification. This is supported by the clinical observation of intrauterine growth retardation, and occasionally congenital microcephaly, in this disease (Andersson *et al*, 1999).

These findings highlight the potential role of dental evaluation in the clinical assessment of patients with MMA and cbl metabolic disorders. In addition to significant esthetic concerns, enamel defects may affect Figure 5 Structural analyses of two affected patient's primary teeth. Tooth picture a, light microscopy (LM) section c, and SEM picture e is of a primary molar donated by a *mut* type methylmalonic acidemias patient. Tooth picture b, LM section d, and scanning electron microscopy (SEM) picture f is of primary incisor donated by a *cblC* patient. Top row: Both teeth (a, b) demonstrated normal clinical morphology. Middle row: Thin sections viewed with light microscope. Normal prismatic enamel (c) compared with increased enamel opacity (d). Bottom row: Sections viewed with SEM. Normal prism morphology (e) compared with altered crystal structure (f)

the caries status of the patient. An association between enamel defects and a higher caries risk has been suggested (William *et al*, 2006). For renal failure patients, however, there may be an association between enamel defects and a lower incidence of caries, either secondary to the dental changes or to the protective influence of altered salivary composition or influence on oral microflora (Lucas *et al*, 2001; Lucas and Roberts, 2005).

The identification of microstructural abnormalities seen in the primary tooth of an affected patient with *cblC* deficiency further enhances our findings. Developmental defects of enamel would be expected to manifest equally on both the incisor and molar sample since the enamel of both types of teeth are expected to have the same microstructure of enamel. Although limited to one tooth sample, the analysis shows ultrastructural anomalies that appear to have occurred during enamel development, rather than postformation attrition or erosion of existing enamel. This suggests an alteration in the control of mineralization during the mineralization and maturation phase of enamel formation (Wright et al, 1993). This type of defect in enamel mineralization is indicative of a decreased mineral content that could occur due to alterations in the formation and processing of the enamel matrix proteins or changes in the control of the microenvironment during formation (Phakey et al, 1995; Hart et al, 2003; Bartlett et al, 2006). Since this finding was in the primary dentition of a *cblC* subject, it may reinforce our findings of severe enamel defects only in the primary dentition of cblC patients. Also, the *mut* type patient who provided the primary molar for microstructural analysis had only moderate levels of serum MMA (167 μ mol l⁻¹).

Analysis of salivary methylmalonic acid levels expands our clinical observations. Methylmalonic acid levels were extremely elevated in the saliva from the two affected *mut* subjects studied compared to controls (21 904 *vs* 427 nmol 1^{-1}), reflecting the subject's matched serum levels (191 886 *vs* 121 nmol 1^{-1}). This raises the possibility of the use of saliva as a noninvasive medium (Tabak, 2001; Kaufman and Lamster, 2002) for metabolic monitoring in these disorders. Elevation of salivary methylmalonic acid may lead to alterations in the oral microflora and salivary pH, which, in addition to the developmental microstructural changes described, may further affect caries risk.

Conclusion

To date, this is the only study that has examined dental manifestations of patients with MMA and cbl disorders. Most US states now screen all newborns using tandem mass spectrometry based methods that detect many inborn errors of metabolism, including the disorders examined here (Therrell *et al*, 2006). It is certain that more individuals will be diagnosed with a wide array of organic acidemias in the future, and a better understanding of dental development in this group will be necessary for comprehensive and interdisciplinary treatment.

This study presents the novel findings of enamel defects in a population of MMA and cbl disorder patients and the demonstration of massively elevated levels of methylmalonate in the saliva of two *mut* subjects. Additionally, the enamel anomalies documented here by clinical and microstructural analysis expands the phenotypic manifestations of these inborn errors of metabolism and suggests that enamel development may be altered in these disorders.

Acknowledgements

This study was supported through the Divisions of Intramural Research of the NHGRI and NIDCR/IRP, NIH, DHHS.

Author contributions

Dr Bassim designed the study, analyzed the data, and drafted the paper. Dr Wright performed the microstructural analysis of enamel. Dr Guadagnini, Dr Muralidharan, and Dr Domingo performed clinical analysis of the patients and data as dentists. Dr Sloan acted as patient coordinator for the MMA cohort. Dr Venditti is the PI for the MMA natural history study and supplied the medical data for the MMA cohort and the salivary data for methymalonic acid. Dr Hart initiated and mentored the study and provided expertise in dental phenotype correlation studies. All authors contributed to the editing of the manuscript and presentation of the data.

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