Case Report

Enamel-renal syndrome associated with hypokalaemic metabolic alkalosis and impaired renal concentration: a novel syndrome?

Xue Jun Fu1, Kandai Nozu1, Katsumi Goji2, Kazushige Ikeda3, Ichiro Kamioka1, Teruo Fujita1, Hiroshi Kaito1, Hisahide Nishio4, Kazumoto Iijima5 and Masafumi Matsuo1

1Department of Pediatrics, Kobe University Graduate School of Medicine, Kobe, 2Department of Endocrinology and Metabolism, Hyogo Children’s Hospital, Kobe, 3Department of Pediatrics, Saiseikai Hyougoken Hospital, Kobe, 4Department of Environmental Health and Safety, Kobe University Graduate School of Medicine, Kobe and 5Department of Nephrology, National Center for Child Health and Development, Tokyo, Japan

Keywords: amelogenesis imperfecta (AI); bartter syndrome; enamel-renal syndrome (ERS); nephrocalcinosis; nephrogenic diabetes insipidus (NDI)

Introduction

Enamel-renal syndrome (ERS, OMIM204690) is characterized by amelogenesis imperfecta (AI) and nephrocalcinosis and has been previously reported in only 10 cases (including four siblings) [1–7]. Cases diagnosed as ERS sometimes show hypocalciuria [2,3,6] or impaired renal concentration [6] and occasionally progress to end-stage renal failure [2,6,7]. On the basis of pedigree analysis results, it has been proposed that ERS is an autosomal recessive inheritance disease.

AI cases show inherited defects of tooth enamel. While AI cases with autosomal dominant, autosomal recessive and X-linked inheritance patterns as well as sporadic cases of AI have been reported, the pathogenesis or molecular base of this condition is not yet fully understood [8].

Bartter syndrome and nephrogenic diabetes insipidus (NDI) are inherited renal tubule disorders and five types of genes responsible for Bartter syndrome (NKCC2, ROMK, CLC-Kb, Barttin and CaSR) and two for NDI (V2R for X-linked and AQP2 for autosomal dominant or recessive form) have been identified. Bartter syndrome shows hypokalaemic metabolic alkalosis and, usually, also mild-to-moderate impairment of renal concentration, but its pathophysiology is not fully understood.

Here, we present the first reported case of ERS complicated with Bartter-like syndrome and mild impaired renal concentration in a child from a consanguineous family.

Case

This report concerns a 14-year-old girl with no siblings, whose parents are first cousins. Severe polyhydramnios was detected antenatally and amniocentesis was administered several times. She was born at 33 weeks gestation with a body weight at birth of 1604 g (normal for the gestational period). At 2 months, she was brought to a paediatric clinic because of poor feeding and failure to thrive. Blood chemical and haematological studies and urinalysis findings disclosed that she had normal renal function (serum creatinine level: 0.3 mg/dl), normal electrolyte levels (Na: 137 meq/l, K: 4.0 meq/l, Cl: 103 meq/l), normal blood gas findings (HCO\textsubscript{3}: 21.6 mmol/l, base excess (BE): −0.6) and normal serum osmolality (280 mOsm/kg), but she had low urinary osmolality (150–250 mOsm/kg). The results of the subsequently administered 4 h water deprivation test and vasopressin administration test (0.2 U/kg, i.m.) conducted when she was 1 year old are shown in Table 1. The initial serum antidiurectic hormone (ADH) level was high in spite of low urinary osmolality. Urine osmolality was less than 300 mOsm/kg in spite of vasopressin administration. Although 4 h water deprivation is not sufficient, our patient was too young to tolerate continuation for more than 4 h. However, her urine osmolality was extremely low as already explained (when serum osmolality is 295 mOsm/kg in normal cases, urine is more than 750 mOsm/kg). Her total urine volume was 236 ml/kg/day and she was thus diagnosed as having an impaired renal concentration. Findings of
Kidney ultrasonography were normal at that time. Treatment with thiazide diuretics based on the treatment for NDI was started, but because failure to thrive persisted, thiazide was discontinued 3 months later. When she was 18 months old, the detection of hypokalaemia (2.3 meq/l), hypercalciuria [urinary calcium creatinine ratio (Ca/Cr): 1.8 mg/mg; normal: 0.1–0.3], and normal blood pressure in spite of high plasma renin activity (14.4 ng/ml/h; normal: 0.3–2.9) and high serum aldosterone levels (750 pg/ml; normal: 36–240) led to the initiation of treatment with indomethacin and spironolacton and these agents produced an improvement in hypokalaemia and the failure to thrive. Blood-gas examination was not done until she was 12 years old. From the age of 12 years, her parents realized that the enamel of her teeth was worm-eaten in appearance and she was thus referred to our department.

Her height is now 145 cm (–1.0SD), appearance and intelligence are normal, secondary sex characteristics have a normal appearance and blood pressure is normal. She was diagnosed as having AI because she had an extremely thin enamel, the surface of which was rough and extensively cracked in her permanent teeth (Figure 1); bilateral nephrocalcinosis was also identified by means of ultrasonography (Figure 2). She now shows hypokalaemia (3.1–3.4 mEq/l) despite taking indomethacin and spironolacton and metabolic alkalosis (HCO₃: 31 mmol/l, BE: 4.5). She shows high plasma renin activity (11.0 ng/ml/h; normal: 0.3–2.9) and high serum aldosterone level (512 pg/ml; normal: 36–240) despite normal blood pressure. Her calcium (9.8 mg/dl) and magnesium (2.1 mg/dl, normal 1.7–2.3) levels are normal. Her renal function [blood urea nitrogen (BUN): 9 mg/dl; Cr: 0.51 mg/dl] and serum osmolality (279 mOsm/kg) are normal, while urinary osmolality (136 mOsm/kg) is low. She shows hypercalciuria (urinary Ca/Cr: 1.1 mg/mg; normal: 0.1–0.3), but her urinary N-acetylglutamate (NAG) and α₁-microglobulin excretion levels were within normal range (urinary NAG: <3 U/l; urinary α₁-microglobulin: 0.9 mg/l; normal: <8.3), while no glucosuria or aminoaciduria was observed. These findings suggest that proximal tubular reabsorption function was normal. In addition, she does not have a family history of diabetes mellitus or hearing loss, and her fundus oculi is normal. Therefore, mitochondrial defects can be ruled out. Her serum intact-parathyroid hormone (PTH) level was slightly high (98.0 pg/ml; normal: 14–66), 1,25-(OH)₂ Vit D level was also slightly high (103 pg/ml; normal: 20–70) and protein induced by vitamin K antagonist II (PIVKAII) was normal (PIVKAII: <1).

In view of the overall findings, the patient was diagnosed as having ERS complicated with Bartter-like syndrome and insufficiency of renal concentration.

### Genetic analysis and results

Having obtained informed consent from the patient and her parents, we performed a genetic analysis of several genes. Genomic DNA was extracted from leucocytes with the standard phenol–chloroform extraction method. We selected previously described primers which amplify all exons and exon–intron boundaries of the NKCC2 gene for type 1 Bartter syndrome, ROMK for type 2, CLC-Kb for type 3, Barttin for type 4, CaSR for type 5, and AQP2 for autosomal dominant and recessive form of NDI. Polymerase chain reaction (PCR) was followed by purification of the PCR products and direct sequencing on both strands. A direct sequencing of the exons of double-strand DNA fragments of the six genes was performed with the aid of the PCR primers, an ABI PRISM Bigdyce Thermal Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA) and an ABI model 310 auto sequencer. The results showed no mutation in any of the six genes.

### Discussion

The syndrome of AI and nephrocalcinosis has been previously reported [1–7] under the name of ERS (OMIM 204690). All cases had thin or absent tooth enamel and bilateral nephrocalcinosis, while some cases progressed to renal insufficiency [2,6,7] and other cases featured certain renal tubular disorders.

### Table 1. Results of the water deprivation test and vasopressin administration tests

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine volume (ml/kg h)</td>
<td>3.8</td>
<td>2.2</td>
<td>3.3</td>
<td>5.9</td>
<td></td>
</tr>
<tr>
<td>Urine osmolality (mOsm/kg)</td>
<td>239</td>
<td>155</td>
<td>204</td>
<td>248</td>
<td>138</td>
</tr>
<tr>
<td>Serum osmolality (mOsm/kg)</td>
<td>285</td>
<td>284</td>
<td>294</td>
<td>273</td>
<td></td>
</tr>
<tr>
<td>Plasma ADH (pg/ml, normal: 0.3–3.5)</td>
<td>13.2</td>
<td>12.3</td>
<td>15.1</td>
<td>5.1</td>
<td></td>
</tr>
</tbody>
</table>

Vasopressin 0.2 U/kg; administered at 4 h.

Fig. 1. Clinical photograph of the case index for permanent dentition showing reduction of enamel thickness and enamel deficit at yellow discoloured spots.
such as hypocalciuria [2,3,6] or urinary concentration disability [6] (Table 2). In addition to ERS, our case also features Bartter-like syndrome and impaired renal concentration, although the concentration insufficiency could be secondary to Bartter-like syndrome. The pathophysiology of impaired renal concentration in Bartter syndrome has been well established: the thick ascending limb of loop of Henle is responsible for 25–30% NaCl reabsorption, this process is a critical step in the generation of medullary hypertonicity, which is necessary for water reabsorption in the collecting duct. In Bartter syndrome, the defect of NaCl reabsorption is responsible for urinary concentrating insufficiency. ERS is usually characterized by various renal tubular disorders. In this case, unknown mechanisms would cause the disorder in the thick ascending limb of the loop of Henle and the Bartter-like syndrome and insufficient renal concentration.

Most patients with ERS, including our case, come from consanguineous families or have siblings with ERS and none of these cases have family histories, which led us to suppose that this syndrome is a single gene disorder and that the inherited form is autosomal recessive.

AI is a group of inherited defects of dental enamel formation characterized by both clinical and genetic heterogeneity. Great progress has been made regarding the definition of the genetic background of AI. To date, mutations in five genes (AMELX, ENAM, DLX3, KLK4 and MMP-20) have been found to cause AI [8]. AMELX is responsible for X-linked AI, ENAM and DLX-3 are autosomal dominant, and KLK4 and MMP-20 are autosomal recessive. Although no mutation in ERS has been reported, it has become clear that many of the dental proteins that were believed to be tissue-specific are expressed in other organs. For example, DLX3, a homeobox protein, is also expressed in the kidney. Further research concerning these proteins is necessary to determine whether they play a pivotal role in the calcium and phosphate metabolism or renal tubular disorders.

AI sometimes occurs following environmental causes, including vitamin D deficiency or intoxication, and the dental appearance of our case due to AI or even fluorosis which occurs by fluorine-treated water supply or toothpaste eating. In the present case, no such environmental problems were responsible for AI or fluorosis.

Since in our case, Bartter-like syndrome and impaired renal concentration were complicated with ERS, we performed a genetic analysis to determine whether mutations of these diseases were involved. Genes responsible for type 1–5 Bartter syndrome (NKCC2, ROMK, CLC-Kb, Barttin and CaSR)

![Fig. 2. Renal ultrasonography of bilateral nephrocalcinosis.](http://ndt.oxfordjournals.org)

<table>
<thead>
<tr>
<th>Author</th>
<th>Issue</th>
<th>Case</th>
<th>AI</th>
<th>Nephrocalcinosis</th>
<th>Renal tubule disorder</th>
<th>Renal insufficiency</th>
<th>Characteristics</th>
<th>Reference</th>
</tr>
</thead>
</table>

AI, amelogenesis imperfecta; IRC, impaired renal concentration.

Table 2. Previous reports of ERS
and NDI (AQP2) were analysed but no mutation was detected. The calcium-sensing receptor (CaSR) gene is specifically suspected of being one of the genes responsible for ERS, because it is involved in calcium and phosphate metabolism and is expressed in both teeth and kidney cells [1]. Moreover, it has recently been reported that gain-of-function mutation in the CaSR gene causes type 5 Bartter syndrome [9]. We therefore suspected that our patient might also have mutations in CaSR, but no mutation was detected in her genomes. From her present history we might suspect that she could have an antenatal Bartter syndrome (type 1 or 2), because she had severe polyhydramnios, but we failed to detect the mutation in NKCC2 and ROMK. We did not conduct a genetic analysis of X-linked form of NDI (V2R) because the patient is a girl and there is no family history of NDI.

Nephrocalcinosis is a common feature of genetically determined disorders with hypercalciuria, such as Dent disease, Bartter syndrome and X-linked hypophosphataemia. However, no case has been reported with those diseases combined with AI. It should further be noted that hypercalciuria is not the only reason for nephrocalcinosis in ERS, because some sibling cases have shown hypocaliuria [2,3,6].

Table 2 lists the previous reports on ERS, showing that only 10 cases (including four sibling cases) have been reported. Since Lubinsky et al. [6] suggested that vitamin K may be related to the cause of ERS, we measured PIVKII but it was in the normal range. Our patient had hypercalciuria and five of these 10 previously reported patients had hypocalciuria, but none of these cases showed Bartter-like laboratory findings. Moreover, given that from her laboratory data and family history, mitochondrial defects are improbable, our patient may represent a novel syndrome. It could be a defect of one or more channels or transporters, for example the basolateral K channel or chloride channel Ka, which can cause hypokalaemia. Our case had normal potassium levels during the first year of her life, but these dropped to below normal after her first year. We cannot explain this unusual phenomenon, but the shift may be associated with the low sodium–potassium-adenosinetriphosphatase (Na+/K+-ATPase) activity seen in low-birth-weight infants [10]. Although ERS may therefore be an extremely rare syndrome, further research into this disease, especially to clarify the genetic defect behind ERS, is very important because the pathogenesis of both AI and nephrocalcinosis remain unclear and our case and previous reports about ERS also suggest the possibility of involvement of the separate but closely linked genes for AI and nephrocalcinosis.

Conclusion

Our report concerns a case of the rare syndrome of AI and nephrocalcinosis (ERS), but what sets it apart from previously reported cases is that the ERS of our case is complicated with hypokalaemic metabolic alkalosis and impaired renal concentration. Since no mutations for Bartter syndrome or NDI could be identified, our case was considered to represent a first case report of ERS complicated with Bartter-like syndrome and mild NDI. To date, no genetic mutation has been identified in ERS, so that further research is needed to clarify the pathogenesis of AI and nephrocalcinosis.

Conflict of interest statement. This work was supported by grants from the Ministry of Education, Culture, Sports, Science and Technology of Japan and Fund of Kidney Disease Research from Hyogo Prefecture Health Promotion Association.

References


Received for publication: 2.11.05
Accepted in revised form: 9.5.06