Recalcitrant Hypocalcaemia in Autoimmune Enteropathy

abstract

Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy syndrome is a monogenic disorder associated with autoimmune destruction of both endocrine and nonendocrine tissues. The classic triad includes candidiasis, hypoparathyroidism, and Addison disease. Up to 25% of patients with autoimmune polyendocrinopathy candidiasis ectodermal dystrophy syndrome also have gastrointestinal manifestations, which can have an impact on the management of other aspects of the disease. The management of the case discussed was challenging because of the complex interplay between the manifestations and treatment of his hypoparathyroidism, Addison disease, and autoimmune enteropathy. Attempts at management of hypocalcemia were largely unsuccessful until the introduction of immunosuppressive therapy for autoimmune enteropathy. This case supports early consideration of immunosuppression in this condition. *Pediatrics* 2014;134:e1720–e1726

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KEY WORDS

autoimmune polyendocrinopathy candidiasis ectodermal dystrophy syndrome, APECED, autoimmune polyendocrinopathy syndrome 1, APS-1, autoimmune enteropathy, enteroendocrine cells, hypocalcaemia

ABBREVIATIONS

17OH—17 hydroxylase APECED—autoimmune polyendocrinopathy candidiasis ectodermal dystrophy syndrome GAD—glutamic acid decarboxylase GH—growth hormone ICA—islet cell antibody IF—intrinsic factor NR—normal range SCC—cholesterol side chain cleavage TPH—tryptophan hydroxylase

Dr Geyer drafted the initial manuscript; Dr Fairchild assisted with drafting sections related to early management of hypoparathyroidism and Addison disease, and reviewed and revised the manuscript; Dr D. Moore assisted with drafting sections relating to autoimmune enteropathy and reviewed and revised the manuscript; Dr L. Moore assisted with staining of biopsies, drafting sections related to histopathology of condition, and reviewed and revised the manuscript; Dr Henning assisted with drafting sections relating to hypocalcaemia and hyperphosphatemia, and reviewed and revised the manuscript; Dr Tham assisted with drafting sections relating to management of complex interplay between enteropathy and endocrinopathies, and reviewed and revised the manuscript; and all authors approved the final manuscript as submitted.

www.pediatrics.org/cgi/doi/10.1542/peds.2013-3308
doi:10.1542/peds.2013-3308

Accepted for publication Jul 10, 2014

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PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275).

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Autoimmune polyendocrinopathy candidiasis ectodermal dystrophy syndrome (APECED; also known as autoimmune polyglandular syndrome type 1) is a rare autosomal recessive condition resulting in autoimmune destruction of a range of tissues.1–4 Mutations in the autoimmune regulator gene on chromosome 21q22.3 are responsible and more than 60 mutations have been identified.1,3,4 These mutations affect negative selection of T cells by the thymus and peripheral immune tissues, resulting in loss of self-tolerance, predisposing to autoimmunity.2,4–6 Prevalence varies from 1:9000 (Iranian Jewish population) to 1:500 000 (North-western France).2

The large spectrum of endocrine and nonendocrine system involvement, with associated antibodies, is listed in Table 1. Candidiasis is the most common initial finding, usually presenting in infancy. Hypoparathyroidism follows in childhood, then adrenal failure in the second decade of life.2,4 More than half the patients in a case series had all 3 manifestations by 15 years of age.1 Gastrointestinal manifestations of APECED are present in ∼25% of patients and can have severe consequences for patient symptomatology and management.8,14,15 The case presented had chronic diarrhea and recalcitrant hypocalcemia despite high supplemental doses of calcium and calcitriol (1,25-dihydroxyvitamin D). Multiple measures were taken to optimize calcium absorption without success until commencement of immunosuppressive therapy to treat autoimmune enteropathy.

### CASE REPORT

A 5-year-old boy presented with a hypocalcemic seizure caused by primary hypoparathyroidism. He was commenced on calcitriol and calcium supplements appropriate for his age and weight. No past history of oral candidiasis was reported; however, groin candidiasis was noted. He was the oldest child of nonconsanguineous parents of Austrian and Australian background. A family history of autoimmune diseases including systemic lupus erythematosus, Sjogren syndrome, ulcerative colitis, and thyroid disease was present in first- and second-degree relatives.

<table>
<thead>
<tr>
<th>TABLE 1 Clinical Components of APECED With Associated Antibodies</th>
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<tr>
<td><strong>Clinical Presentation</strong></td>
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<tr>
<td><strong>APECED</strong></td>
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<tr>
<td>Candidiasis</td>
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<tr>
<td>Endocrine</td>
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<tr>
<td>Hypoparathyroidism</td>
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<td>Addison disease</td>
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<td>Hypogonadism</td>
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<td>Type 1 diabetes</td>
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<tr>
<td>Hypothyroidism</td>
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<tr>
<td>Hypophysis-GH,</td>
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<tr>
<td>GtN, ACTH deficiency</td>
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<tr>
<td>Gastrointestinal</td>
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<td>Pernicious anemia</td>
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<tr>
<td>Enteropathy</td>
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<td>Alopecia</td>
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<td>Vitiligo</td>
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<td>Keratitis</td>
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210H, 21 hydroxylase; AADC, aromatic L-amino acid decarboxylase; ACTH, adrenocorticotropic hormone; CaSR, calcium sensing receptor; GnT, gonadotropin; HDC, histidine decarboxylase; IA2, insulinoma antigen 2; LKM-A, liver kidney microsomal antibodies; NALPS, NACHT leucine rich repeat protein 5; PC, parietal cell; SOX9 and SOX10, transcription factors; TDRD6, Tudor domain containing protein 6; TG, thyroglobulin; TH, tyrosine hydroxylase; TPO, thyroid peroxidase.1,5–7,15

* Interferon antibodies positive in 98% of patients with APECED and thought to be diagnostic markers.

Addison disease was diagnosed 6 months later after an acute salt wasting adrenal crisis. Glucocorticoids (hydrocortisone) and mineralocorticoids (fludrocortisone acetate) were commenced. APECED was diagnosed clinically and confirmed with genetic studies. DNA sequencing revealed heterozygosity for 2 separate mutations in the autoimmune regulator gene: c.769C>T(p.Arg257X), one of the most common mutations associated with APECED and c.789delC (p.Ala264LeufsX114) a frameshift mutation reported once previously, in an Australian family.16 Both parents carried 1 gene. Investigations revealed positive interferon-omega (ω) antibodies in addition to antibodies directed to the adrenal cortex (21 hydroxylase, 17 hydroxylase [170H], cholesterol side chain cleavage [SCC]), pancreas (glutamic acid decarboxylase [GAD], islet cell antibody [ICA]), skin and liver (aromatic L-amino acid decarboxylase), intrinsic factor (IF), gonads (SCC, 170H), and intestine (tryptophan hydroxylase [TPH], GAD). Parathyroid antibodies (NACHT leucine rich repeat protein 5) were negative.

He subsequently developed dental enamel hypoplasia, alopecia, mucocutaneous candidiasis, and significant gastrointestinal manifestations including autoimmune gastritis, pernicious anemia, cheilitis, and intermittently elevated liver enzymes.

Recurrent diarrhea, morning nausea, and intermittent abdominal pain occurred from age 9, and he became Cushingoid because of frequent stress doses of hydrocortisone. Stool microscopy was initially positive for Cryptosporidium, but no pathogens were found in subsequent stool samples, despite ongoing diarrhea. Pernicious anemia was diagnosed with a vitamin B12 of 80 pmol/L (normal range [NR], 100–700), positive IF antibodies, and elevated serum gastrin (510 pg/mL, NR <100 pg/mL). He commenced vitamin B12 injections. A
trial of esomeprazole gave some improvement to nausea.

Episodes of diarrhea increased in frequency and severity and were associated with symptomatic hypocalcemia, hyperphosphatemia, and hypokalemia. Stool microscopy was positive for fat globules and fatty acid crystals. Fecal elastase was borderline, 210 μg/g (NR >200 μg/g). Celiac screen was negative, and a lactulose hydrogen breath test excluded the possibility of bacterial overgrowth. Pancreatic enzyme replacement was trialed for borderline exocrine function with initial but not ongoing improvement.

Upper gastrointestinal endoscopy and biopsy revealed no evidence of candidiasis or *Helicobacter pylori*. Gastric biopsies revealed chronic inflammatory change, some atrophy, and an absence of parietal cells. Hematoxylin and eosin staining of duodenal biopsy was normal with no excess inflammatory cells (Fig 1A). Chromogranin A staining revealed absent enteroendocrine cells in duodenal mucosa (<1 per 20 crypts; Fig 1B). This, along with positive TPH antibodies, confirmed the diagnosis of autoimmune enteropathy. Normal staining of enteroendocrine cells with chromogranin A is shown as brown stained cells in Fig 1C.

By age 12, he had persistent hypocalcemia, with continued diarrhea and growth failure (Fig 2). Thyroid function and growth hormone (GH) stimulation testing were normal (thyrotropin 0.71 mU/L, Free thyroxine 14 pmol/L, peak GH 32.8 mIU/L). Gonadotropins were prepubertal (luteinizing hormone <1.0 IU/L, follicle stimulating hormone <1 IU/L, testosterone < 0.7 nmol/L), and bone age was 4 years delayed. Growth failure was attributed to chronic illness and iatrogenic Cushing’s syndrome. Pubertal induction with oral testosterone was commenced at 13 years and 4 months and changed to 3 monthly depot intramuscular injections at 14 years and 5 months.

**FIGURE 1**
Duodenal biopsies. A, Normal appearance of duodenal mucosa of the case with hematoxylin and eosin stain. B, Chromogranin A stain of same duodenal biopsy revealing significantly reduced enteroendocrine cells (brown color; <1:20 crypts), compared with (C) normal control duodenal chromogranin A stain with 29:20 crypts enteroendocrine cells.
FIGURE 2
Growth charts.26
At 14 years of age, it became difficult to maintain his ionized calcium above 0.9 mmol/L despite calcitriol of 0.06 μg/kg per day (3 μg/day) and calcium supplements between 4000 mg and 7000 mg elemental calcium (carbonate) titrated to symptoms. Timing of calcium carbonate administration was changed and esomeprazole ceased to improve gastric acidity and thereby calcium absorption. When this failed to have an effect, calcium supplements were changed to calcium citrate as absorption is less dependent on gastric acidity. Increased doses of hydrocortisone were avoided where possible, to optimize calcium absorption. Ongoing diarrhea and lower hydrocortisone contributed to weight loss. Hypomagnesaemia was managed with magnesium supplements to further support calcium absorption.

With increased calcitriol doses and ongoing hypocalcemia, phosphate levels increased to 3.63 mmol/L. Urinary calcium was low and 1,25-dihydroxyvitamin D elevated at 175 pmol/L. Renal function was normal. This was managed with low phosphate diet, phosphate binders (aluminum based followed by sevelamer), and reducing calcitriol to 0.04 μg/kg per day (2 μg/day).

Despite the above measures, calcium levels were unable to be maintained (4000 mg/day calcium citrate), and he became symptomatic with difficulty breathing with exercise, muscle cramping, and prolonged QTc on electrocardiograph. Parathyroid hormone injections were not considered an option because of the possible risk of osteosarcoma. A decision to trial immunosuppressive therapy for autoimmune enteropathy was made.

Cyclosporin was commenced at a dose of 3.5 mg/kg per day (divided) and titrated to a serum trough level of 100 ng/mL at 15 years and 4 months. Within 3 months, his diarrhea, hypocalcemia, and alopecia improved, enabling cessation of calcium supplements and reduction of calcitriol dose to 0.02 μg/kg per day.

Hypertension developed 3 months after commencing cyclosporin. Investigations revealed normal renin and electrolytes indicating appropriate fluidocortisone dose. Two years after commencement of therapy, screening renal biopsy revealed significant scarring attributed to cyclosporin. This was weaned over 3 months, and he commenced azathioprine, on which he has remained for 3 years without relapse of hypocalcemia or malabsorption.

Subsequently, a younger sibling developed symptomatic hypocalcemia and was diagnosed with hypoparathyroidism. He was found to have the same mutations and adrenal cortex antibodies. He went on to develop Addison disease, enamel hypoplasia, and oral candidiasis, without other gastrointestinal manifestations of APECED. Thyroid peroxidase, thyroglobulin, GAD, ICA, and insulinoma antigen 2 antibodies were negative. Other antibodies were not tested given absence of clinical indication.

**DISCUSSION**

Hypocalcemia in the setting of primary hypoparathyroidism is usually easily treated with oral calcitriol.4,5 We have described a case of recalcitrant hypocalcemia, attributed to autoimmune enteropathy. Other contributors to hypocalcemia, both pathologic and iatrogenic are discussed.

Chronic diarrhea and malabsorption seen in APECED have been attributed to a number of causes including bacterial overgrowth, celiac disease, intestinal infections, and pancreatic exocrine insufficiency.1,2,8,18 Hypocalcemia can also lead to malabsorption through reduction of cholecystokinin, decreased gall bladder emptying, and pancreatic insufficiency.2,5,14

More recently, autoimmune enteropathy has been described as a significant contributor with destruction of enteronecocrine cells by TPH antibodies.12,19 TPH is an enzyme involved in the synthesis of serotonin by enteronecocrine cells. These cells assist gut growth, circulation, motility, and secretion of pancreatic enzymes and bile.2,14,18 Lack of enteronecocrine cells in small bowel biopsies confirms autoimmune enteropathy and is demonstrated with chromogranin A and serotonin staining. Hematoxylin and eosin stains are often normal, as shown in this case. There are multiple other gastrointestinal manifestations of APECED including constipation, gastrointestinal candidiasis, and autoimmune hepatitis with which there is significant morbidity and mortality.8,12,14

The small intestine is primarily responsible for calcium absorption, predominantly the duodenum through active transport stimulated by 1,25-dihydroxyvitamin D. When this system is saturated (high calcium intake), passive absorption occurs in the jejenum and ileum.19,20 Widely available calcium carbonate is best absorbed in a highly acidic environment. When administered with meals or other factors that increase stomach pH (proton pump inhibitors, pernicious anemia) absorption is reduced. Calcium citrate absorption is not affected by acidity and therefore is preferable in autoimmune enteropathy.20,21

Glucocorticoids reduce intestinal calcium absorption, renal calcium resorption, and bone remodelling6; therefore, the need for stress dosing must be carefully assessed. In this case, recurrent diarrhea necessitated stress doses of hydrocortisone to prevent adrenal crisis and at times resulted in short-term improvement of diarrhea, suggesting immunosuppressive benefits.
There is currently no consensus on the treatment of autoimmune enteropathy. The largest case series published by Perheentupa recommends the use of immunosuppressive agents to treat autoimmune hepatitis, autoimmune enteropathy, and keratoconjunctivitis. Despite the efficacy of cyclosporin, the risk of nephropathy is well described and requires screening. A possible adverse effect of immunosuppressives is generalized candidiasis, which has been reported in 1 patient. This requires ongoing investigation. Other immunosuppressive agents have been used successfully such as pulse methylprednisolone, cyclophosphamide, and azathioprine, with smaller studies involving mycophenolate, 6-mercaptopurine, tacrolimus, sirolimus, infliximab, and rituximab.

**CONCLUSIONS**

Primary hypoparathyroidism in children is a rare presentation, and consideration of APECED should be made, particularly if associated with family history of autoimmunity. This instructive case highlights multiple factors involved in calcium absorption including calcium formulation, gastric acidity, enterocendocrine cells, and glucocorticoids. Chromogranin A staining to demonstrate reduced enterocendocrine cells and TPH antibody testing have enabled more accurate diagnosis of autoimmune enteropathy in children with APECED. Small case series have shown improvement with immunosuppression, and the swift and persistent improvement in enteropathy in this case highlights the need for consideration of early commencement of immunosuppression and further studies balancing their risks with regression of the aspects of the disease.

**ACKNOWLEDGMENTS**

The authors thank Elizabeth Thompson for assistance with genetic investigations, Hamish Scott for assistance with antibody and genetic investigations, and Jaakko Perheentupa for advice regarding initiation of immunosuppression. Antibody testing was performed by Eystein Husebye’s research group, Bergen, Norway. Genetic studies were performed by Institute of Medical and Veterinary Science, Adelaide, Australia.

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FINANCIAL DISCLOSURE: Dr David Moore is principal investigator in a trial with pegylated interferon sponsored by Roche, of no relevance to this case report; the other authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: No external funding.

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.
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*Pediatrics* originally published online November 17, 2014;

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Pediatrics originally published online November 17, 2014;

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