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Long-term Parathyroid Hormone 1-34 Replacement Therapy in Children with Hypoparathyroidism

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Abstract

Objective.—To determine whether multiple daily injections of PTH 1–34 are safe and effective as long-term therapy for children with hypoparathyroidism.

Study design.—Linear growth, bone accrual, renal function, and mineral homeostasis were studied in a long-term observational study of PTH 1–34 injection therapy in 14 children.

Methods.—Subjects were 14 children with hypoparathyroidism due to autoimmune polyglandular syndrome type 1 (APS-1, N=5, ages 7–12 y) or calcium receptor mutation (CaR, N=9, ages 7–16 y). Mean daily PTH 1–34 dose was 0.75 ± 0.15 $\mu\text{g/kg/day}$. Treatment duration was 6.9 ± 3.1 y (range: 1.5 – 10 y). Patients were evaluated semiannually at the NIH Clinical Center.

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Results.—Mean height velocity and lumbar spine, whole body, and femoral neck bone accretion velocities were normal throughout the study. In the first two years, distal 1/3 radius bone accrual velocity was reduced compared with normal children ($P < 0.003$). Serum alkaline phosphatase correlated with PTH 1–34 dose ($P < 0.006$) and remained normal (235.3 ± 104.8 [SD] U/L, N: 51–332 U/L). Mean serum and 24-h urine calcium levels were 2.05 ± 0.11 mmol/L (N: 2.05–2.5 mmol/L) and 6.93 ± 1.3 mmol/24h (N: 1.25–7.5 mmol/24h), respectively—with fewer high urine calcium levels vs. baseline during calcitriol and calcium treatment ($P < 0.001$). Nephrocalcinosis progressed in 5 of 12 subjects who had repeated renal imaging although renal function remained normal.

Conclusions.—Twice-daily or thrice-daily subcutaneous PTH 1–34 injections provided safe and effective replacement therapy for up to 10 years in children with hypoparathyroidism due to APS-1 or CaR.

Keywords

Hypocalcemia; Hypomagnesemia; Vitamin D; PTH 1–34; teriparatide; APECED; APS-1; autosomal dominant hypocalcemia; ADH; calcium receptor

Hypoparathyroidism remains among the few classic hormonal insufficiency states for which replacement therapy remains widely unavailable (1–3). Although conventional calcitriol and calcium treatment can maintain normal serum calcium, this therapy entails multiple daily pills of calcitriol, calcium, and/or magnesium, and may produce nephrocalcinosis and impair renal function (4,5). These renal complications arise because calcitriol lacks parathyroid hormone's renal calcium-retaining effects and maintains serum calcium principally by increasing gastrointestinal calcium absorption (6–8). To avoid hypercalciuria-induced renal damage, calcitriol dosage must be adjusted to maintain serum calcium at the lowest tolerated level. Hypocalcemia, however, may lead to tetany, laryngospasm, seizures, and potential brain injury, and thus conventional management involves a delicate balance between hypercalciuria and hypocalcemia.

Genetic etiologies of hypoparathyroidism pose unique treatment challenges that, in many cases, cannot be overcome with conventional therapy. Patients with autoimmune polyglandular syndrome type 1 (APS-1)—also called autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy (APECED)—may have intermittent malabsorption causing an inability to absorb conventional therapy (9, 10). Patients with an activating calcium receptor (CaR) mutation exhibit excessive urine calcium even when serum calcium is low and, thus, do not respond well to conventional therapy (11).

PTH 1–34 treatment in adults with CaR, when given once-daily, failed to normalize urine calcium excretion, whereas twice-daily injection provided improved, albeit still abnormal, calcium homeostasis (5). Similar results were observed in a randomized controlled study in children (12). A subsequent study showed safe and effective treatment with twice-daily PTH 1–34 for up to 3 years—with normal linear growth, weight gain, and bone accretion, and high-normal or just above normal bone turnover markers in children (13).

Randomized studies comparing pump delivery of PTH 1–34 with twice-daily injections—in adults (14) and children (15)—showed normal serum and urine calcium and bone turnover markers with pump delivery. Pump delivery seemed particularly promising for future long-term study in children with APS-1 or CaR, in whom concurrent normalization of serum and urine calcium has been difficult to achieve. These and other studies showed that patients with congenital hypoparathyroidism require larger PTH 1–34 dosage (expressed as mcg/kg/day) than patients with post-surgical hypoparathyroidism (5,14,15). In each study, however, PTH 1–34 daily dose requirement decreased as dose frequency was increased from once-daily to twice-daily to pump delivery.

Available long-term PTH treatment data in adults with hypoparathyroidism—treated with twice-daily PTH 1–34 for 3 years (16) or with alternate-day PTH 1–84 plus concurrent conventional therapy for 6 years (17)—have shown normal or slightly below normal serum calcium levels with persistently elevated bone turnover markers.

The current report presents results over a prolonged duration of PTH 1–34 replacement therapy in children with hypoparathyroid—data that are essential because the need for therapy is life-long, PTH 1–34 may have long-term effects on bone accrual, and the assessment of any adverse impact on renal function may take many years to confirm. In this report we describe linear growth, bone accrual rates, mineral homeostasis, and renal function—observed longitudinally for up to 10 years—in 14 PTH 1–34-treated children with hypoparathyroidism due to APS-1 or CaR.

Methods

This IRB-approved observational study was conducted at the National Institutes of Health (NIH), Clinical Center, Bethesda, MD. All participants had hypoparathyroidism due to APS-1 or an activating CaR mutation and were treated with synthetic human PTH 1–34 as previously described (6). Mutational analysis was performed in commercial laboratories (Gene DX, Athena Diagnostics), except for patients A, C, and I, whose mutations were identified at NIH Clinical Center (Table 1). Renal CT and ultrasound scans were rated by two radiologists from the NIH Clinical Center who reviewed and graded all scans independently and then together reviewed a subset of scans a third time to resolve inconsistencies. The following levels of calcifications were attributed to each scan according to methods previously described (18, 19): 0, no calcifications; 1, mild, 2, moderate, and 3, severe nephrocalcinosis. Repeated renal imaging was not included prospectively in the study protocol, but results of repeat renal imaging were analyzed for the 12 subjects in whom they were available. Imaging results for cerebral calcification were also analyzed when available.

At study initiation, subjects had a 1-week inpatient stay for baseline evaluation, discontinuation of conventional therapy, and start of twice-daily PTH 1–34 injections. Follow-up outpatient evaluations, performed semiannually, included review of interim history, a physical examination and repeat of the pre-PTH baseline studies, including height (mean of 3 Harpenden Stadiometer measurements), bone density by dual energy radiograph absorptiometry (DXA), and 3 days of morning blood (pre-PTH injection) and 24-h urine collection for mineral levels (calcium, phosphate, and magnesium) and measurement of

bone turnover markers (serum alkaline phosphatase, and urine pyridinoline and deoxypyridinoline). Measurements were performed at NIH Clinical Center or Mayo Medical Labs, Rochester, MN as previously described (15). Between NIH visits, blood and urine mineral levels were measured monthly by the patient's local physician, with results sent to the study PI for PTH 1–34 dose adjustment as needed.

Height Z-scores were determined according to CDC reference data (20). Adult height criterion was bone age ≥ 16 in boys or ≥ 15 in girls and height velocity ≥ 2 cm/y. Bone mineral density of the lumbar spine, radius, hip and whole body were measured on a QDR 4500 scanner (Hologic, Inc. Bedford MA). Bone density and content Z-scores, and bone-accrual velocity and height-velocity Z-scores, were determined from Bone Mineral Density in Childhood Study (BMDCS) standards, and height adjustment was applied to all Z-score results (21–23).

Statistical Analyses

Sex- and pubertal status-specific BMC and aBMD velocity Z-scores—adjusted for baseline age, height, weight, BMC/aBMD, and interval weight and height velocity—were generated from the BMDCS (21, 22); prediction equations were based upon generalized mixed effects models with an independent correlation structure (submitted for publication). Height velocity Z scores were based on age, sex, and pubertal timing specific reference ranges (23). Differences between PTH-treated hypoparathyroid patients and BMDCS reference standards—in height velocity Z-score and in BMC and aBMD velocity Z-scores for each yearly interval—were assessed using generalized estimating equations with independent correlation structure. Patients A and I were not included in these bone mineral accretion analyses because most of their DXA scans were performed using Hologic 2000 scanner.

Longitudinal trajectories for each outcome measure were estimated using the linear mixed model approach, which can account for both the mean trajectories (estimated fixed effects) and individual trajectories (addition of fixed and individual patient's random effects). Time of study was divided into two intervals—baseline to 1 year and >1 to 10 years. A dosage fixed effect accounted for varying PTH 1–34 dosage. A diagnostic subgroup effect accounted for potential differences between APS-1 and CaR. Urine excretion values were adjusted to BSA of 1.73 m^2 . Between-group APS-1 vs. CaR distributional differences (testing mean differences) in the calcium-phosphate product ($\text{Ca} \times \text{P}$), and in serum and urine calcium levels, were analyzed by two-sample t-test.

Results

The subjects were 14 children with severe hypoparathyroidism (Table 1). Except for patient I, who was receiving 1-alpha calcidiol at baseline, all were receiving calcium and calcitriol at study entry.

Five patients had APS-1 (ages 7–12 y; 1 male) and 9 had CaR (ages 7–16 y; 4 male). Among APS-1 patients, A, B, and D had the common AIRE gene mutation c.967_979del13, and A and E had the R257X mutation. Patients B and E were homozygous for AIRE mutations, and A and D, who had a novel mutation (24), were compound heterozygotes.

AIRE mutations were not detected in patient C, who had hypoparathyroidism and Addison's disease, as did an older brother. Sequencing included AIRE exomes, flanking intron-exon regions, and copy number variation to identify deletions not found by Sanger sequencing (10). Among CaR patients, all had activating mutations, which were novel in F, I, J, and N. Hypoparathyroidism was diagnosed at age 2–5 y in all APS-1 patients, and in infancy in all CaR patients except J and N. Patients K and L are brothers. Before medical diagnosis, hypoparathyroidism was accompanied by tetany (A, B, C, I) and/or seizures (A, B, D, E, I, J, M, N) for extended periods.

At study baseline, all patients had normal renal function and all who had renal imaging (B–N), had nephrocalcinosis. Patient I was diagnosed with nephrocalcinosis by ultrasound prior to study entry. Greater height deficits were observed in APS-1 versus CaR patients ($P < .04$). Patients B, C, D, G, H had a history of poor linear growth, with B, G, and H having height Z scores < -1.5 . At baseline visit, patient C was diagnosed with growth hormone deficiency and treated with growth hormone. All patients except A had chronic magnesium deficiency.

After baseline evaluation, patients A and I participated in a six-month randomized crossover study (12) comparing once (QD) and twice-daily (BID) PTH 1–34. The sequence for patient A was QD/BID PTH and for patient I was BID/QD PTH. This dose study was followed by a 3-year randomized, parallel NIH study comparing twice-daily PTH 1–34 with calcitriol plus calcium (13). They (A, I) received twice-daily PTH 1–34 injections for 3 years before continuing twice-daily therapy in this observational study.

Data for current analysis ended when participants reached age 21 (patients A, C), discontinued the study (patient I—after 6.5 y), or were discharged from the study at its conclusion (patients B, D–H; J–N). Subsequently, most patients have continued receiving PTH 1–34 as rhPTH1–34 (teriparatide, Forteo®, approved for osteoporosis treatment).

PTH Therapy

Twice-daily subcutaneous PTH 1–34 injections were begun at the dose of 0.5 µg/kg body weight/day. Magnesium supplements were continued as needed to correct hypomagnesemia. Calcium supplements were discontinued—but permitted emergently for tetany—except for patient N who was given 300 mg elemental calcium carbonate with meals because of milk protein allergy and low dietary calcium.

Four patients with CaR, and patient B with APS-1, received thrice-daily PTH 1–34 during some or all of the study. Thrice-daily treatment began at baseline in patient B, at study year 0.5 in patients K and N, at year 2 in L, and at year 6.5 in H. Twice daily PTH injections led to bone pain in patient K which disappeared with smaller PTH doses given thrice daily. Patient B, a 9 yo child with APS-1, including Addison's disease, hypoparathyroidism, malabsorption, nephrocalcinosis and lithiasis had a history of multiple hospitalizations for hypocalcemia, asthma, and adrenal insufficiency. The purpose of thrice daily was hypothetically to provide a more a physiologic approach to therapy by decreasing the total daily dose and potentially to lower urine calcium excretion and reduce serum calcium fluctuation. In patient H, with the most data on each regimen, twice-daily PTH 1–34 (0.5–6 y) vs. thrice-daily (6.5–10 y) reduced mean PTH 1–34 dose from 40.2 µg/day (1.4 µg/kg/

day) to 19.5 µg/day (0.54 µg/kg/day, $P<0.0003$), reduced serum calcium, magnesium, and alkaline phosphatase (from 224 to 154 U/L) and 24-h urine magnesium (data not shown, all $P<0.01$), without significant change in 24-h urine calcium (8.7 to 7.7 mmol/24h, $P=NS$).

Mean PTH 1–34 dose was 0.35 ± 0.08 µg/kg/dose or 0.75 ± 0.15 µg/kg/day. PTH dose range per injection was 4–54 µg (0.1–0.75 µg/kg/injection), with daily total 8–108 µg (0.2–1.49 µg/kg/day), given subcutaneously twice or thrice daily. Mean dose did not differ according to diagnostic group. Individual PTH 1–34 dose requirements appeared to be influenced by severity of hypoparathyroidism for APS-1 patients, and by functional severity of CaR mutation for the CaR patients. Mean PTH 1–34 treatment duration was 6.9 ± 3.1 y (range: 1.5 – 10 y).

PTH 1–34 dose was adjusted to the lowest urine calcium level that could be achieved without symptomatic hypocalcemia, which resulted in serum calcium levels at the lower normal or slightly below normal range. Injection sites included arm, thigh, or abdomen—depending on patient preference—with instruction to rotate sites. During initial inpatient dose adjustment, serum and urine calcium were measured daily or twice-daily. Thereafter, outpatient monitoring frequency was reduced progressively to monthly, if feasible. However, hypo- or hypercalcemia episodes often prompted more frequent blood calcium monitoring.

Participation in this long-term observational study was interrupted for 6 months in 9 patients—between study year 0.5–1 in patient F and study years 4–10 in patients B, C, E, G, H, K, L, and M—to take part in a 6-month randomized, crossover study comparing PTH 1–34 delivery by pump vs twice-daily injection (15). The current analyses excluded data from these 3 months of PTH 1–34 pump delivery.

Linear Growth and Bone Mineral Accrual

APS-1 and CaR patients did not differ significantly in linear growth or bone mineral accrual Z-scores during PTH 1–34 treatment, and data for these outcomes have been combined for presentation (Figure 1). Mean height velocity (Ht-Vel) was normal for age throughout the study.

All patients except B, I, and J reached adult height during PTH 1–34 treatment, and patients B and I attained adult height (measured at NIH) while receiving rhPTH1–34 (Forteo) after study conclusion. Patient J had not attained adult height at time of report. APS-1 patients B, C, and D were 8–12 cm below their sex-adjusted mid-parental height (MPH) at adult height. By contrast, all CaR patients except G attained MPH, and patients H and M exceeded MPH by 4 and 6 cm, respectively.

Lumbar spine, whole body, and femoral neck bone accretion velocities remained normal throughout treatment. In study years 1 and 2, bone mineral density accrual velocity at the distal 1/3 radius was reduced vs. age-matched reference values (1/3 Radius aBMD Vel Z, Figure 1, $P<0.05$) but normalized in years 3–5. During study years 2 and 4, total hip bone accrual Z-scores were greater than average ($P<0.01$).

Bone Turnover Markers and Vitamin D

Mean $\pm$ SD serum alkaline phosphatase remained normal (235.31 ± 104.758 U/L, N: 51–332 U/L). Urine pyridinoline and deoxypyridinoline did not change significantly during treatment (494.6 ± 237.4 and 209.9 ± 99.5 nmol/mmol creatinine, respectively). All three bone markers correlated with the PTH 1–34 dose ($P < 0.003$) and there was no diagnostic group effect. Mean $\pm$ SD 1,25-dihydroxyvitamin D₃ (63.5 ± 8.4 pg/mL, N: 24–86 pg/mL) and 25-hydroxyvitamin D₃ (38 ± 7.1 ng/mL, N: 25–80 ng/mL) levels remained normal without significant change during treatment and both were significantly higher in APS-1 vs. CaR ($P < 0.02$; Table 2 [available at www.jpeds.com]). Mean 25-hydroxyvitamin D₃ was inversely related to PTH dose ($P < 0.05$).

Serum and Urine Mineral Levels

Serum and 24-h urine calcium correlated with the PTH 1–34 dose ($P < 0.001$). Mean $\pm$ SD serum and 24-h urine calcium levels during treatment were 2.05 ± 0.11 mmol/L (N: 2.05–2.5 mmol/L) and 6.93 ± 1.3 mmol/d (N: 1.25–7.5 mmol/d), respectively. Mean serum calcium decreased significantly from study baseline to 1 y (-0.13 mmol/L, $P < 0.04$), as did urine calcium (-1.6 mmol/d, $P < 0.03$), and did not change significantly thereafter (> 1 –10 y).

During calcitriol and calcium treatment at study baseline, analysis of the distribution of serum and urine calcium showed more frequent low, and fewer normal, serum calcium levels in CaR vs. APS-1 patients—with only minor differences in the frequency of elevated urine calcium levels between diagnostic groups (Figure 2, A). PTH 1–34 treatment (0.5–10 y) produced fewer normal or high, and more low, serum calcium levels ($P < 0.001$), with less frequent hypercalciuria—from 58% to 24% of measurements for APS1 and from 61% to 52% for CaR—and more frequent normal urine calcium (Figure 2, B, $P < 0.001$).

Mean serum phosphate and magnesium levels decreased significantly during PTH 1–34—with most of the decrease occurring after year 1 (data not shown, $P < 0.01$). Serum phosphate levels were related inversely to PTH 1–34 dose ($p < 0.01$), with no diagnostic group effect. By contrast, urine phosphate and serum and urine magnesium levels were not related to PTH 1–34 dose, and urine phosphate and magnesium did not change significantly during treatment.

APS-1 vs CaR Serum and Urine Mineral Levels

Serum calcium and magnesium were significantly higher, and serum phosphate significantly lower, for APS-1 vs CaR patients—mean $\pm$ SD serum calcium, 2.08 ± 0.18 and 1.95 ± 0.22 mmol/L, respectively ($P < 0.0001$); serum magnesium, 0.72 ± 0.07 and 0.65 ± 0.14 mmol/L ($P < 0.0001$); and serum phosphate, 1.55 ± 0.35 and 1.69 ± 0.36 mmol/L ($P < 0.02$). Correspondingly, urine calcium, magnesium and phosphate were significantly lower for APS-1 vs. CaR patients (all $P < 0.001$; Table 2)

Calcium-Phosphorus (Ca x P) Product

Mean Ca x P product was 41 ± 8 , and 93% of determinations were < 55 mg²/dL² (< 4.4 mmol²/L²)—a threshold used in managing hypoparathyroid adults to lower renal calcification risk (25, 26). However, Ca x P product did not correlate with 24-h calcium excretion, and high 24-h urine calcium values were observed with both high and low Ca x P

values (Table 3). The distribution of Ca x P values was skewed toward higher levels for CaR vs. APS-1 patients, which produced higher mean Ca x P in the CaR group (42 ± 7 vs. 39 ± 9 ; $P < 0.02$, Figure 3 [available at www.jpeds.com]).

Creatinine Clearance

Mean $\pm$ SD creatinine clearance, corrected for body surface area (102.63 ± 23.15 mL/min/ 1.73 m²), showed no significant change over time, and no dose or diagnostic group effect.

Nephrocalcinosis

Seven patients with CaR (G, H, I, K, L, M, N) had renal ultrasounds (US) showing nephrocalcinosis before study entry, between ages 1.3–5 y for all patients except L (8 y) and G (12 y). At study baseline, kidney computed tomography (CT) in eight patients with CaR (G–N) showed nephrocalcinosis in all except Pt I whose baseline CT scan was missing and not available for this analysis. She was diagnosed with nephrocalcinosis by ultrasound 2 years prior to protocol entry by her local nephrologist. Patient F with CaR had renal US at baseline showing moderate nephrocalcinosis. CT scans at baseline in APS-1 patients B–E showed mild nephrocalcinosis.

Repeat renal imaging in 12 patients showed progression of nephrocalcinosis in 5 patients (3 CaR and 2 APS-1). APS-1 patients C and E showed no progression at study year 8.5 and 9.5, respectively and patients B and D progressed to moderate nephrocalcinosis at years 4 and 9, respectively. CaR patients H and K, with moderate nephrocalcinosis at baseline, had severe nephrocalcinosis at year 6 and 3.5, respectively. Patient M with mild nephrocalcinosis progressed to moderate at year 4.5 years. By contrast, CaR patients F, G, J, L, N showed no progression of renal calcifications. Patient G, with moderate nephrocalcinosis (at 0,1,3,5 yrs) by CT or US, had a pattern of mild nephrocalcinosis on US at study year 6. Four patients had nephrolithiasis diagnosed at baseline (B, F) or prior to study entry (E, H). Patients C, G, H, K, N had nephrolithiasis seen on ultrasound between study years 4–10.

Both renal CT and ultrasound imaging were performed during this study; concurrent CT and ultrasound in patients H (baseline) and K (3.5 yr) revealed moderate nephrocalcinosis by CT but severe nephrocalcinosis by US. Concurrent CT and ultrasound in patient M at study year 4.5 revealed severe nephrocalcinosis by CT and moderate nephrocalcinosis by ultrasound.

Cerebral Calcifications

Patient B, with normal baseline brain CT, showed a small calcification in the left globus pallidus at year 4. Patient M, who had evidence of brain calcifications from early childhood, had repeat brain scans (at study years 1 and 4) showing similar calcifications in basal ganglia and thalami, and patient F had normal brain scans at baseline and year 4. Other patients had brain CT scans at various timepoints throughout the study. Only patients E and F had no evidence of calcifications. (Table 4; available at www.jpeds.com)

Adverse Events Related to APS-1

APS- 1 is characterized by at least two of the following major criteria: chronic mucocutaneous candidiasis, adrenal insufficiency, and hypoparathyroidism (9,10). Patients

A, B, D had chronic fungal infections diagnosed between 6–24 months. Patients A–E had Addison’s disease and malabsorption which were diagnosed between ages 5 to 13 yo. APS-1 patients C and D had poor weight gain associated with intermittent malabsorption during study years 3 and 9, respectively. Patient D required TPN and a feeding tube during years 9–10. Patients A and E were diagnosed with ovarian failure (elevated FSH) at ages 11 and 10 y, respectively. Both were placed on estradiol to initiate breast development and later placed on hormonal replacement therapy by pills or patch. Patient B had reactive-airway disease, diagnosed at age 3. CT imaging of the chest for evidence of autoimmune pneumonitis was negative at age 9 yo. Patient C, who had failure to thrive and malabsorption, received 5 years of GH treatment for GH deficiency beginning at study entry. Patient C also had pubertal delay with a 2–3-year bone age delay, and received monthly testosterone injections from age 14 until onset of puberty at age 17. Three patients (B, D, E) had biopsy proven chronic active hepatitis, diagnosed at 8, 10, and 4 years old, respectively.

In patients with intermittent malabsorption, therapies that bypass the GI tract were used to manage endocrinopathies. Pubertal initiation in patients A and E was done with a low-dose transdermal estrogen and, in patient C, with IM testosterone. Vitamin D deficiency was treated with IM vitamin D. Addison’s disease was treated with thrice-daily oral steroid doses at a higher dose (15 mg/m²) than typically required for physiologic replacement.

The inclusion of regular screening for autoantibodies helped us to identify a higher risk of autoimmune diseases associated with APS-1. We relied on classic hormonal testing to diagnose Addison’s disease, gonadal failure, or thyroiditis. Patient D had a positive adrenal antibody panel at age 4 yo and was diagnosed with Addison’s disease at age 11 yo. Patients C and D had positive thyroid antibodies when thyroiditis was diagnosed. Patient A had elevated thyroglobulin antibodies at age 17 yo with no other biochemical evidence of thyroid disease.

Adverse Events

No study-related serious adverse events occurred during the study. Patient K developed tibial bone pain when given PTH 1–34 twice daily which resolved with thrice daily PTH 1–34. Patients A, C, E, M experienced recurrent headaches and A, C, D complained of muscle weakness. Patient L suffered a finger fracture while playing basketball during the first year of PTH 1–34 therapy. Patients B and I had asthma which was exacerbated by upper respiratory infections and exercise. Enamel hypoplasia was observed in all patients (A–N) at study baseline.

Quality of Life

Quality of life measures were not included in this study, but distinct differences were observed between CaR and APS-1 groups. Most CaR patients did well academically—F, G, H, I, and M were among the top students in their class, and all went to college. APS-1 patients did not attain the academic level of CaR patients. Multiple co-morbidities led to poor attendance at school; patients B, C, and D had extended home schooling. APS-1 patients reported more fatigue, especially those with Addison’s disease—a symptom, however, which has also been reported in adults with hypoparathyroidism (27).

Discussion

In up to a decade of observational study in 14 children with severe genetic hypoparathyroidism, we have shown that PTH 1–34 given by twice- or thrice-daily subcutaneous injection is safe and effective—with normal linear growth, bone accrual, and kidney function, and normal or slightly below normal serum calcium values in nearly all measurements. 24-Hour urine calcium excretion was normal in only 40% of values at baseline on conventional therapy. During PTH therapy, 76% of urine calcium in APS-1 and 48% of urine calcium measurements in CaR patients were within normal limits. Nephrocalcinosis was present at baseline in all patients who had imaging and progressed in 5 of 12 patients (2 with APS-1, and 3 with CaR) who underwent repeated renal imaging, which highlights the difficulty in normalizing calcium homeostasis with twice-daily or thrice-daily PTH 1–34 injections. This observed progression in nephrocalcinosis, however, was not accompanied by a decrease in renal function.

PTH 1–34 dose requirements during this study were consistent with prior studies, which have shown that treatment of genetic hypoparathyroidism requires higher PTH 1–34 dosage—whether administered twice-daily or by pump—compared with treatment of postsurgical hypoparathyroidism (5,14,15).

During the first two years of PTH 1–34 treatment, a transient reduction in bone accretion rate was observed at the distal radius. This skeletal area is comprised mostly of cortical bone, which exhibits decreased bone density in adults with PTH excess (28). Future studies with pQCT, a measure of volumetric BMD, may permit distinction between cortical vs trabecular bone effects and provide a greater understanding of the effects of PTH 1–34 in hypoparathyroid children who, unlike adults with hypoparathyroidism (29), have normal bone turnover and do not manifest high cortical bone density. From a clinical perspective, the subsequent normal trajectory of bone accrual—at all observed sites, and to adult height in most subjects—is reassuring.

A serum Ca x P product $<55 \text{ mg}^2/\text{dL}^2$ ($<4.4 \text{ mmol}^2/\text{L}^2$) has been advocated to lower risk of renal calcification in adults with hypoparathyroidism and risk of arterial calcification in chronic kidney disease (2,25,26). However, because variable serum phosphate levels primarily determine Ca x P product, Uhlig et al have advised against using Ca x P product for decision-making in renal insufficiency (30). Further, Ca x P product has not proven to be a better predictor than blood and urine Ca for renal calcification (31), and has not been used in pediatric hypoparathyroidism management in this or other studies. The current study showed no correlation between the morning pre-PTH 1–34 dose Ca x P product and 24-h urine calcium—some of the highest urine calcium levels were associated with the lowest Ca x P product, and $>90\%$ of Ca x P values were $<55 \text{ mg}^2/\text{dL}^2$, despite persisting hypercalciuria in 24% (APS-1) to 52% (CaR) of urine calcium measurements.

Rodent PTH carcinogenicity studies indicating dose-dependent osteogenic sarcoma has led to black box warnings for both currently marketed forms of PTH—Forteo® (rhPTH1–34) and Natpara® (rhPTH1–84) (32). Although no increased osteosarcoma risk has appeared during 15 years of rhPTH 1–34 use in $>500,000$ patients with osteoporosis, the toxicology

data and black box warning remain a continuing concern and an obstacle to pediatric use outside of research settings. Nonetheless, such research is needed because—in severe pediatric hypoparathyroidism—recurrent ER visits, tetany, seizures, and renal damage remain an ongoing problem with conventional treatment. Although pediatric exposure to PTH 1–34 remains limited (96 patient-years in the current study and 217 patient-years for all studies from Winer et al, including several who were followed into early adulthood), to our knowledge there have been no cases of osteosarcoma during pediatric use by our group or others.

Despite the overall long-term safety and efficacy of PTH 1–34 in this study, multiple PTH injections are pharmacologic, not physiologic, replacement. In short-term studies, we have shown that continuous delivery by insulin pump produces more physiologic calcium profiles in blood and urine, reduces markers of bone turnover and the need for magnesium supplementation, and substantially reduces the overall PTH 1–34 dose requirement (14,15). We hypothesize that such an approach will represent a further advance toward the goal of truly physiologic replacement.

The progression of nephrocalcinosis observed in 5 of our patients had at least two potential causes—the cumulative effect of IV calcium administration during ER visits for tetany (or for malabsorption or adrenal insufficiency in APS-1 patients), and the unphysiologic nature of intermittent PTH 1–34 replacement by multiple daily injection. Early evidence suggests that the second of these causes may be improved by pump delivery. To address the problem of intermittent hypo- or hypercalcemia, however, a self-measured blood calcium device—analogue to the self-measured blood glucose devices for diabetes—is an urgent unmet need. Such a device could permit frequent dose adjustment, reduce overtreatment, and reduce IV calcium administration during ER visits for tetany or seizure.

In conclusion, PTH 1–34 administered twice or thrice daily represents a safe and effective long-term therapy for children with severe hypoparathyroidism due to APS-1 or CaR.

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Bibliography

1. Horowitz MJ, Stewart AF. Hypoparathyroidism: Is it time for replacement therapy? *J Clin Endocrinol Metab* 2008; 93:3307–9 [PubMed: 18772463]
2. Bollerslev J, Rejnmark L, Marococchi C, Shoback D, Sitges-Serra A, Van Biesen W, et al. European Society of Endocrinology Clinical Guideline: Treatment of Chronic Hypoparathyroidism in adults. *European Journal of Endocrinology* 2015;173: G1–G20. [PubMed: 26160136]
3. Winer KK. Perspectives on the Search for a True Physiologic Replacement Therapy for Hypoparathyroidism. *European Endocrinology* 2016; 12:47–8. [PubMed: 29632590]

4. Mancilla EE, De Luca F, Ray K, Winer KK, Fan GF. A Ca (2+) sensing receptor mutation causes hypoparathyroidism by increasing receptor sensitivity to CA2+ and maximal signal transduction. *Pediatr Res*. 1997; 42:443–7
5. Winer KK, Yanovski JA, Sarani B, Cutler GB, Jr. A randomized, crossover trial of once-daily versus twice-daily human parathyroid hormone 1–34 in the treatment of hypoparathyroidism. *J Clin Endocrinol Metab* 1998; 83:3480–3486. [PubMed: 9768650]
6. Winer KK, Yanovski JA, Cutler GB, Jr. Synthetic human parathyroid hormone 1–34 vs calcitriol and calcium in the treatment of hypoparathyroidism: Results of a randomized crossover trial. *JAMA* 1996; 276:631–636 [PubMed: 8773636]
7. Underbjerg L, Sikjaer T, Mosekilde L, Rejnmark L. Cardiovascular and renal complications to postsurgical hypoparathyroidism: a Danish nationwide controlled historic follow-up study. *J Bone Miner Res* 2013;28:2277–85. [PubMed: 23661265]
8. Mannstadt M, Bilezikian J, Thakker R., Hannan FM, Clarke BL, Rejnmark L, et al. Hypoparathyroidism. *Nature Disease Primers* 2017; 97:4507–14.
9. Husebye ES, Perheentupa J, Rautemaa R, Kämpe O. Clinical manifestations and management of patients with autoimmune polyendocrine syndrome type I. *J Intern Med* 2009;265:514–29 [PubMed: 19382991]
10. Ferre EM, Rose SR, Rosenzweig SD, Burbelo PD, Romito KR, Niemela JE, et al. Redefined clinical features and diagnostic criteria in autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. *JCI Insight* (2016). doi:10.1172/jci.insight.88782.
11. Lienhardt A, Bai M, Lagarde JP, Rigaud M, Zhang Z, Jiang Y, et al. Activating mutations of the calcium-sensing receptor: management of hypocalcemia. *J Clin Endocrinol Metab* 2001; 86:5313–23. [PubMed: 11701698]
12. Winer KK, Sinaii N, Peterson D, Sainz B, Jr, Cutler GB, Jr. Effects of Once Versus Twice-Daily Parathyroid Hormone 1–34 Therapy in Children with Hypoparathyroidism. *J Clin Endocrinol Metab* 2008; 93:3389–95. [PubMed: 18492754]
13. Winer KK, Sinaii N, Reynolds J, Peterson D, Dowdy K, Cutler GB, Jr. Long-Term Treatment of 12 Children with Chronic Hypoparathyroidism: A Randomized Trial Comparing Synthetic Human Parathyroid Hormone 1–34 *versus* Calcitriol and Calcium. *J Clin Endocrinol Metab* 2010; 95:2680–2688. [PubMed: 20392870]
14. Winer KK, Zhang B, Shrader JA, Smith M, Albert PS, Cutler GB Synthetic Human Parathyroid Hormone 1–34 Replacement Therapy: A Randomized Crossover Trial Comparing Pump Versus Injections in The Treatment of Chronic Hypoparathyroidism. *J Clin Endocrinol Metab* 2012; 97:391–9. [PubMed: 22090268]
15. Winer KK, Fulton K, Albert PS, Cutler GB. Effects of Pump versus Twice-Daily Injection Delivery of Synthetic Parathyroid Hormone 1–34 in Children with Severe Congenital Hypoparathyroidism. *J Pediatr* 2014; 165:556–653. [PubMed: 24948345]
16. Winer KK, Ko CW, Reynolds J, Dowdy K, McGarvey C, Cutler GB. Long-term treatment of hypoparathyroidism: a randomized controlled study comparing parathyroid hormone (1–34) *versus* calcitriol and calcium. *J Clin Endocrinol Metab* 2003; 88:4214–20. [PubMed: 12970289]
17. Rubin MR, Cusano NE, Fan WW, Delgado J, Zhang C, Costa AG, et al. Therapy of hypoparathyroidism with PTH (1–84): a prospective six-year investigation of efficacy and safety. *J Clin Endocrinol Metabol* 2016; 101:2742–2750.
18. Patriquin H, Robitaille P Renal calcium deposition in children: sonographic demonstration of the Anderson-Carr progression. *Am J Roentgenol*.1986; 146:1253–6. [PubMed: 3518370]
19. Boyce AM, Shawker TH, Hill SC, Choyke PL, Hill MC, James R, Yovetich NA, Collins MT, Gafni RI. Ultrasound is superior to computed tomography for assessment of medullary nephrocalcinosis in hypoparathyroidism. *J Clin Endocrinol Metab*. 2013 98:989–94. [PubMed: 23348401]
20. Kuczmarski RJ, Ogden CL, Guo SS, Grummer-Stawn LM, Flegal KM, Mei Z, et al. 2000 CDC Growth Charts for the United States: methods and development. *Vital Health Statistics* 2002;246:1–190.
21. Zemel BS, Kalkwarf HJ, Gilsanz V, Lappe JM, Oberfield S, Shepherd JA, et al. Revised reference curves for bone mineral content and areal bone mineral density according to age and sex for black

- and non-black children: results of the bone mineral density in childhood study. *J Clin Endocrinol Metab* 2011;96:3160–9. [PubMed: 21917867]
22. Zemel BS, Leonard MB, Kelly A, Lappe JM, Gilsanz V, Oberfield S, et al. Height adjustment in assessing dual energy x-ray absorptiometry measurements of bone mass and density in children. *J Clin Endocrinol Metab* 2010; 95:1265–73. [PubMed: 20103654]
23. Kelly A, Winer KK, Kalkwarf H, Oberfield SE, Lappe J, Gilsanz V et al. Age-based reference ranges for annual height velocity in US children. *J Clin Endocrinol Metab* 2014;99:2104–12. [PubMed: 24601728]
24. Huibregtse KE, Wolfgram P, Winer KK, Connor EL Polyglandular autoimmune syndrome type I - a novel AIRE mutation in a North American patient. *J Pediatr Endocrinol Metab* 2014; 27:1257–60. [PubMed: 24945421]
25. Mannstadt M, Clarke BL, Vokes T, Brandi ML, Ranganath L, Fraser WD, et al. Efficacy and safety of recombinant human parathyroid hormone (1–84) in hypoparathyroidism (REPLACE): a double-blind, placebo-controlled, randomized phase 3 study. *Lancet Diabetes Endocrinol* 2014; 1:275–83.
26. Bilezikian JP, Brandi ML, Cusano NE, Mannstadt M, Rejnmark L, Rizzoli R, et al. Management of Hypoparathyroidism. *J Clin Endocrinol Metabol* 2016; 101:2313–2324.
27. Cusano NE, Rubin MR, McMahon DJ, Irani D, Anderson L, Levy E, et al. PTH (1–84) is associated with improved quality of life in hypoparathyroidism through 5 years of therapy. *J Clin Endocrinol Metabol* 2014;99: 3694–99.
28. Duan Y, DeLuca V, Seeman E Parathyroid Hormone deficiency and Excess: Similar Effects on Trabecular Bone but Differing Effects on Cortical Bone. *J Clin Endocrinol Metabol* 1999; 84:718–22.
29. Rubin MR, Dempster DW, Zou H, Shane E, Nickolas T, Sliney J, Jr, et al. Dynamic and structural properties of the skeleton in hypoparathyroidism. *J Bone Miner Res* 2008; 23:2018–2024. [PubMed: 18684087]
30. Uhlig K, Berns JS, Kestenbaum B, Kumar R, Leonard M, Martin KJ et al. KDOQI US Commentary on 2009 KDIGO Clinical Practice Guideline for the Diagnosis, Evaluation and Treatment of CKD Mineral and Bone Disorder (CKD-MBD). *A J Kidney Dis* 2010; 55:773.
31. O'Neill WC. The fallacy of the calcium-phosphorus product. *Kidney Int* 2007; 72:792–796. [PubMed: 17609689]
32. Tashjian AH, Jr, Golzman D Perspective on the Interpretation of Rat Carcinogenicity Studies for Human PTH 1–34 and Human PTH 1–84. *J Bone Min Res* 2008; 23:803–811.

Figure 1.

Boxplots show height velocity and 5 measures of bone accrual velocity, shown as velocity Z scores (Vel-Z) vs study year. Boxplots indicate median, interquartile intervals, and maximum and minimum values for each measure, except where outlier values (> 3 SD from remaining values) have been shown individually as circles. HT (annualized height velocity); WBBMC (whole body bone mineral content); aBMD (areal bone mineral density); LS (lumbar spine); FN (femoral neck); 1/3 radius (distal 1/3 radius).

Figure 2b

Figure 2.

The distribution of serum calcium (left panel) and 24-h urine calcium (right panel) among low, normal, and high values for each diagnostic group (APS-1 vs. CaR). Figure 2a represents serum and urine calcium distributions measured during baseline evaluation while patients received vitamin D analogs and calcium supplementation. Figure 2b represents serum and urine calcium distributions from 0.5 to 10 years of PTH 1–34 therapy.

Figure 3.
The distribution of serum calcium x phosphorus (Ca x P) product for the two diagnostic groups from 0.5 to 10 years of PTH 1–34 therapy.

Table 1

Baseline Characteristics of Fourteen Children with APS-1 or CaR

Patient Letter	Age (Y)	Disease Duration (Y)	Sex	Time on Protocol (Y)	Calcitriol dose (mcg/d ay)	Alkaline Phosphatase (SI-332 U/L)	Calcium (2.05-2.5 mmol/L)	Phosphorus (0.8-1.65 mmol/L)	Magnesium (0.65-1.05 mmol/L)	25-OH Vitamin D (25-80 ng/mL)	Urinary Calcium (1.25-7.5 mmol/24 h)	Urinary Phosphorus (12.9-42 mmol/24h)	Urinary Magnesium (3.0-4.25 mmol/24h)	Coarctate Clearance (90-125 mL/min)	Mutation	Weight Z score	Height Z score	LS RMD B-A/J Z
A	11	9	F	10	0.5	154.7	2.3	1.8	0.8	NA	9.7	18.4	6.3	113.9	R257X δ	1.26	-0.76	1.89
B	9	7.5	F	5.5	1.0	138.2	1.7	2.5	0.7	24	7.5	23.8	5.5	118.4	c.967_979del13	-1.07	-1.84	0.41
C	12	7	M	9	2.6	115.0	2.3	2.0	0.8	29.5	12.8	38.6	3.2	123.9	ϕ	-0.92	-0.74	1.11
D	7	4	F	10	0.5	178.3	2.1	2.2	0.8	19.7	7.8	35.4	5.8	89.8	c.20_1154del96 $\delta\delta$	-1.23	-1.15	0.78
E	8	5	F	10	0.8	268.0	2.1	2.4	0.7	NA	12.6	18.7	1.9	115.5	R257X	-0.39	-0.31	0.01
F	16	15.5	F	1.5	0.5	50.2	2.0	1.7	0.7	39	8.3	24.8	6.0	117.4	A840V δ	1.19	-0.03	2.06
G	14	13.5	F	6.5	0.8	149.7	2.0	2.4	0.9	19	10.6	51.5	5.7	112.0	N802I	-1.49	-2.08	-0.59
H	7	7	F	10	0.4	113.5	1.8	2.0	0.6	NA	8.9	12.9	4.1	129.7	F832S	-0.65	-1.56	0.05
I	6	6	F	6.5	$\delta\delta$	167.5	2.1	2.2	0.5	NA	9.7	30.9	6.7	76.0	F806S δ	0.09	0.49	1.15
J	9	1	M	1.5	0.5	206.0	2.2	1.4	0.8	36	9.6	26.4	8.1	127.9	A784V δ	-0.12	0.06	0.70
K	14	14	M	4	0.7	312.5	2.6	1.5	0.7	32	9.8	40.9	5.8	98.4	E604K	0.29	-0.31	3.30
L	15	14.5	M	4	1.3	167.7	2.4	1.6	0.7	37	4.6	34.8	5.1	100.7	E604K	1.87	0.82	0.90
M	12	11	F	7.5	2.0	284.0	2.1	2.8	0.6	26	10.4	16.1	12.93	106.0	E127K	-0.53	-0.60	-0.33
N	7	5	M	10	0.6	202.0	2.3	1.9	0.7	NA	15.8	21.9	8.3	114.1	F128C δ	-1.31	-1.29	-1.01
Mean	10.5	8.57		6.86	0.94	179.09	2.14	2.03	0.71	29.13	9.87	28.22	6.11	110.26				
SEM	0.90	1.19		0.85	0.18	19.11	0.06	0.11	0.03	1.99	0.71	2.93	0.69	3.99				

Y= years

* =Novel Mutation

** = 4 mcg alphacalcidol

ϕ =no mutation found

δ = c.967_979del13 second mutation

Normal range shown in parentheses

A-E APS-1

F-N CaR

Table 2:

Comparison of Biomarkers in 14 Children with APS-1 vs CaR during PTH 1–34 Therapy

Biomarker	APS-1: Mean (SD)	CaR: Mean (SD)	P-value
Serum Calcium (2.05–2.5mmol/L)	2.08 (0.18)	1.95 (0.223)	<0.0001
Serum Phosphorus (0.8–1.65 mmol/L)	1.55 (0.35)	1.69 (0.36)	<0.02
Serum Magnesium (0.65–1.05 mmol/L)	0.72 (0.07)	0.65 (0.139)	<0.0001
Urine Calcium (1.25–7.5 mmol/24h)	6.07 (2.60)	7.68 (2.97)	<0.001
Urine Phosphorus (12.9–42 mmol/24h)	21.0 (8.7)	27.78 (7.43)	<0.0001
Urine Magnesium (3–4.25 mmol/24h)	5.55 (2.41)	7.41 (2.57)	<0.0001
25-Hydroxy Vitamin D (25–80 ng/mL)	39.68 (21.01)	32.38 (11.70)	<0.01
1,25-Hydroxy Vitamin D (24–86 pg/mL)	70.32 (29.88)	60.45 (20.58)	<0.02
Alkaline Phosphatase (51–332 U/L)	233.24 (103.55)	237.12 (106.31)	0.80

Calcium-Phosphate Product (Ca x P) and Urine Calcium Excretion in 14 Children with Hypoparathyroidism

Table 3

Ca x P range mg ² /dL ²	Urine Ca excretion range (mmol/24 h)	Mean Urine Ca excretion (mmol/24h)	SD
15–24	4.14–9.47	6.56	1.9
25–34	2.14–13.0	6.54	2.54
35–44	1.75–16.3	7.25	3.14
45–54	1.56–14.9	7.57	3.28
55–65	2.30–13.1	5.44	2.78

Ca x P was measured in morning serum samples before PTH dose. Range and mean $\pm$ SD of urine calcium excretion were determined from the corresponding 24-h urine calcium collections.

Table 4

Cerebral Calcifications Detected by Cranial Computerized Tomography Imaging

Subject	Study Year	Location of Ca
B	Baseline	ϕ
B	4.5	BG
C	8.5	BG, FL
D	9	BG, FL, PL
E	9.5	ϕ
F	Baseline	ϕ
F	1	ϕ
J	Baseline	FL
K	3.5	BG, FL
L	3.5	BG, Th
M	1	BG, Th
M	4.5	BG, Th
N	10	BG

ϕ No calcifications; BG: Basal Ganglia; FL: Frontal Lobe

PL: Parietal Lobe; Th: Thalami.