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Cinacalcet sustainedly prevents pancreatitis in a child with a compound heterozygous *SPINK1/AP2S1* mutation

I. Scheers [a, *], E. Sokal [a], N. Limaye [b], C. Denoncin [c], X. Stephenne [a], Y. Pirson [d, 1], N. Godefroid [e, 1]

[a] Pediatric gastroenterology, Hepatology and nutrition Unit, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium
[b] Genetics of Autoimmune Diseases and Cancer, de Duve Institute, Université Catholique de Louvain, Brussels, Belgium
[c] Pediatric Unit, Clinique Sud Luxembourg, Site Saint-Joseph, Arlon, Belgium
[d] Nephrology Unit, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium
[e] Pediatric nephrology Unit, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels, Belgium

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ABSTRACT

Familial hypocalciuric hypercalcemia is an autosomal dominant genetic disorder characterized by hypercalcemia associated with inappropriate hypocalciuria and normal parathyroid hormone levels.

Acute recurrent pancreatitis (ARP) is rare in children. Predisposing factors include hypercalcemia and mutations in the serine protease inhibitor Kazal-type 1 (*SPINK1*) gene. The disease carries a heavy morbidity and preventive treatment options are scant.

Here, we report a child with a novel genetic/metabolic form of ARP associated with compound heterozygous *SPINK1/AP2S1* (adaptor protein-2 σ1-subunit) mutations, recurrence of which was completely abrogated for 6 years by cinacalcet treatment.

Introduction

Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant inherited disease caused by a mutation in either the calcium-sensing receptor (*CASR*: FHH1), guanine nucleotide-binding protein (G-protein) subunit α_{11} (*GNA11*: FHH2) or adaptor-related protein complex 2 (AP2) σ_1 subunit (*AP2S1*: FHH3). A loss of function mutation in one of these three genes leads to decreased sensitivity of the calcium sensor receptor to ionized calcium (FHH1) or altered receptor signal transduction (FHH2 and 3), ultimately resulting in hypercalcemia, reduced urinary calcium secretion, renal tubular calcium reabsorption and increased circulating parathormone (PTH) levels. While FHH1 is usually asymptomatic and therefore considered a benign condition, patients with FHH3 have a more severe phenotype [1]. Cinacalcet is an allosteric activator of the calcium sensing receptor that increases the receptor's sensitivity to circulating calcium. The drug has been shown to ameliorate symptoms of hypercalcemia in patients with FHH1 and FHH3[2].

Acute recurrent pancreatitis (ARP) is a rare disease entity. Recent data suggest that genetic, metabolic, immune and anatomic anomalies, or a combination of these factors, are involved in the development of ARP in children [3]. Hypercalcemia and mutations in the serine protease inhibitor Kazal-type 1 (*SPINK1*) gene are recognized predisposing factors for pancreatitis. Calcium overload induces premature intrapancreatic activation of trypsinogen [4], while loss of function of *SPINK1* hinder prematurely activated trypsin inhibition, allowing for pancreatic cellular damage by the active enzyme [5]. Treatment options to prevent disease recurrence are scant.

We report here the first case of FHH3 presenting with recurrent episodes of pancreatitis in a child with a compound heterozygous mutation in the adaptor protein-2 σ_1-subunit (*AP2S1*) and *SPINK1* genes. Cinacalcet was found to sustainably reduce serum calcium and completely prevent pancreatitis recurrence.

* Corresponding author. Isabelle Scheers Pediatric gastroenterology, hepatology and nutrition unit, Cliniques Universitaires Saint-Luc, Av Hippocrate 10, Brussels, 1200, Belgium.
E-mail address: isabelle.scheers@uclouvain.be (I. Scheers).
[1] Authors contributed equally.

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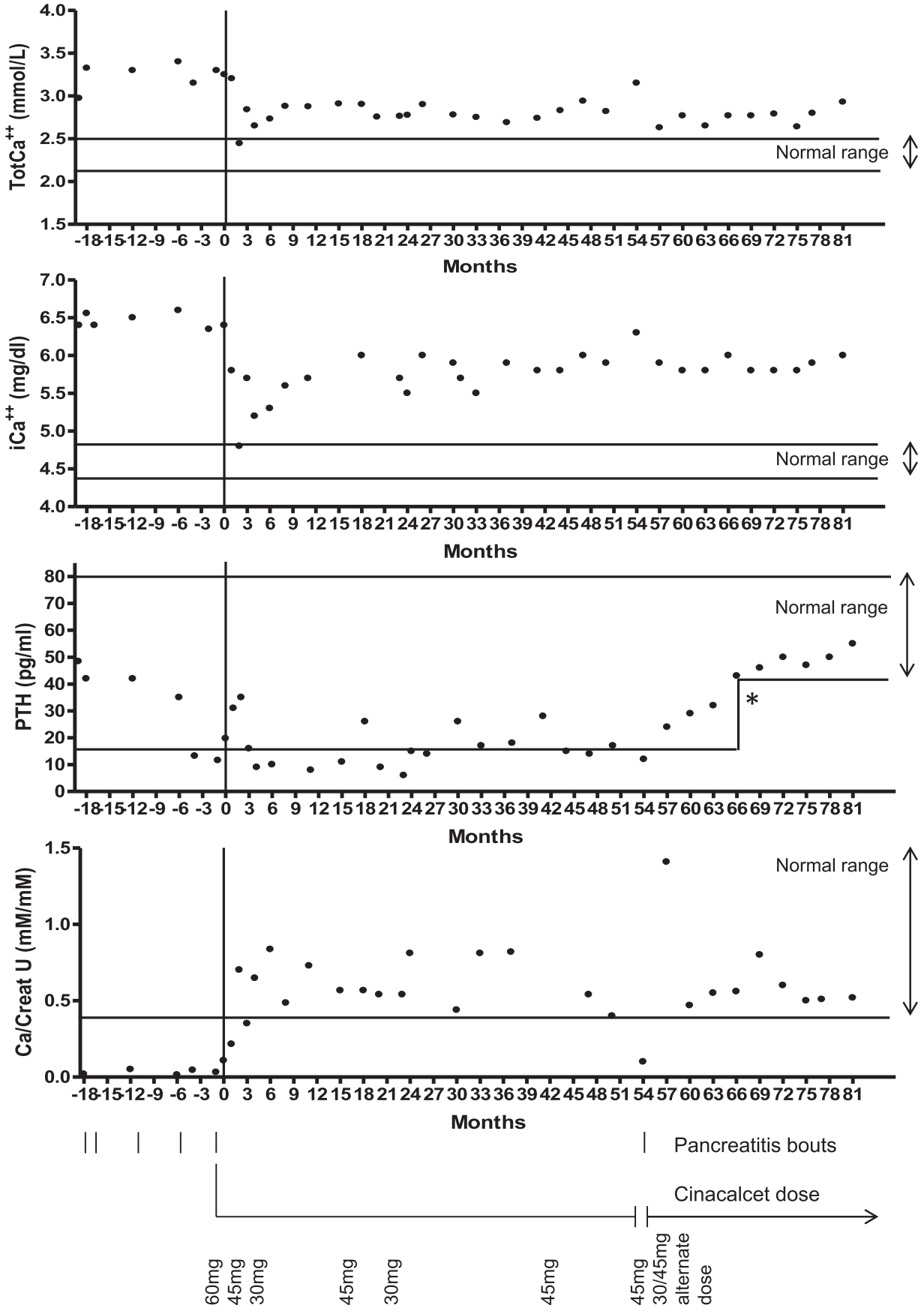
TotCa++ (mmol/L)
Normal range
Months
iCa++ (mg/dl)
Normal range
Months
PTH (pg/ml)
Normal range
*
Months
Ca/Creat U (mM/mM)
Normal range
Months
Pancreatitis bouts
Cinacalcet dose
60mg
45mg
30mg
45mg
30mg
45mg
45mg
30/45mg
alternate
dose

Material and methods

Patients

The patient and her parents gave written informed consent for the publication of this manuscript.

Targeted Next Generation Sequencing (NGS)

DNA was extracted from a venous blood sample (Qiagen, Antwerp, Belgium) and quantified using the Qubit system (Thermo Fisher Scientific, Merelbeke, Belgium). Targeted Next Generation Sequencing (NGS) of all protein coding exons (along with 25 base-pairs of flanking introns) of candidate genes (listed below) was performed by an accredited laboratory of clinical genetics (ISO15189 - Cliniques Universitaires Saint Luc, Brussels, Belgium), using a custom DNA capture-kit (Sophia Genetics, Saint Sulpice, Switzerland) and the KAPA HyperPlus library-preparation kit (Roche, Brussels, Belgium), on an Illumina MiSeq v3 machine. Sequence alignment, variant-calling, annotation and visualization were performed using Sophia DDM software (Sophia Genetics). The candidate genes (with reference transcripts) screened by targeted NGS included: *AP2S1 (ENST00000263270.10)*, *CASR* (ENST00000639785.1), *CEL* (ENST00000372080.6), *CFTR* (ENST00000003084.10), *CLDN2* (ENST00000541806.5), *CPA1* (ENST00000011292.8), *CTRC* (ENST00000375949.4), *CTSB* (ENST00000353047.11), *GNA11* (ENST00000078429.9), *PRSS1 (ENST00000311737.12)*, *SPINK1* (ENST00000296695.9). In addition, CFTR intragenic deletions/duplications were tested using a commercial Multiplex Ligation-dependent Probe Amplification kit (P091−C1, MRC-Holland, Amsterdam, The Netherlands) run on an ABI3130xl Genetic Analyzer (Thermo Fisher), with fragment analysis performed using Genemarker 2.2 software.

Mutation confirmation

Two variants-of-interest identified by targeted NGS (*SPINK1: ENST00000296695.9* c.200G > A, p.Arg67His; *AP2S1: ENST00000263270.10* c.44G > T, p.Arg15Leu) were confirmed by PCR-amplification (primers and annealing temperatures listed below), followed by Sanger-sequencing of the purified products (Agencourt AMPure XP beads, Beckman Coulter, Suarlée, Belgium) on an ABI3130xl Genetic Analyzer (Thermo Fisher). *SPINK1*: Forward primer: TTTTCCCCCTGTTTTTCTCC, Reverse primer: AAGTCCCCTGACCCTGGTAT (annealing temperature: 60 °C, product size: 269 base-pairs). *AP2S1*: Forward primer: TTCCTCCTTCTCCTCCTGCT, Reverse primer: AGAGGGTCCAAGGGGTTCT (annealing temperature: 60 °C, product size: 278 base-pairs).

Case report

A 13 year-old girl, was referred for 5 episodes of pancreatitis over the last 2 years. Family history couldn't be retrieved as the child was adopted. A typical pancreatitis episode started with heavy supra-umbilical abdominal pain. Symptoms were associated with raised lipase levels (>1000 IU/L, normal range (NR) < 60) and pancreatic edema on ultrasound. The patient had a short stature (Z-score −1.9). Laboratory investigations revealed normal triglycerides (0.9 g/L; NR: 0.7−1.7) but hypercalcemia (ionized calcium 6.5 mg/dl; NR: 4−4.8 and total calcium 3.32 mmol/L; NR: 2.1−2.55) with normal serum phosphate (2.8 mg/dl; NR: 2.4−4.7) and slightly raised magnesium (1 mmol/L; NR: 0.73−0.95) levels. Parathyroid hormone was inappropriately normal (42 pg/ml; NR: 16−81) and the urinary calcium to creatinine ratio was extremely low (0.007 mg/mg) (Fig. 1). Ultrasound and a MIBI-^{123}I scintigraphy of the parathyroid glands were unremarkable. Bone mineral density was low (lumbar spine Z-score −2.8; left hip Z-score −2.8; whole body Z-score −2.7). Detailed imaging by ultrasound and magnetic resonance cholangiopancreatography performed at referral and repeated 5 years later didn't show any biliary stones or pancreatic abnormality. Besides pancreatitis, the patient also had severe gastroesophageal reflux, recurrent peptic ulcers, constipation and learning disabilities. Because a diagnosis of familial hypocalciuric hypercalcemia (FHH) was suspected, genes involved in calcium homeostasis were sequenced: calcium sensor receptor (*CASR*), adaptor protein-2 σ1-subunit *(AP2S1)* and G-protein subunit α (*GNA11*) [6]. A heterozygous p.Arg15Leu (c.44G > T) mutation in *AP2S1* was identified and reported in Hannan's [1] cohort as case 02/11. Because of the presentation with recurrent pancreatitis and the previously reported association of this clinical finding with both *CASR* and *SPINK1* genes, the latter was initially screened but no mutation was found (sequencing of exon 2 and 3). Later, a complete gene sequencing identified a heterozygous p.Arg67His (c.200G > A) mutation in the pancreatitis susceptibility gene *SPINK1* (Supplementary Fig. 1). No mutations were identified in other pancreatitis susceptibility genes sequenced: *CTRC*, *CFTR*, *PRSS1*, *CTSB*, *CEL*, *CLDN2* and *CPA1*. Treatment with cinacalcet at a dose of 40 mg was initiated. However, a constellation of symptoms mimicking hypocalcemia repeatedly appeared when serum iCa^{++} levels dropped below 5.5 mg/dl (total calcium 2.4 mmol/L), prompting reduction of the dose to 30 mg. Remarkably, only one episode of pancreatitis occurred since treatment onset 6 years earlier (Fig. 1), which correlated with a brief trial of cinacalcet interruption.

Discussion

FHH is a genetic form of hypercalcemia caused by mutations in either *CASR*, *AP2S1* or *GNA11* [1,6]. It is of note that patients with FHH3, particularly those harboring the p.Arg15Leu substitution in *AP2S1*, have significantly higher serum calcium and magnesium levels and reduced urinary calcium to creatinine clearance ratios than those with other mutations [1,6]. AP2σ_1, a key component of clathrin coated vesicles, has an important role in clathrin-mediated endocytosis, which internalizes and recycles among others the calcium sensor receptor. It is believed that mutations in AP2σ_1 leads to hypercalcemia by altering signal transduction of CaSR (altered receptor endocytosis) and reducing cell sensitivity to calcium (altered receptor recycling) [7].

Acute recurrent pancreatitis is a rare condition in children. Predisposing factors include hypercalcemia, and, among others, mutations in genes promoting trypsinogen activation into trypsin such as *SPINK1*, *PRSS1* and *CTRC*. Nevertheless, only a fraction of FHH patients will ever develop pancreatitis [8−13] suggesting that hypercalcemia alone is not sufficient to induce pancreatic injury [8]. Indeed, several studies have shown that pancreatitis mainly occurred in patients harboring combined *SPINK1/CASR* [9,12,13]. To

Fig. 1. Effect of cinacalcet in a child with acute recurrent pancreatitisEvolution
of pancreatitis bouts, serum levels of ionized calcium (iCa^{++}), total calcium (TotCa^{++}), parathormone (PTH), urinary calcium to creatinine ratio (Ca/Creat U) before and after treatment onset with cinacalcet. FHH3 is characterized by hypercalcemia with inappropriate hypocalciuria and normal PTH levels. Cinacalcet modulates the calcium receptor by increasing it's sensitivity to extracellular calcium; consequently, the drug reduces PTH secretion and serum iCa^{++} (as well as TotCa^{++}) concentration. Low PTH prevents renal calcium reabsorption. *New quantification method.

the best of our knowledge, pancreatitis occurring in the context of *SPINK1/AP2S1* mutations has never been described.

SPINK1 encodes the pancreatic secretory trypsin inhibitor (PSTI), an important defense mechanism to inhibit prematurely activated trypsin. The p.Arg67His mutation has been experimentally proven to abolish PSTI expression [5,14].

The underlying pathophysiologic mechanisms of pancreatitis in patients with hypercalcemia are still incompletely understood. Experiments demonstrated that increased Ca^{++} levels in the apical compartment of the acinar cells promote (1) inappropriate intracellular trypsinogen activation and (2) postexocytic endocytic vacuole formation [4,15,16]. Nevertheless, severe hypercalcemia is responsible, in a concentration-dependent matter, of a sustained rise in cytosolic Ca^{++} which alone seems insufficient in vitro to induce pancreatitis [16]. Furthermore, pancreatitis is an unusual complication of FHH. There is increasing evidence that ARP is a multifactorial disease where the effect of a single mutation in different predisposing genes might sum up to favor pancreatitis development. Thus, hypercalcemia seems to trigger pancreatitis solely when associated with other disease risk factors such as *SPINK1* mutations.

Cinacalcet, a calcimimetic drug acting as an allosteric activator of *CASR*, has been shown to correct (FHH1) or reduce (FHH2 and FHH3) serum hypercalcemia and improve symptoms in FHH patients [2,17]. In this child, cinacalcet was able to reduce serum calcium to sufficient levels to prevent pancreatitis recurrence. The threshold for hypercalcemia to induce pancreatitis in patients with a *SPINK1/AP2S1* mutation is not known and may be influenced by the residual expression/function of the mutant proteins. In our patient, pancreatitis only occurred when iCa^{++} levels rose above 6 mg/dl; suggesting that aiming for calcium levels within the normal range might not be required to prevent pancreatitis.

In summary, we describe a unique case of acute recurrent pancreatitis related to a novel compound heterozygous *SPINK1/AP2S1* mutation. Cinacalcet successfully prevented pancreatitis recurrence over a follow-up period of 6 years while treatment withdrawal led to disease relapse.

Authorship contribution (ICMJE recommendations)

IS – a-b-c-d; ES – a-b-c; NL – a-b-c; CD – a-b-c; XS – a-b-c; YP – a-b-c-d; NG – a-b-c-d.

Conflict of interest

The authors have no conflict of interest.

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The authors have approved the final manuscript.

Written informed consent was obtained from the patient and her parents.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pan.2019.07.045.

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