Immune Checkpoint Inhibitor-Induced Autoimmune Hypoparathyroidism Associated with Calcium-Sensing Receptor-Activating Autoantibodies

Paramarajan Piranavan,[1] Yan Li,[1] Edward Brown,[3] E. Helen Kemp,[4] and Nitin Trivedi[2]

[1]Department of Medicine and [2]Division of Endocrinology Saint Vincent Hospital, Worcester, MA, USA; [3]Division of Endocrinology, Diabetes and Hypertension, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA; and [4]Department of Oncology and Metabolism, University of Sheffield, Sheffield, UK

Short Title: Nivolumab therapy and autoimmune hypoparathyroidism

Address correspondence and reprint requests to: Dr Paramarajan Piranavan, Department of Medicine, Saint Vincent Hospital, Worcester, MA, USA; Tel: +1 508-363-6208; Fax: +1 508-363-9798; Email: piranavan19@gmail.com

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Abbreviations: APS1, autoimmune polyglandular syndrome type 1; CaSR, calcium-sensing receptor; cpm, counts per minute; CTLA-4, cytotoxic T lymphocyte antigen-4; ELISA, enzyme-linked immunosorbent assay; ICI, immune checkpoint inhibitors; IFN, interferon; IL, interleukin; PBS, phosphate-buffered saline; PD-1, programmed cell death protein 1; PD-L1, programmed cell death protein 1 ligand 1; PTH, parathyroid hormone; RLBA, radioligand binding assays; SCLC, small cell lung cancer.

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Precis: A patient with small cell lung cancer undergoing treatment with nivolumab developed hypocalcemia due to hypoparathyroidism caused by calcium-sensing receptor-activating autoantibodies.

Abstract

Context: While therapy with immune checkpoint inhibitors such as nivolumab have substantially improved survival in several types of cancer, increased attention has been given to adverse immune events associated with their use, including the development of endocrine autoimmunity.

Objectives: First, to describe a patient with a two-year history of metastatic small cell lung cancer who had been treated with nivolumab a few months prior to presentation with the signs and symptoms of severe hypocalcemia and hypoparathyroidism. Second, to investigate the etiology of the patient's hypoparathyroidism, including the presence of activating autoantibodies against the calcium-sensing receptor (CaSR), since humoral and cellular immune responses against the CaSR have been reported in patients with autoimmune hypoparathyroidism.

Case and Results: A 61-year-old female was admitted with persistent nausea, vomiting, epigastric pain, constipation, and generalized weakness. Laboratory analyses showed low total serum calcium, ionized calcium, and parathyroid hormone (PTH). The patient was diagnosed with severe hypocalcemia as a result of autoimmune hypoparathyroidism after testing positive for CaSR-activating autoantibodies. She was treated with intravenous calcium gluconate infusions followed by a transition to oral calcium carbonate plus calcitriol which normalized her serum calcium. Her serum PTH remained low during her hospitalization and initial outpatient follow-up despite adequate repletion of magnesium.

Conclusions: This case illustrates autoimmune hypoparathyroidism induced by immune checkpoint inhibitor-blockade. As immune checkpoint inhibitors are now used to treat many cancers, clinicians should be aware of the potential risk for hypocalcemia that may be associated with their use.

Introduction

Immunotherapies targeting the immune checkpoint molecules, such as cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed cell death protein 1 (PD-1), and its ligand PD-L1, are now commonly used in clinical practice to treat malignancies (1). For example, nivolumab is an anti-PD-1 monoclonal antibody that works as an immune checkpoint inhibitor (ICI). It blocks the interaction between PD-1 on the surface of activated T cells and PD-L1 produced by cancer cells, a signalling process that would normally prevent T lymphocytes from attacking tumors. While therapy with ICIs such as nivolumab has substantially improved cancer survival, increased attention has been called to immune-related adverse events associated with their use including endocrine autoimmunity (1, 2). ICI-induced autoimmune endocrinopathies involving the pituitary and thyroid are frequent being reported in up to 10% of patients treated with antibodies against CTLA-4 or PD-1/PD-L1 (1-5). In contrast, primary adrenal insufficiency and type 1 diabetes are uncommon (1-3), while ICI-induced autoimmune involvement of the parathyroid gland is exceedingly rare (4). To date, there have been two reported cases of ICI-induced hypocalcemia, but the etiology of their low calcium levels was not reported (6).

A common cause of hypocalcemia is hypoparathyroidism. Normally, low blood calcium concentrations are detected by the parathyroid-expressed calcium-sensing receptor (CaSR), which responds by stimulating parathyroid hormone (PTH) secretion from the parathyroid glands (7). There is a resulting normalization of serum calcium as it is reabsorbed by the kidneys, released by bone, and absorbed by the intestine. However, in circumstances of absent, reduced or ineffective PTH, this regulatory function is lost and hypocalcemia ensues. Hypoparathyroidism can be due to surgical or autoimmune destruction of the parathyroid glands (8). In addition, autoantibodies that stimulate CaSR activity, even when blood calcium levels are lower than normal, can cause the inhibition of PTH secretion from the parathyroid, such that calcium remains below the critical concentration that would normally stimulate PTH secretion

87 (9). Such CaSR-activating autoantibodies have been identified in patients with idiopathic
88 hypoparathyroidism and autoimmune polyendocrine syndrome type 1 (APS1), in which
89 hypoparathyroidism is a prominent manifestation (10, 11).

90 The aims of this study were to describe a patient with a two-year history of metastatic small cell
91 lung cancer (SCLC), who had been treated with nivolumab a few months prior to presentation
92 and developed the signs and symptoms of severe hypocalcemia and hypoparathyroidism, and
93 to investigate a possible autoimmune etiology of the patient's hypoparathyroidism by testing for
94 the presence of CaSR autoantibodies. In addition, NALP5 and cytokine autoantibodies were
95 evaluated since these are indicative of APS1.

Materials and Methods

Case description

A 61-year-old woman with metastatic SCLC was admitted to our hospital with persistent nausea, vomiting, epigastric pain, constipation and generalized weakness in July 2017. In addition to these symptoms, she also reported bilateral distal lower limbs paresthesias but no muscle spasms or cramps. She had three prior admissions at other institutions within the last two months for similar symptoms. Low serum calcium levels were the most notable laboratory finding during these prior hospital admissions. The patient's hypocalcemia was treated during these hospitalizations, and the patient was instructed to take over-the-counter calcium and vitamin D3 supplementation at discharge. She was not taking the recommended calcium and vitamin D supplementation at the time of admission to our hospital.

The patient was diagnosed with SCLC in July 2015, which was initially treated with multiple cycles of platinum-based chemotherapy and radiation therapy. Given the progression of the disease despite the initial treatment, nivolumab therapy was begun in March 2017. The patient received six intravenous infusions of nivolumab, which led to partial remission of her SCLC. The last infusion of nivolumab was two months prior to the current hospitalization.

At our hospital, the patient reported no family history of autoimmune endocrinopathies, neck irradiation or neck surgery. Her physical examination was unremarkable except for mild gait ataxia. Chvostek and Trousseau signs were negative. Laboratory findings upon admission are summarized in Table 1 and showed low total serum calcium, ionized calcium, and PTH levels. A CT scan of her neck, chest and abdomen revealed a primary left lung mass with extensive lymph nodes and bone metastases without any identifiable lesions in the parathyroid glands.

During the current hospitalization, her serum calcium level normalized after she received intravenous calcium gluconate infusions. The patient experienced marked improvement in her

symptoms following normalization of serum calcium. Magnesium sulfate was infused to treat hypomagnesaemia. Serum PTH levels remained persistently suppressed despite normalization of serum calcium and magnesium (Fig. 1). Treatment with oral calcium carbonate and calcitriol was started as maintenance therapy for hypocalcemia. At two weeks following discharge from the hospital and continued treatment with calcium and calcitriol, her serum calcium was normal. However, her serum PTH level remained persistently low.

Due to the terminal nature of her disease and the inconvenience of additional medical appointments, the patient decided on regularly scheduled follow-ups only with her oncologist at another institution. We called the patient and her oncologist, learning that nivolumab therapy was replaced with a different chemotherapy regimen in March 2018 due to progression of mesenteric nodal metastatic disease. The oncologist reported that the patient's serum calcium was low in March 2018 after the patient stopped taking calcitriol for a few days. Given the nature of her illness, the patient was reluctant to obtain regular laboratory testing.

Written consent was obtained from the patient for blood tests and publication.

Measurement of CaSR-binding antibodies

CaSR immunoprecipitation assays for detecting CaSR antibodies were carried out as before (12). The patient's serum and healthy control sera (n = 10) were stored at -80°C. Human embryonic kidney 293 (HEK293) cells were transiently transfected with plasmid pcCaSR-FLAG, and cell extracts were prepared containing expressed CaSR-FLAG protein (11). Aliquots (50-µl) of GammaBind® Sepharose beads (Amersham Biosciences, Little Chalfont, UK) were mixed with patient serum, control sera or positive control anti-CaSR antiserum (Alpha Diagnostic International, San Antonio, TX) in duplicate at 1:100 dilution in 1 ml of immunoprecipitation buffer and incubated for 1 h at 4°C. The bead/IgG complexes were collected by centrifugation and incubated with cell extract containing CaSR-FLAG protein at 4°C for 16 h. The bead/IgG/protein complexes were collected by centrifugation, washed, denatured, and

subjected to SDS-PAGE in 7.5% polyacrylamide gels. The separated proteins were transferred onto Trans-Blot® Transfer Membranes (Bio-Rad Laboratories Ltd., Hemel Hempstead, UK) using standard protocols. Immunoprecipitated CaSR-FLAG protein was detected using anti-FLAG® M2-Peroxidase Conjugate (Sigma-Aldrich, Poole, UK) and an ECL™ Western Blotting Analysis System (Amersham Biosciences) with a final exposure to pre-flashed x-ray film for 5 min. Densitometry of the developed films was performed in a Bio-Rad GS 690 Scanning Densitometer with Multi-Analyst Version 1.1 Software (Bio-Rad Laboratories Ltd.), which produced a densitometry value for each individual band. A CaSR antibody index for each serum sample in the immunoprecipitation assay was calculated as the densitometry value of the tested serum/mean densitometry value of 10 control sera. The upper normal limit for the assay was calculated using the mean CaSR antibody index + 3 SD of these control individuals. A CaSR antibody index above the upper normal limit was designated as positive for CaSR antibody reactivity. All assays were run blinded to avoid operator bias.

Measurement of CaSR-activating antibodies

As detailed elsewhere (11), the response of the CaSR to Ca^{2+} was assessed in HEK293 cells stably expressing the receptor (HEK293-CaSR) by measuring intracellular inositol-1-phosphate (IP1) accumulation using an IP-One ELISA Kit (CIS Bio International, Gif-sur-Yvette, France). Monolayer HEK293-CaSR cells were cultured in 24-well plates. The cells were washed with serum-free medium and then Ca^{2+}-free assay buffer containing 10 mM lithium chloride. For investigating antibody effects, cells were pre-incubated for 10 min at 37°C with duplicate IgG samples (1:100 in assay buffer), which had been prepared from the patient's and control sera (n = 10) by standard methods (11). Assay buffer containing varying concentrations of calcium chloride (0-5 mM final concentration) was added to the cells followed by incubation for 60 min at 37°C. HEK293-CaSR cells without pre-incubation with IgG were also included in experiments as controls. Following incubation, cells were lysed for 30 min at 37°C with 50 µl of 2.5% IP-One

ELISA Kit Lysis Reagent (CIS Bio International). The accumulation of intracellular IP1 was measured according to an IP-One ELISA Kit, an immunoassay based on competition between free IP1 and IP1-horse-radish peroxidase (HRP) conjugate for binding to anti-IP1 monoclonal antibody. The results for IP1 accumulation were expressed as: percentage inhibition of IP1-HRP binding = [1 - IP1-HRP binding in stimulated cells/IP1-HRP binding in unstimulated cells] x 100. Increasing IP1 accumulation in the HEK293-CaSR cells is reflected by an increase in the percentage inhibition of IP1-HRP binding.

Measurement of NALP5 antibodies

A TnT® T7-Coupled Reticulocyte Lysate System (Promega, Southampton, UK) was used to produce [^{35}S]-labelled NALP5 protein *in vitro* from the transcription-translation of NALP5 cDNA in plasmid pCMV6-XL5-NALP5 (a gift from Professor Olle Kampe, University Hospital, Uppsala University, Uppsala, Sweden), as detailed in the manufacturer's protocol. Radioligand binding assays (RLBA) were carried out as detailed elsewhere (13). In brief, for each RLBA, an aliquot of NALP5 *in vitro* transcription-translation reaction containing 100,000 counts per minute (cpm) of trichloroacetic acid-precipitable material, as determined per the manufacturer's instructions, was suspended in 50 µl of IP buffer (20 mM Tris-hydrochloride, pH 8.0, 150 mM sodium chloride, 1% Triton X-100, and 10 mg/ml aprotinin (Bayer, Newbury, UK)). A sample of patient or healthy control serum was then added to a final dilution of 1:100. Anti-NALP5 polyclonal goat antibody (Santa Cruz Biotechnology Inc., Dallas, TX) was used at 1:200 a dilution as a positive control. All serum and antibody samples were tested in duplicate. After overnight incubation at 4 C, 50 µl of protein G Sepharose™ 4 Fast Flow (GE Healthcare Life Sciences, Little Chalfont, UK), prepared according to the manufacturer, were added followed by incubation for 1 h at 4°C. Subsequently, the protein G Sepharose-antibody-antigen complexes were collected by centrifugation and washed six times for 15 min in immunoprecipitation buffer at 4°C. The complexes were then transferred to 1 ml of Ultima-Gold® XR scintillation fluid (Packard

Bioscience, Groningen, The Netherlands) and immunoprecipitated cpm evaluated in a Beckman LS 6500 Multi-Purpose Scintillation Counter (Beckman Coulter, Inc., Fullerton, CA). A NALP5 antibody index for each serum was calculated as: cpm immunoprecipitated by tested serum/mean cpm immunoprecipitated by 10 healthy control sera. Each serum was tested in duplicate in two experiments and the mean NALP5 antibody index calculated. The upper limit of normal for the NALP5 antibody RLBA was calculated using the mean NALP5 antibody index + 3SD of 10 healthy controls. Any serum sample with NALP5 antibody index above the upper limit of normal was designated as NALP5 antibody-positive.

Measurement of cytokine antibodies

Antibodies against cytokines were detected in enzyme-linked immunosorbent assays (ELISAs) (14, 15). Interleukin (IL)-22, IL-17A, IL-17F (R and D Systems, Minneapolis, MN), interferon (IFN)-omega, IFN-alpha2A and IFN-lamda1 (IL-29) (Sigma-Aldrich) were prepared according to the manufacturer's instructions. For ELISAs, the required cytokine was diluted in phosphate-buffered saline (PBS) to 0.1 µg/ml and 100-µl samples used to coat the wells of a Corning polystyrene 96-well microtitre plate (Bibby Sterilin Ltd., Bargoed, UK). The plates were then incubated overnight at 4°C. Excess peptide was removed by decanting, and the wells were blocked with blocking buffer (PBS containing 0.1% Tween-20 and 3% bovine serum albumin) for 30 min at 37°C. Plates were washed four times with washing buffer (PBS containing 0.1% Tween-20). Aliquots (100 µl) of serum at a 1:100 dilution in blocking buffer were added to the wells. PBS was applied as a control. The plates were incubated at room temperature for 1 h and then washed four times with washing buffer. Aliquots (100 µl) of anti-human IgG alkaline phosphatase-conjugate (Sigma-Aldrich) diluted to 1:2000 in blocking buffer were added to the wells for 1 h at room temperature. After washing five times with washing buffer, 100 µl of alkaline phosphatase substrate Sigma Fast *p*-Nitrophenyl Phosphate (Sigma-Aldrich) were applied to each well and plates incubated at room temperature to allow colour development. A

LabSystems Integrated EIA Management System (Life Sciences International, Basingstoke, UK) was used to read absorption of the wells at 405 nm. All sera were tested in duplicate and the average OD_{405} value taken. The binding reactivity of each patient and control sera to each cytokine was expressed as an antibody index calculated as: mean OD_{405} of tested serum/mean OD_{405} of 10 healthy control sera. Each serum was tested in two experiments and the mean antibody index calculated. The upper limit of normal for each ELISA was calculated using the mean antibody index + 3SD of 10 healthy control sera. Patient sera with an antibody index greater than the upper limit of normal were regarded as positive for cytokine antibodies.

Results

Detection of CaSR autoantibodies in the patient's serum

Initially, a CaSR immunoprecipitation assay, as detailed in Materials and Methods, was used to analyse the patient's serum for CaSR autoantibodies. The upper limit of normal for the CaSR immunoprecipitation assay was estimated as a CaSR antibody index of 2.81, calculated from a population of 10 healthy control sera (Fig. 2). The serum sample from the patient tested positive for CaSR autoantibodies with a CaSR antibody index of 40.1 (Fig. 2). The positive control anti-CaSR antiserum had a CaSR antibody index of 20.5 (Fig. 2).

Subsequently, the response to Ca^{2+} of the CaSR expressed in HEK293 cells was assessed by measuring intracellular IP1 accumulation as described in Materials and Methods. To determine the effects of IgG from the patient and healthy controls (n = 10) on the response of the CaSR to Ca^{2+}, HEK293-CaSR cells were incubated with IgG samples at a 1:100 dilution prior to no stimulation or stimulation with a range of Ca^{2+} concentrations (0.5, 1.5, 3.0, and 5.0 mM). Each experiment included HEK293-CaSR cells stimulated with Ca^{2+} alone. IP accumulation was measured using the IP-One ELISA Kit as detailed in Materials and Methods and the percentage inhibition of IP1-HRP binding was calculated.

Of the 10 control IgG samples analyzed, none had any effect upon the levels of IP1 accumulation when compared with stimulation by Ca^{2+} alone (Fig. 3). In contrast, the IgG sample from the patient significantly increased the levels of IP1 accumulation at Ca^{2+} concentrations of 0.5, 1.5 and 3.0 mM when compared with Ca^{2+}-stimulation alone (*P* values < 0.001, one-way ANOVA) (Fig. 3). The IgG from the patient had no detectable effect on IP1 accumulation, however, when no Ca^{2+} was added to the buffer, indicating that some degree of receptor activation by its natural ligand, Ca^{2+}, was needed to see the functional effect of the IgG. The results indicated the presence of CaSR-stimulating activity in the patient IgG sample.

Detection of NALP5 and cytokine autoantibodies in the patient's serum

The patient's serum sample was evaluated for NALP5 and cytokine antibodies using RBLAs and ELISAs, respectively, as detailed in Materials and Methods. The upper limits of normal for antibody assays against IL-22, IL-17F, IL-17A, IFN-omega, IFN-alpha2A, and IFN-lamda1, and NALP5 were antibody indices of 1.29, 1.25, 1.33, 1.89, 2.03, 1.70, and 1.81, respectively. The patient was negative for autoantibodies against NALP5 and all the cytokines tested; antibody indices were 1.01, 0.98, 0.91, 0.75, 1.02, 1.11, and 1.07 for assays measuring autoantibodies against IL-22, IL-17F, IL-17A, IFN-omega, IFN-alpha2A, and IFN-lamda1, and NALP5, respectively.

Discussion

The biochemical investigations carried out here strongly suggest hypoparathyroidism as the cause of the patient's hypocalcemia. As there was no history of neck radiation, surgery or infiltration of the parathyroids on imaging, an autoimmune etiology of hypoparathyroidism seemed most likely. Furthermore, persistently low PTH despite normalization of serum magnesium excluded the possibility of hypomagnesemia as the cause of hypocalcemia and hypoparathyroidism.

Although the parathyroid glands are not the target of most autoimmune diseases, autoimmunity is an important cause of hypoparathyroidism, either as an isolated endocrinopathy or as a component of APS1 (16, 17). Autoimmune hypoparathyroidism can be caused by permanent hypoparathyroidism owing to irreversible, immune-mediated damage to the parathyroid glands or functional hypoparathyroidism due to antibody-induced activation of signaling pathway(s) regulating parathyroid function (18). In APS1, common autoantibody targets include parathyroid-expressed NALP5 and cytokines. However, testing carried out here revealed that the patient was not positive for autoantibodies against NALP5, a marker for parathyroid autoimmunity, or a panel of interferons and interleukins which are diagnostic for APS1 (13-15). As such, the patient was not diagnosed with multiple endocrine autoimmune disease to account for her low parathyroid function. Autoantibodies directed against the parathyroid-expressed CaSR have been recognized to be present in the serum of patients with autoimmune hypoparathyroidism (16), including autoantibodies that can activate the receptor, thereby causing reduced PTH secretion (10, 11).

Antibody tests of the patient's serum confirmed our diagnosis of hypoparathyroidism caused by the development of CaSR-activating autoantibodies. Autoantibody-activation of the CaSR was indicated by increased inositol-1-phosphate levels in a cell line expressing the CaSR following treatment with the patient's IgG; the phosphoinositide pathway being a key intracellular mediator

of CaSR activation. Thus, by shifting the calcium-PTH curve to the left and decreasing the set-point of the CaSR, PTH is not released at lower serum calcium concentrations resulting in hypocalcemia. The temporal relationship between the initiation of nivolumab infusions suggests that this drug is likely responsible for the development of the CaSR-activating autoantibodies, although was cannot rule out with certainty that the development of anti-CaSR antibodies and hypoparathyroidism were coincidental. Although the patient's hypoparathyroidism appears persistent despite discontinuation of nivolumab, it is possible that improvement could take place with longer follow-up. To the best of our knowledge this is the first report illustrating a case of autoimmune hypoparathyroidism induced by ICI-blockade as the underlying cause.

The reasons for differential autoimmune involvement of the endocrine glands between anti-PD1 and anti-CTLA-4 therapy are not entirely clear (2). Higher risk of hypophysitis with anti-CTLA-4 therapy such as ipilimumab compared to anti-PD1 pembrolizumab may be due to the expression of CTLA-4 in the normal pituitary gland (2). In contrast to the high risk of hypophysitis with anti-CTLA-4 therapy, autoimmune thyroiditis appears to be more common in anti-PD1 therapy. It has been speculated that in addition to effects on T cell immunity, anti-PD1 therapy may augment humoral immunity, thus boosting anti-thyroid antibody formation (2). Careful studies of ICIs *in vitro* and in animal models have also shown that their complex role in T cell immunity and blockade may involve the inhibition of regulatory T cells, which could be a factor in the induction of autoimmunity (19).

Given the high risk of autoimmune diseases, including endocrinopathy, during treatment with ICIs, the risk of toxicity should always be balanced against the benefit that may derive from the ICI-blockade. As ICIs are now used to treat many cancers, clinicians should be aware of the potential risk for hypocalcemia associated with their use.

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Figure Legends

Figure 1. Time course for changes in biochemical laboratory tests. The patient's ionized calcium, magnesium, and PTH levels during admission to our hospital are shown. The normal range is indicated by the hatched boxes: ionized calcium, 1.12-1.31 mmol/L; magnesium, 1.62-2.62 mg/dL; and PTH, 12-65 pg/mL.

Figure 2. Detection of patient CaSR autoantibodies. Serum from the patient, healthy controls (n = 10), and positive control anti-CaSR antiserum were tested in CaSR immunoprecipitation assays at a 1:100 dilution and assigned a CaSR antibody index as detailed in Materials and Methods. The upper limit of normal for the CaSR immunoprecipitation assay was calculated using the mean CaSR antibody index + 3 SD of 10 control sera, which gave a CaSR antibody index value of 2.81. A CaSR antibody index above the upper normal limit was designated as positive for anti-CaSR antibody reactivity. The CaSR antibody index of the patient's sample (40.1) and the positive control anti-CaSR antiserum (20.5) are shown and are positive.

Figure 3. Effect of patient IgG on CaSR activity. Intracellular IP1 accumulation in HEK293-CaSR cells was measured in response to Ca^{2+}-stimulation (final concentrations of 0-5 mM) after cells were pre-incubated with the patient's IgG sample or healthy control IgG at a 1:100 dilution. Cells without pre-incubation with IgG were also included. The accumulation of intracellular IP1 was measured using an IP-One ELISA Kit, an immunoassay based on competition between free IP1 and IP1-horse-radish peroxidase (HRP) conjugate for binding to anti-IP1 monoclonal antibody. The results for IP1 accumulation were expressed as: percentage inhibition of IP1-HRP binding = [1 - IP1-HRP binding in stimulated cells/IP1-HRP binding in unstimulated cells] x 100. Increasing IP1 accumulation in the HEK293-CaSR cells is reflected by an increase in the percentage inhibition of IP1-HRP binding. The results show IP1 accumulation (mean ± SD of six experiments) in Ca^{2+}-stimulated HEK293-CaSR cells that were pre-incubated with either IgG from the patient or IgG from a single control, or that were not pre-incubated with IgG. Only the

401 patient's IgG sample significantly increased the levels of IP1 accumulation when compared with
402 Ca^{2+}-stimulation (at 0.5, 1.5, and 3 mM) of HEK293-CaSR cells not pre-incubated with IgG: *P
403 values were < 0.001 (One-way ANOVA).

Table 1. Laboratory Test Results Upon Admission to Our Hospital

Test	Results	Normal range
Calcium	5.8 mg/dL (Low)	8.3-10 mg/dL
Albumin	4.2 g/dL (Normal)	3.6-4.8 g/dL
Ionized calcium	0.85 mmol/L (Low)	1.12-1.32 mmol/L
PTH	7.77 pg/mL (Low)	12-65 pg/mL
Magnesium	1.4 mg/dL (Low	1.6-2.6 mg/dL
Potassium	3.4 mEg/L (Low)	3.6-5.6 mEq/L
Phosphate	4.3 mg/dL (Normal)	2.5-4.5 mg/dL
25-hydroxy vitamin D	23.8 ng/mL (Low)	30-200 ng/mL
1,25-dihydroxy vitamin D	10.6 pg/mL (Low)	19.9-79.3 pg/mL
TSH	0.99 mIU/mL (Normal)	0.45-4.5 mIU/mL
EKG-QTc	492 sec (High)	431-470 sec (women)

Figure 1

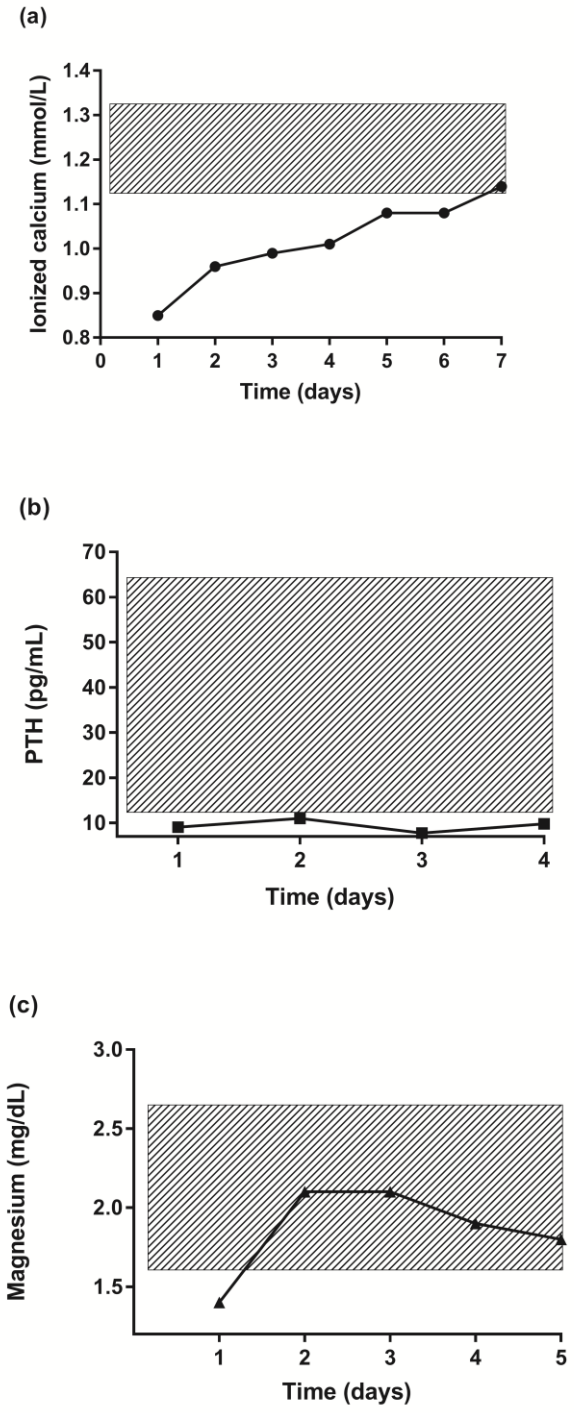

(a)
Ionized calcium (mmol/L)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0 1 2 3 4 5 6 7
Time (days)
(b)
PTH (pg/mL)
70
60
50
40
30
20
10
1 2 3 4
Time (days)
(c)
Magnesium (mg/dL)
3.0
2.5
2.0
1.5
1 2 3 4 5
Time (days)

Figure 2

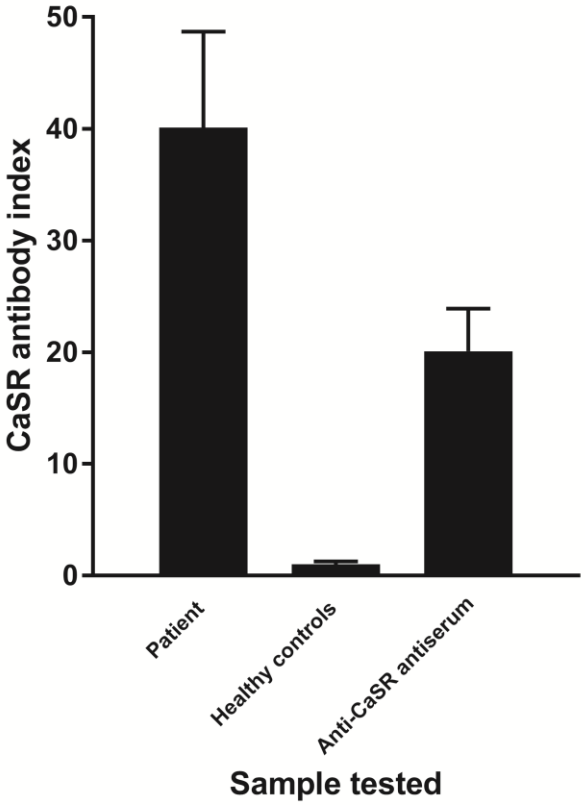
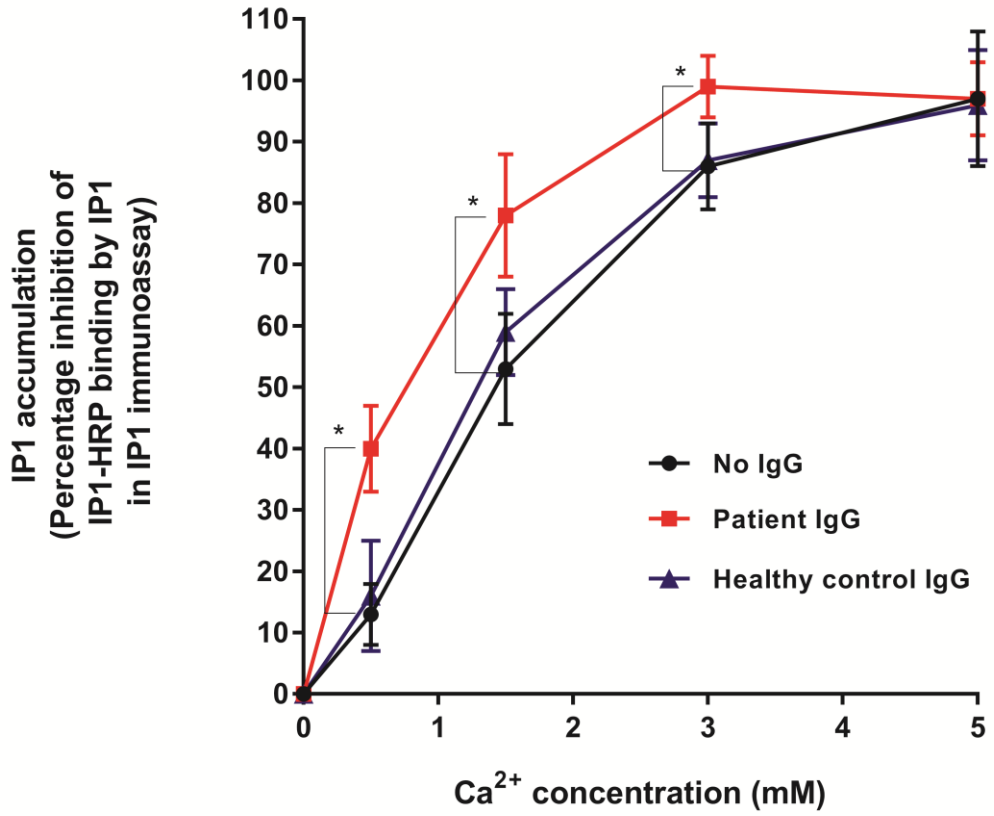

50
40
30
20
10
0
CaSR antibody index
Patient
Healthy controls
Anti-CaSR antiserum
Sample tested

Figure 3


IP1 accumulation
(Percentage inhibition of
IP1-HRP binding by IP1
in IP1 immunoassay)
110
100
90
80
70
60
50
40
30
20
10
0
0
1
2
3
4
5
Ca2+ concentration (mM)
No IgG
Patient IgG
Healthy control IgG
*
*
*