Glucocorticoid-responsive lymphocytic parathyroiditis and hypocalciuric hypercalcemia due to autoantibodies against the calcium-sensing receptor: a case report and literature review

Li Song^{1,2}, Liping Liu^{1,3}, R Tyler Miller^{1,3,4,5}, Shirley X Yan⁶, Nancy Jackson⁷, Shelby A Holt⁷ and Naim M Maalouf^{1,2,5}

¹Department of Internal Medicine, ²Divisions of Mineral Metabolism and Endocrinology and ³Division of Nephrology, University of Texas Southwestern Medical Center, Dallas, Texas, USA, ⁴Dallas VA Medical Center, Dallas, Texas, USA,

⁵Charles & Jane Pak Center for Mineral Metabolism & Clinical Research, and Departments of ⁶Pathology and

⁷Surgery, University of Texas Southwestern Medical Center, Dallas, Texas, USA

Correspondence should be addressed to N M Maalouf

Email

naim.maalouf@utsouthwestern.edu

Abstract

Objective: Autoimmune lymphocytic parathyroiditis and acquired hypocalciuric hypercalcemia associated with autoantibodies against the calcium-sensing receptor (anti-CaSR) are rare and poorly understood conditions. Here, we describe a patient with acquired parathyroid hormone (PTH)-dependent hypercalcemia with associated hypocalciuria, found to have true lymphocytic parathyroiditis on histopathology, and circulating anti-CaSR antibodies in serum.

Design and methods: A 64-year-old woman was referred to our clinic for persistent hypercalcemia after a subtotal parathyroidectomy. She was normocalcemic until the age of 63 years when she was diagnosed with primary hyperparathyroidism. She underwent subtotal parathyroidectomy with appropriate intraoperative PTH decline. Two weeks post-parathyroidectomy, she presented with persistent hypercalcemia and hyperparathyroidism. Urine studies revealed an inappropriately low 24-h urine calcium (Ca)/creatinine clearance ratio. Surgical pathology was consistent with true lymphocytic parathyroiditis with lymphoid follicles. The presence of circulating anti-CaSR antibodies was detected by immunoprecipitation of CaSR by the patient's serum. After a 4-week course of prednisone, serum Ca and PTH normalized, and her anti-CaSR titers declined. She remains normocalcemic 10 months after the discontinuation of glucocorticoid therapy. We present this patient in the context of the relevant published literature on lymphocytic parathyroiditis and acquired hypocalciuric hypercalcemia related to anti-CaSR antibodies.

Conclusions: Autoimmune lymphocytic parathyroiditis and acquired hypocalciuric hypercalcemia associated with anti-CaSR antibodies is a very rare yet important condition to be considered in a patient with acquired PTH-dependent hypercalcemia with inappropriate hypocalciuria. Although subtotal parathyroidectomy is unlikely to correct the hypercalcemia, this entity may respond to a short course of prednisone therapy.

European Journal of Endocrinology (2017) 177, K1–K6

Introduction

Autoimmune lymphocytic parathyroiditis is a rare cause of parathyroid hormone (PTH)-dependent hypercalcemia (1, 2). Non-specific lymphocytic infiltrates in the

parathyroid glands are found in 6% of nearly 1000 autopsy cases, whereas true lymphocytic parathyroiditis, defined by more stringent criteria of interstitial lymphocytic

Case Report	L Song and others	Acquired hypocalciuric hypercalcemia	177:1	K2
-------------	-------------------	--------------------------------------	-------	----

infiltrates with plasma cells or germinal centers with or without tissue damage, is found in no more than 0.5% of autopsies of apparently healthy individuals (3). The mechanism underlying autoimmune lymphocytic parathyroiditis is unclear.

A few case reports have described patients with PTH-dependent hypercalcemia with inappropriate hypocalciuria mediated by autoantibodies against the calcium-sensing receptor (anti-CaSR) (2, 4, 5, 6, 7). This condition is also termed acquired hypocalciuric hypercalcemia. The natural course and management of this entity are poorly understood. Parathyroidectomy and bisphosphonate treatment are usually unsuccessful (2, 4, 7). Glucocorticoids have been reported to reduce anti-CaSR antibody titers and normalize serum PTH and calcium (Ca) in one case (2), but not in another (7). Cinacalcet was shown to be effective in one case (5).

Here, we describe a patient with autoimmune lymphocytic parathyroiditis and acquired hypocalciuric hypercalcemia due to anti-CaSR antibodies whose serum PTH and Ca normalized and anti-CaSR antibodies were reduced after a short course of glucocorticoids; providing support for the pathogenic nature of the autoantibodies.

Subject and methods

Case report

A 64-year-old woman with type 2 diabetes (T2D), hypertension and depression was referred to our Mineral Metabolism Clinic for evaluation of persistent hyperparathyroidism after a subtotal parathyroidectomy. She had normocalcemia until the age of 63 years (Fig. 1A), when she presented with chronic nausea, constipation and 'cloudy thinking', and she was diagnosed with primary hyperparathyroidism with secondary hypercalcemia. She had no history of nephrolithiasis or fractures. Family history was only significant for osteoporosis in her mother. Her pre-operative laboratory studies revealed serum Ca level of 3.08 mmol/L and PTH level of 10.82 pmol/L, which were consistent with PTH-dependent hypercalcemia (Table 1; Pre-op). Her dual-energy X-ray absorptiometry showed osteopenia. Due to the progressive hypercalcemia (highest serum Ca: 3.35 mmol/L), she was referred to endocrine surgery for parathyroidectomy. Her neck ultrasound and Sestamibi scan were non-localizing, so she underwent subtotal parathyroidectomy with resection of three of the four parathyroid glands. Her intraoperative PTH declined from

8.23 pmol/L to 2.29 pmol/L at 15 min. Serum PTH and Ca normalized with symptomatic improvement 1 day after parathyroidectomy, but nausea, constipation and anorexia recurred a few days thereafter accompanied by laboratory evidence of persistent hyperparathyroidism (Table 1; POD 13). Surgical pathology showed hypercellular parathyroid tissue with multiple lymphoid follicles consistent with lymphocytic parathyroiditis in all the three resected glands (Fig. 1B and C). She was referred to our clinic 7 weeks post-parathyroidectomy for further evaluation. Her physical examination was unremarkable except for a well-healed parathyroidectomy scar. Her laboratory studies showed an elevated serum Ca level of 2.70 mmol/L, PTH level of 7.42 pmol/L and 24-h urine Ca to creatinine clearance ratio of 0.005 (Table 1; pre-glucocorticoid).

Lymphocytic infiltrate in the parathyroid glands has been associated with systemic inflammatory conditions such as sepsis, acute myocardial infarction, vascular congestion from heart failure, malignancies and autoimmune conditions. As the patient did not have any evidence of systemic inflammatory conditions, infections, cardiovascular disease or neoplasm, it was thought that her lymphocytic parathyroiditis could be autoimmune in nature. Her serum antinuclear antibody (ANA), thyroid function test and blood counts were unremarkable (Table 1; pre-glucocorticoid). Her inappropriately low 24-h urine Ca to creatinine clearance ratio and high normal serum magnesium level in the setting of acquired PTH-dependent hypercalcemia were suggestive of a potential CaSR abnormality. In combination with the pathological findings of lymphoid follicles in her parathyroid glands, we set out to search for possible anti-CaSR antibodies.

Informed consent for the relevant experiments and publication of this manuscript including the accompanying images was obtained from the patient.

Anti-CaSR antibodies

The presence of circulating anti-CaSR antibodies was detected by immunoprecipitation of CaSR by the patient's serum. Madin-Darby canine kidney (MDCK) epithelial cells that do not express native CaSR and MDCK cells stably expressing transfected CaSR were lysed and incubated with either normal mouse IgG (Santa Cruz Biotechnology), the patient's serum or a normal control human serum at a 1:200 dilution at 4°C overnight. The next morning, 100 µg protein A/G beads was added to each sample and incubated at room temperature for 4 h

Figure 1

Biochemical and histopathological findings and detection of anti-CaSR antibodies in serum. (A) Biochemical response to parathyroidectomy (arrow) and prednisone (black blocks: taller block represents prednisone 40mg daily for 2 weeks and shorter block represents prednisone 20mg daily for 2.5 weeks). The dotted lines represent the upper and lower limits of the reference range; (B and C) H&E stain of one of patient's parathyroid glands at 40× (B) and 200× (C) magnifications showing hypercellular parathyroid tissue with multiple lymphoid follicles consistent with lymphocytic parathyroiditis; (D) immunoprecipitation assay of the patient's serum prior to the glucocorticoid therapy and demonstration of the specificity of her anti-CaSR antibodies. MDCK cells that do not express native CaSR (lanes 1, 2 and 3) and MDCK cells stably expressing transfected CaSR (lanes 4, 5 and 6) were lysed and incubated with either normal mouse IgG (lanes 1 and 4), the patient's serum (lanes 2 and 5) or a normal control human serum (lanes 3 and 6) at a 1:200 dilution at 4°C overnight. The next morning, 100µg protein A/G beads were added to each sample and incubated at room temperature for 4 h followed by elution to separate the immunoglobulin fraction from serum. The eluted samples were run on a 10% SDS-PAGE gel and probed with a specific anti-CaSR antibody; (E) immunoprecipitation assay of the patient's serum prior to, and after the glucocorticoid therapy. MDCK cells stably expressing the CaSR were harvested for the total cell lysate and divided into 10 equal aliquots, which were incubated with different dilutions (1:200, 1:400, 1:600, 1:800 and 1:1000) of the pre-glucocorticoid or post-glucocorticoid serum from our patient at 4°C overnight. The next morning, 50µg protein A/G beads were added to each sample and incubated at room temperature for 4 h followed by elution. The eluted samples were run on a 10% SDS-PAGE gel and probed with a specific anti-CaSR antibody.

followed by elution to separate the immunoglobulin fraction from serum. The eluted samples were run on a 10% SDS-PAGE gel and probed with a specific anti-CaSR antibody (Sigma Aldrich).

The anti-CaSR antibody assay was performed again on the patient's serum prior to, and after glucocorticoid therapy to assess the response in anti-CaSR antibody

titers after the glucocorticoid therapy. MDCK cells stably expressing the CaSR were harvested for the total cell lysate and divided into 10 equal aliquots, which were incubated with different dilutions (1:200, 1:400, 1:600, 1:800 and 1:1000) of the pre-glucocorticoid or post-glucocorticoid serum from the patient, and the immunocomplexes were blotted for CaSR as described above.

Case Report	L Song and others	Acquired hypocalciuric hypercalcemia	177:1	K4
-------------	-------------------	--------------------------------------	-------	----

Table 1 Timeline of laboratory studies.

	Reference	Pre-op	POD 1	POD 13	Pre-glucocorticoid	Post-glucocorticoid [†]
Serum						
Calcium (mmol/L)	2.10–2.63	3.08		3.13	2.70	2.60
Ionized calcium (mmol/L)	1.13–1.33	1.68	1.33		1.35	
Phosphorus (mmol/L)	0.77–1.45				0.81	
PTH (pmol/L)	1.59–6.90	10.82	1.93	7.73	7.42	4.46
25-OH vitamin D (nmol/L)	75–200	85			69	
1,25-OH vitamin D (pmol/L)	43–187				146	
Alkaline phosphatase (U/L)	35–104	100			100	
Albumin (g/L)	35–52	43		45	46	43
Magnesium (mmol/L)	0.66–1.07		0.82		0.99	
Creatinine (μmol/L)	45.1–84.0	61.9			69.8	63.6
Anti-nuclear antibodies (ANA)	Negative				Negative	
Serum protein electrophoresis	Normal pattern				Normal pattern	
Hemoglobin (g/L)	120–150				142	
Hematocrit	0.34–0.44				0.43	
WBC (×10 ⁹ /L)	4.00–11.00				7.80	
Eosinophil (×10 ⁹ /L)	0.00–0.70				0.24	
24-h urine						
Calcium (mmol/day)					2.28	3.90
Magnesium (mmol/day)					6.53	7.73
Phosphorus (mmol/day)					27.0	34.8
Creatinine (mmol/day)					11.7	12.4
Ca/Cr clearance ratio [‡]					0.005	0.008

POD, post-operative day; [†]post-glucocorticoid laboratory studies were after discontinuation of prednisone for about 3 months; [‡]24-h urine calcium to creatinine clearance ratio = (serum creatinine × 24-h urine calcium)/(24-h urine creatinine × serum calcium).

Results

The patient's pre-glucocorticoid serum showed the presence of anti-CaSR antibodies (Fig. 1D). She was started on prednisone 40 mg daily for autoimmune lymphocytic parathyroiditis. Her serum PTH and Ca normalized after 2 weeks. Further tapering of prednisone to 20 mg daily was associated with continued normal serum PTH and Ca values, and the patient discontinued prednisone completely after 2 weeks of prednisone 20 mg daily. Ten months after the last prednisone dose, the patient's serum PTH and Ca have remained within the reference range and urine Ca excretion remained low but increased compared to that prior to glucocorticoid therapy (Table 1; post-glucocorticoid). A repeat assay on the patient's serum showed a decreased serum anti-CaSR antibody titer (Fig. 1E). To date, the patient's serum PTH and Ca values remain in the reference range (Fig. 1A).

Discussion

True lymphocytic parathyroiditis, defined by evidence of interstitial lymphocytic infiltrates with plasma cells or germinal centers with or without tissue damage, is a rare clinical entity (3). It has been associated with variable parathyroid functions (Fig. 2) (1, 8, 9, 10), and various

conditions including multiple endocrine neoplasia type 1 (3) and IgG4-related diseases (2). Rarely, lymphocytic parathyroiditis is associated with the presence of anti-CaSR antibodies (Fig. 2) (2).

Anti-CaSR antibodies can be activating (11, 12) or inhibitory (4, 5, 6, 7). Neutral anti-CaSR autoantibodies likely exists but will never be searched for. The functional nature of these antibodies will impact on parathyroid function and Ca status: anti-CaSR antibodies have been described in PTH-dependent hypocalcemia (autoimmune polyendocrine syndrome type 1 (11), and isolated autoimmune hypoparathyroidism (12, 13)), PTH-dependent hypercalcemia (autoimmune or acquired hypocalciuric hypercalcemia (2, 4, 5, 6, 7, 14) and autoimmune polyendocrine syndrome type 1 (15)) and normocalcemia with normal PTH in two patients with Graves' disease (16). The presence of anti-CaSR antibodies does not always correlate with lymphocytic infiltrate in the parathyroid glands (17). This intricate relationship is demonstrated in Fig. 2.

Our patient has true lymphocytic parathyroiditis based on her surgical pathology. Her biochemical profile mimics that of familial hypocalciuric hypercalcemia (FHH) with PTH-dependent hypercalcemia and hypocalciuria. FHH is unlikely in this patient given normocalcemia until the age of 63 years and absence of family history

Figure 2

Association of parathyroid function, anti-CaSR antibody and parathyroiditis.

of hypercalcemia. Thus, she most likely has acquired hypocalciuric hypercalcemia. Her serum anti-CaSR antibodies can be presumed to be inhibitory antibodies leading to increased PTH release and increased renal Ca resorption despite hypercalcemia. Despite the detection of antibodies to CaSR in patient's serum, we were unable to test whether these antibodies truly inhibited CaSR signaling.

A handful of cases of acquired hypocalciuric hypercalcemia associated with anti-CaSR antibodies have been reported (2, 4, 5, 6, 7), and only one case with both anti-CaSR and pathological evidence of lymphocytic infiltrate in the parathyroid gland in the setting of IgG4-related diseases (2). To the best of our knowledge, there is no case reported to date of a patient with both acquired hypocalciuric hypercalcemia and pathological evidence of true lymphocytic parathyroiditis with lymphoid follicles associated with anti-CaSR antibodies as described in our patient.

The inciting event for autoimmunity to the parathyroid glands, the natural course of this condition, its potential complications and the optimal treatment are poorly understood due to the rarity of the condition. Based on the few case reports, parathyroidectomy and bisphosphonate treatment are usually unsuccessful (2, 4, 7, 18). Glucocorticoids have been shown to normalize serum PTH and Ca (2, 14, 18) and reduce the titers of anti-CaSR (2) in three cases, but not in another (7). Two of the

three glucocorticoid-responsive cases described patients with underlying IgG4-related diseases (2, 18) with one patient with predominant anti-CaSR IgG4 antibody subclass (2). Responsiveness to glucocorticoids in patients with acquired hypocalciuric hypercalcemia has been suggested to reflect the fact that IgG4-related diseases typically respond to glucocorticoids (2). The parathyroid glands of our patient did not have the histopathological or immunohistochemical-staining pattern typical for IgG4-related diseases with an IgG4/IgG plasma cell ratio <40% and no storiform fibrosis. Thus, IgG4-related disease is unlikely in our patient, and serum IgG4 level was not measured. Nonetheless, she responded to a short course of glucocorticoids with normalized serum Ca and PTH levels and decreased anti-CaSR antibody titers. Thus, it is still unclear what predicts glucocorticoid responsiveness in this condition. In addition, the duration of remission after the glucocorticoid therapy in this condition is unclear. Long-term follow-up data was only available in one of the three glucocorticoid-responsive cases (2, 14, 18), which reported normal serum PTH and Ca levels at least 2 years after the cessation of glucocorticoid therapy (18). Glucocorticoid use is not an optimal long-term solution for our patient given its adverse effects on bone health with her pre-existing osteopenia and T2D. Fortunately, she remains normocalcemic to date, more than 10 months after the discontinuation of glucocorticoids. Cinacalcet, an allosteric activator of CaSR, was shown to be effective in one case (5) and may be a potential option for our patient if her hyperparathyroidism recurs.

Conclusions

Autoimmune lymphocytic parathyroiditis and acquired hypocalciuric hypercalcemia associated with anti-CaSR antibodies is a very rare, yet important condition to be considered in a patient with acquired PTH-dependent hypercalcemia with inappropriate hypocalciuria. This entity may respond to a short course of prednisone therapy, whereas parathyroidectomy is unlikely to correct the hypercalcemia.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this case report.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements

The authors are grateful to Orson Moe, for his assistance in providing insights to the diagnostic studies involved in this patient's care and reviewing this manuscript.

References

- 1 Furuto-Kato S, Matsukura S, Ogata M, Azuma N, Manabe T, Shigeno C, Asato R, Tanaka K, Komatsu Y & Nakao K. Primary hyperparathyroidism presumably caused by chronic parathyroiditis manifesting from hypocalcemia to severe hypercalcemia. *Internal Medicine* 2005 **44** 60–64. (doi:10.2169/internalmedicine.44.60)
- 2 Pallais JC, Kifor O, Chen YB, Slovik D & Brown EM. Acquired hypocalciuric hypercalcemia due to autoantibodies against the calcium-sensing receptor. *New England Journal of Medicine* 2004 **351** 362–369. (doi:10.1056/NEJMoa040008)
- 3 Talat N, Diaz-Cano S & Schulte KM. Inflammatory diseases of the parathyroid gland. *Histopathology* 2011 **59** 897–908. (doi:10.1111/j.1365-2559.2011.04001.x)
- 4 Kifor O, Moore FD Jr, Delaney M, Garber J, Hendy GN, Butters R, Gao P, Cantor TL, Kifor I, Brown EM *et al.* A syndrome of hypocalciuric hypercalcemia caused by autoantibodies directed at the calcium-sensing receptor. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 60–72. (doi:10.1210/jc.2002-020249)
- 5 Kuo E, Kemp EH, Sandhu HK, Brown EM, Weetman AP & Huang CL. Acquired hypocalciuric hypercalcemia in a patient with CKD. *American Journal of Kidney Diseases* 2013 **62** 1151–1154. (doi:10.1053/j.ajkd.2013.04.023)
- 6 Makita N, Sato J, Manaka K, Shoji Y, Oishi A, Hashimoto M, Fujita T & Iiri T. An acquired hypocalciuric hypercalcemia autoantibody induces allosteric transition among active human Ca-sensing receptor conformations. *PNAS* 2007 **104** 5443–5448. (doi:10.1073/pnas.0701290104)
- 7 Pallais JC, Kemp EH, Bergwitz C, Kantham L, Slovik DM, Weetman AP & Brown EM. Autoimmune hypocalciuric hypercalcemia unresponsive to glucocorticoid therapy in a patient with blocking autoantibodies against the calcium-sensing receptor. *Journal of Clinical Endocrinology and Metabolism* 2011 **96** 672–680. (doi:10.1210/jc.2010-1739)
- 8 Vaizey CJ, Ali M & Gilbert JM. Chronic parathyroiditis associated with primary hyperplastic hyperparathyroidism. *Journal of the Royal Society of Medicine* 1997 **90** 336–337.
- 9 Bondeson AG, Bondeson L & Ljungberg O. Chronic parathyroiditis associated with parathyroid hyperplasia and hyperparathyroidism. *American Journal of Surgical Pathology* 1984 **8** 211–215. (doi:10.1097/0000478-198403000-00007)
- 10 Chetty R & Forder MD. Parathyroiditis associated with hyperparathyroidism and branchial cysts. *American Journal of Clinical Pathology* 1991 **96** 348–350. (doi:10.1093/ajcp/96.3.348)
- 11 Kemp EH, Gavalas NG, Krohn KJ, Brown EM, Watson PF & Weetman AP. Activating autoantibodies against the calcium-sensing receptor detected in two patients with autoimmune polyendocrine syndrome type 1. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 4749–4756. (doi:10.1210/jc.2009-1080)
- 12 Kifor O, McElduff A, LeBoff MS, Moore FD Jr, Butters R, Gao P, Cantor TL, Kifor I & Brown EM. Activating antibodies to the calcium-sensing receptor in two patients with autoimmune hypoparathyroidism. *Journal of Clinical Endocrinology and Metabolism* 2004 **89** 548–556. (doi:10.1210/jc.2003-031054)
- 13 Li Y, Song YH, Rais N, Connor E, Schatz D, Muir A & Maclaren N. Autoantibodies to the extracellular domain of the calcium sensing receptor in patients with acquired hypoparathyroidism. *Journal of Clinical Investigation* 1996 **97** 910–914. (doi:10.1172/JCI118513)
- 14 Seino A, Iguchi A, Ito T, Saeki T, Makita N, Iiri T & Yamazaki H. Case report; a case of acquired hypocalciuric hypercalcemia due to autoantibody against the calcium-sensing receptor. *Nihon Naika Gakkai Zasshi* 2014 **103** 1180–1182. (doi:10.2169/naika.103.1180)
- 15 Pelletier-Morel L, Fabien N, Mouhoub Y, Boitard C & Larger E. Hyperparathyroidism in a patient with autoimmune polyglandular syndrome. *Internal Medicine* 2008 **47** 1911–1915. (doi:10.2169/internalmedicine.47.1355)
- 16 Gavalas NG, Kemp EH, Krohn KJ, Brown EM, Watson PF & Weetman AP. The calcium-sensing receptor is a target of autoantibodies in patients with autoimmune polyendocrine syndrome type 1. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 2107–2114. (doi:10.1210/jc.2006-2466)
- 17 Charrie A, Chikh K, Peix JL, Berger N, Decaussin M, Veber S, Biennu J, Lifante JC & Fabien N. Calcium-sensing receptor autoantibodies in primary hyperparathyroidism. *Clinica Chimica Acta* 2009 **406** 94–97. (doi:10.1016/j.cca.2009.05.023)
- 18 Hasegawa S, Mine S & Hagiwara S. IgG4-related disease combined with autoimmune hemolytic anemia and steroid-responsive transient hypercalcemia. *Clinical Medicine Insights: Case Reports* 2015 **8** 51–55. (doi:10.4137/ccrep.s25553)

Received 28 February 2017

Revised version received 16 April 2017

Accepted 21 April 2017