Case Report

A Novel Mutation of the Calcium-Sensing Receptor Gene Causing Familial Hypocalciuric Hypercalcemia Complicates Medical Followup after Roux-en-Y Gastric Bypass: A Case Report and a Summary of Mutations Found in the Same Hospital Laboratory

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Heterozygous inactivating mutations in the calcium-sensing receptor (CaSR) gene are known to cause familial hypocalciuric hypercalcemia (FHH), usually a benign form of hypercalcemia without symptoms of a disrupted calcium homeostasis. FHH can be mistaken for the more common primary hyperparathyroidism (PHP), for which surgical treatment may be needed. We describe a case of a 36-year-old woman with hypercalcemia and elevated PTH, initially suspected of having PHPT. Sequencing of the CaSR-gene revealed a mutation in nucleotide 437, changing the amino acid in position 146 from Glycine to Aspartate. The mutation was previously undescribed in the literature, but a very low calcium:creatinine clearance ratio supported the diagnosis FHH. A few years later, the patient's two daughters were tested and the association between mutation and hypercalcemia could be confirmed. The patient was gastric bypass-operated and therefore, due to malabsorption and increased risk of fracture, was in need of adequate calcium supplementation. The chronically elevated calcium levels challenged medical followup, as calcium sufficiency could not be monitored in a traditional manner. Eventually the patient developed elevated alkaline phosphatase, a further increased PTH and a decreased DXA T-score indicating calcium deficiency and bone resorption. As a supplement, all CaSR-mutations found at our hospital, 2005-2018.

1. Introduction

Calcium homeostasis is crucial for many biological functions and is regulated by direct and indirect effects of parathyrine (PTH) on kidney, bone, and intestine, released as a reaction to sensing of hypocalcemia in the parathyroid gland, mediated through the calcium-sensing receptor (CaSR) [1]. Calcium is usually absorbed throughout the whole intestine, where an adequate vitamin D-level is required for optimal uptake [2]. If the supply is insufficient, increased PTH stimulates urinary calcium reabsorption and resorption of calcium from the skeleton. In addition, through their own calcium-sensing receptors, the kidneys promote urinary calcium reabsorption independently of PTH [1].

Heterozygous inactivating mutations in the CaSR-gene are known to cause familial hypocalciuric hypercalcemia (FHH), also called benign hypercalcemia due to its mild, often asymptomatic phenotype [3, 4]. Neonatal severe hyperparathyroidism and autosomal dominant hypocalcemia (ADH) are other more severe clinical
manifestations of mutations in the same gene. So far, more
than 130 FHH-causing mutations of the CaSR-gene have
been described and novel mutations keep being reported [1].

In patients with hypercalcemia, the main reason for
sequencing the CaSR-gene in search for mutations is to
distinguish patients with FHH from patients with primary
hyperparathyroidism (PHPT), who otherwise have a similar
presentation biochemically [4, 5]. Parathyroidectomy, a
removal of one or several of the parathyroid glands is needed
to treat symptomatic PHPT that typically arises as a sporadic
mutation in an adenoma [6], while FHH rarely requires
treatment and cannot be cured surgically, unless a total
parathyroidectomy is performed [7]. Most patients with FHH
have a mutation in the CaSR-gene (FHH 1) but occasionally
the cause is a mutation in two other genes, GNAI1 (FHH2)
or AP2S1 (FHH3), the latter associated with a more severe
phenotype [1, 5, 8, 9]. A similar clinical presentation in
patients without mutations related to the CaSR-gene may
also be caused by CaSR-blocking autoantibodies, as seen in
autoimmune hypocalciuric hypercalcemia (AHH) [3]. Apart
from genetic sequencing, the calcium:creatinine clearance
cratio is important in distinguishing between PHPT and FHH
[10].

Calcium deficiency and secondary hyperparathyroidism
are also problematic and commonly seen in patients treated
for obesity with Roux-en-Y gastric bypass, where a part
of the stomach and proximal intestine is excluded from
food passage [11]. These patients are at increased risk of
getting a fracture compared to the general population [12]
and an adequate supplementation of calcium and vitamin
D, monitored by measurements of calcium-ion, 25-hydroxy-
vitamin D, and PTH, is important to reduce this risk [13].

We describe a case of a 36-year-old gastric bypass-
operated woman with hypercalcaemia and elevated PTH.
 Sequencing of the CaSR-gene revealed a previously unde-
scribed mutation in nucleotide 437, changing the amino acid
in position 146 from Glycine (G) to Aspartate. Six years later, her
two daughters were tested. One of the daughters was found to
carry the same mutation.

2. Case Presentation

A 36-year-old woman was referred to the department of
endocrinology for further examination of hypercalcaemia,
which was discovered during routine blood tests after gastric
bypass operation 1 year earlier. There was no history of
kidney stones, fractures, or osteoporosis that may be a result
of hypercalcaemia, and she had no known hyperthyroidism,
Addison’s disease, malignancy, sarcoidosis, or any other
granulomatous disease that could explain the hypercalcaemia.
She had lost contact with her mother and sister, her only
living relatives. Thus, a family history of hypercalcaemia could
not be investigated.

The patient inconsistently took calcium and vitamin D3
supplements in addition to iron, cobalamine, and multi-
vitamins after the gastric bypass operation. She did not
take thiazide diuretic or any other medications. She had
symptoms of depression, anxiety, and tiredness and was later
prescribed antidepressant medication. She also had recurrent
episodes of dizziness, tremor, sweating, and fatigue, which
resolved with the ingestion of carbohydrate and was related
to hypoglycemia. Reactive hypoglycemia is a known late
complication of gastric bypass operation induced by inappro-
priate hyperinsulinemia after the intake of rapidly absorbed
carbohydrates [13]. The reactive hypoglycemia responded to
dietitian instructions.

Repeated blood tests showed Ca-ion between 1.42 and
1.47 mmol/l (ref: 1.18 – 1.32 mmol/l), PTH between 6.3
and 8.9 pmol/l (ref: 1.7 – 71 pmol/l), and 25-hydroxy vitamin
D between 43 and 58 nmol/l (ref: > 50 pmol/l). Alkaline
phosphatase and thyroid function were normal. Dual-energy
X-ray absorptiometry (DXA) showed T=-0.6 and T=-0.2
at the lumbar spine and total hip, respectively (Table 1).

Based on the mild hypercalcaemia and the high normal to
slightly elevated PTH, the patient was suspected of having
primary PHPT. Before referral to a surgeon, FHH, the
rare differential diagnosis to PHPT, had to be excluded.
Genetic testing for mutations in the CaSR-gene showed
a heterozygous mutation in nucleotide 437, changing
the amino acid in position 146 from Glycine (G) to Aspartate
(D) (c.[437G>A];[=], p.[(G146D)];[=]), reference sequence
NM_000388.3 OMIM 601199), here called G146D. The found
mutation was not formerly associated with FHH. However,
urine calcium:creatinine clearance ratio was very low, 0.0029
and 0.0017 on two occasions, which verified the diagnosis
of FHH, at the same time excluding the more common PHPT.

The CaSR is a member of the subfamily C of G-protein-
coupled receptors which have seven transmembrane domains
and function as disulfide-linked homodimers [14]. Its gene
is located on chromosome 3ql3.3-21.1 [15] and includes
seven exons where exon 2-7 are protein coding [1]. The
mutation G146D is located in exon three, in the coding
region of the receptor’s extracellular Venus flytrap domain,
more specifically as a part of LB1, a domain important for
the ligand binding of calcium ions (Ca$^{2+}$) and the amino
acid L-tryptophan (L-Trp) that functions as an allosteric
activator, enhancing the sensitivity of the receptor towards
Ca$^{2+}$ [14]. The binding of Ca$^{2+}$ at this position seems to
be important for maintaining the structural integrity of the
receptor whereas other binding positions for Ca$^{2+}$ are of
more importance for receptor activity. Mutations in amino
acids positioned as number 145 and 147, flanking number 146
on each side, have both been shown to eliminate the Ca$^{2+}$
induced receptor activity. Based on this and the fact that
the amino acid in position 146 is a part of the L-Trp binding
cleft, it is likely that a mutation in position 146 reduces the
receptor activity. Indeed, predictions from public in silico
evaluation tools (Mutation Taster, PolyPhen, Provean) that
were used to assist in the interpretation of the DNA-variant
G146D all predicted a homozygous mutation G146D to be
disease causing when factors like the location of the mutation
regarding change of splice site, active site, or amino acid, as
well as the nucleotide conservation between species, were
taken into consideration. This knowledge about the CaSR-
structure and consequence of mutations in neighboring
amino acid positions was however not available at the time
the patient was diagnosed.
Six years later, the patient’s two daughters, 12 and 17 years old, were tested for the mutation. The youngest daughter did not have any mutation in the CaSR-gene, but she had a low level of D-vitamin associated with secondary hyperparathyroidism, low Ca-ion and elevated alkaline phosphatase. The eldest daughter was found to be carrying the same mutation in the CaSR-gene as her mother, associated with hypercalcemia and inappropriately high PTH. She, too, had a low level of D-vitamin associated with secondary hyperparathyroidism, low Ca-ion and elevated alkaline phosphatase. The eldest daughter was found to be carrying the same mutation, the diagnosis meant that she, like her mother, was not considered for surgery. In general, it is important to test females of FHH families, because calcium gets across the placenta and, in FHH mothers, leads to fetal hypercalcemia with a subsequent suppression of fetal parathyroid function resulting in the risk of transient, neonatal, hypocalcemic tetanus [16]. Apart from this, FHH is usually asymptomatic with rarely seen complications such as pancreatitis, gallstones, or chondrocalcinosis [3, 4, 10]. As complications are correlated to the degree of hypercalcemia, most distinctly in patients with FHH3 [1], simply monitoring the calcium level without treatment often suffice. Extreme values or symptoms of hypercalcemia might be treated with calcimetics [17].

Chronic hypercalcemia is associated with kidney stones and osteoporosis (the latter not in FHH), but also neuromuscular and psychiatric symptoms [6]. The patient in the case vignette reported fatigue and depression, which could be caused by hypercalcemia. To our knowledge, this is the first case report of a patient with FHH treated for obesity with gastric bypass surgery, a combination that challenges medical investigation and followup. Especially, monitoring of calcium homeostasis needs a broader approach, as plasma levels of calcium and PTH are chronically elevated. In this case, malabsorption after gastric bypass surgery resulting in calcium deficiency could be identified by an increased level of PTH, compared to the habitual level of PTH, eventually combined with an increased level of alkaline phosphatase and a reduced bone mineral density (BMD). This complexity emphasizes the importance of qualified medical followup after gastric bypass surgery.

Interestingly, this case report also illustrates another complexity, namely, the large variation within the spectrum of hyperparathyroidism, here with several variants seen in the same family. First, we have our patient with FHH and mineral supplementations. For the eldest daughter, who is a carrier of the same mutation, the diagnosis meant that she, like her mother, was not considered for surgery. In general, it is important to test females of FHH families, because calcium gets across the placenta and, in FHH mothers, leads to fetal hypercalcemia with a subsequent suppression of fetal parathyroid function resulting in the risk of transient, neonatal, hypocalcemic tetanus [16]. Apart from this, FHH is usually asymptomatic with rarely seen complications such as pancreatitis, gallstones, or chondrocalcinosis [3, 4, 10]. As complications are correlated to the degree of hypercalcemia, most distinctly in patients with FHH3 [1], simply monitoring the calcium level without treatment often suffice. Extreme values or symptoms of hypercalcemia might be treated with calcimetics [17].

### 3. Discussion

We have described a case story of a woman with hypercalcemia and permanently elevated PTH, where sequencing of the CaSR-gene revealed a probable disease-causing mutation in nucleotide 437, changing the amino acid in position 146 from Glycine to Aspartate. Mutations in the same locus had not been previously described, but the clinical presentation with a low calcium:creatinine clearance strongly supported the diagnosis FHH. Six years later, following CaSR-gene sequencing in two daughters, we were finally able to confirm that the novel mutation found most likely is the cause of the hypocalciuric hypercalcemic phenotype in this family. At this time, updated mutation databases and a better understanding of CaSR-structure and function were helpful in predicting the mutation to be disease causing, inactivating the CaSR.

In hyperparathyroidism, a correct diagnosis is important for several reasons. For our patient, the diagnosis of FHH1 ensured that she avoided unnecessary parathyroidectomy and finally enabled prescription of the proper vitamin and mineral supplementations. For the eldest daughter, who is a carrier of the same mutation, the diagnosis meant that she, like her mother, was not considered for surgery. In general, it is important to test females of FHH families, because calcium gets across the placenta and, in FHH mothers, leads to fetal hypercalcemia with a subsequent suppression of fetal parathyroid function resulting in the risk of transient, neonatal, hypocalcemic tetanus [16]. Apart from this, FHH is usually asymptomatic with rarely seen complications such as pancreatitis, gallstones, or chondrocalcinosis [3, 4, 10]. As complications are correlated to the degree of hypercalcemia, most distinctly in patients with FHH3 [1], simply monitoring the calcium level without treatment often suffice. Extreme values or symptoms of hypercalcemia might be treated with calcimetics [17].

### 4. Conclusion

The patient’s two daughters, 12 and 17 years old, were tested for the mutation. The youngest daughter did not have any mutation in the CaSR-gene, but she had a low level of D-vitamin associated with secondary hyperparathyroidism, low Ca-ion and elevated alkaline phosphatase. The eldest daughter was found to be carrying the same mutation, the diagnosis meant that she, like her mother, was not considered for surgery. In general, it is important to test females of FHH families, because calcium gets across the placenta and, in FHH mothers, leads to fetal hypercalcemia with a subsequent suppression of fetal parathyroid function resulting in the risk of transient, neonatal, hypocalcemic tetanus [16]. Apart from this, FHH is usually asymptomatic with rarely seen complications such as pancreatitis, gallstones, or chondrocalcinosis [3, 4, 10]. As complications are correlated to the degree of hypercalcemia, most distinctly in patients with FHH3 [1], simply monitoring the calcium level without treatment often suffice. Extreme values or symptoms of hypercalcemia might be treated with calcimetics [17].

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Interestingly, this case report also illustrates another complexity, namely, the large variation within the spectrum of hyperparathyroidism, here with several variants seen in the same family. First, we have our patient with FHH and...
calcium deficiency, second the eldest daughter with FHH and normal level of PTH, and last the youngest daughter with secondary hyperparathyroidism due to deficiency of vitamin D and calcium. In common, all family members have low levels of D-vitamin.

Compared to the relatively high prevalence of PHPT, FHH is rare [4]. At our hospital, we started in 2005 to perform genetic testing by polymerase chain reaction (PCR) and Sanger sequencing of the six protein coding exons (exon 2-7) and intron boundaries of the CaSR-gene. Since the beginning, we have sequenced DNA from 822 patients suspected of having a parathyroid adenoma. Altogether, we have found 56 different mutations in heterozygous form (Supplementary Table S1), a majority of which as of September 2018 had been described in the medical literature [5, 18–26] and/or reported in mutation databases like ClinVar [27]. Most of the mutations found were inactivating mutations. In total, a mutation was found in 91 (11%) of the patients tested. The real mutation prevalence in this population is, although, most certainly higher. This is due to the limitations of the method that only sequence the coding exons and the intron boundaries of the CaSR-gene but not the promoter region, the deep intron regions, and the 3’ untranslated region. Moreover, large genomic deletions and sequence variants in primer binding sites can be missed, resulting in false-negative test results. Mutations in other genes, for example, GNA11 or AP2S1 where mutations can result in FHH2 and FHH3, are not examined.

Important to note and as illustrated in this case report is that the interpretation of the clinical consequence of the mutation can change depending on the data available for a found mutation at the given time. The clinical picture, supported by results from routine biochemical analyses and diagnostic imaging, will always be of great value in diagnosis.

Consent

Informed consent for publication was obtained from the patient involved.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors’ Contributions

Elin Rebecka Carlsson collected and interpreted the biochemical data and wrote the article. Mai-Britt Toft Nielsen collected the clinical data, wrote parts of the case report section, and contributed to discussion. Anne Mette Høgh and Rikke Veggerby Grønlund interpreted mutation analysis results. Mogens Fenger was medically responsible for the analysis and contributed to discussion. Louise Ambye was responsible for the analytical method and quality of the analysis, contributed with information about the CaSR-receptor and mutation data and contributed also to discussion. All authors read and approved the final manuscript.

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Supplementary Materials

Supplementary Table S1. A summary of all CaSR-mutations found at our hospital laboratory between 2005 and 2018, their associated phenotypes (based on available laboratory results), in silico predictions, and references to the literature. (Supplementary Materials)

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