Multiple endocrine neoplasia phenocopy revealed as a co-occurring neuroendocrine tumor and familial hypocalciuric hypercalcemia type 3

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Key Clinical Message
Familial hypocalciuric hypercalcemia type 3 should be considered as differential diagnosis in patients with suspected primary hyperparathyroidism and/or suspected multiple neoplasia syndrome, as correct diagnosis will spare the patients for going through multiple futile parathyroidectomies and for the worry of being diagnosed with a cancer susceptibility syndrome.

Keywords
AP2S1, familial hypocalciuric hypercalcemia type 3, multiple endocrine neoplasia type 1, neuroendocrine tumors, primary hyperparathyroidism.

Introduction
Familial hypocalciuric hypercalcemia (FHH) is a heterogeneous, autosomal dominantly inherited disorder characterized by hypercalcemia with (relative) hyperparathyroidism, and hypocalciuria [1]. Classically, FHH is caused by inactivating mutations in the gene encoding the calcium-sensing receptor (CASR) on chromosome 3q13, termed FHH type 1 (FHH1) [2–4]. Recently, further two FHH phenotypes have been identified; FHH2 have been shown to be caused by inactivating mutations in the gene encoding subunit alpha-11 of the G protein (GNA11) on chromosome 3q13, termed FHH type 2 (FHH2) [5]. FHH3 by mutations affecting codon 15 (p.Arg15Leu, p.Arg15Cys, and p.Arg15His) in the gene encoding the adaptor protein 2 sigma subunit (AP2S1) on chromosome 19q13.32 [5, 6]. FHH is generally considered a benign, symptomless condition [7–9]. However, it is an important differential diagnosis to primary hyperparathyroidism (PHPT). Due to the biochemical similarity to mild PHPT, patients are at risk of being referred to parathyroidectomy (PTX) which does not benefit patients with FHH1. New data, however, suggest that FHH3 is associated with hypercalcemic symptoms in >20% of patients [10], thus giving rise to suspicion that mutations in AP2S1 have more deleterious effects. At the time of writing, 55 index cases of FHH3 have been reported [6, 10–13].

We now report a family with FHH3 among whom two brothers had PTX performed due to suspected PHPT with one of the brothers having a concomitant diagnosis of a neuroendocrine tumor (NET). The study is conducted in accordance with the Helsinki Declaration and approved by The Health Research Ethics Committee in the Central Denmark Region (no. 1-10-72-174-14) and by the Danish Data Protection Agency (no. 1-16-02-466-14). The patients gave informed consent to have their disease story published.
Clinical Report

The index case, a male born in 1976, was diagnosed with hyperparathyroid hypercalcemia at the age of 36 (Table 1). Despite only moderate hypercalcemia (ionized calcium 1.48 mmol/L), he had numerous symptoms possibly attributable to hypercalcemia including tiredness, a progressive feeling of muscle weakness, and pain in the upper extremities. Renal imaging (CT scan) showed no renal calcifications. DXA scans showed a normal BMD at the hip (T-score = −0.3) and mild osteopenia at the lumbar spine (T-score = −1.3) and forearm (T-score = −1.3) [14]. 25-hydroxyvitamin D was just below the normal range. Due to a low calcium-creatinine clearance ratio (CCCR = 0.005), a genetic test was performed for FHH1, which is in accordance with the diagnostic guidelines for PHPT [15]. However, no mutations were identified in the CASR. Family screening revealed a father with intermittently elevated serum levels (total calcium 2.52–2.62 mmol/L). The oldest brother (born 1971) refused testing, whereas another brother (born 1973) had normocalcemia.

Besides hypercalcemic symptoms, the index patient also had gastrointestinal symptoms (4–6 loose stools per day), leading to a colonoscopy, which showed an intussusception of the terminal ileum. Abdominal CT showed thickened intestinal wall in the distal ileum and enlarged lymph glands. The patient underwent hemicolectomy and removal of the distal ileum. Histological examination showed a radically excised intermediate-grade NET, which was immunohistochemically positive for CD56, chromogranin A, and synaptophysin. Ki-67 proliferation index was 3%. Five of 22 lymph glands contained metastases. The co-occurrence of hyperparathyroid hypercalcemia and a NET leads to suspicion of a multiple endocrine neoplasia (MEN) syndrome, but additional genetic tests did not reveal mutations in CDC73, RET, proto-oncogene, or MEN1. Biochemical testing and scans of the pituitary gland and the pancreas showed no signs of additional tumors.

Due to the patient’s young age and the clinical suspicion of a MEN1 syndrome, the hyperparathyroid hypercalcemia was considered as a state of PHPT and he was referred to PTX. Prior to surgery, neck ultrasound suggested an enlarged parathyroid gland on the right inferior side of the thyroid gland, but a parathyroid scintigraphy (pertechnetate/99mTc-MIBI) did not support the finding. Subtotal PTX was performed in March 2014 with removal of the upper right and lower left glands, as well as half of the upper left gland and the thymus gland. The lower right gland was not identified. Intraoperatively, PTH decreased from 8.7 to 3.8 pmol/L. However, PTH increased to 8.8 pmol/L in the days following surgery and the patient remained hypercalcemic. He was re-operated 11 days later with removal of the right thyroid lobe. The histopathological examination revealed an intrathyroid parathyroid adenoma. Following the second operation, calcium and PTH levels remained elevated and the patient had sustained complaints of hypercalcemic symptoms. Accordingly, treatment with cinacalcet was initiated causing normalization of serum calcium levels and symptomatic relief. Due to the improved well-being following normalization of calcium levels, a third PTX was performed 6 months later with removal and partial autoimplantation of the remaining half-gland on the right thigh (vastus lateralis, m. quadriceps femoris). Subsequently, the patient developed hypoparathyroidism, but despite consistent needs for treatment, he reported an improved well-being.

Due to the complicated course of the index patient, his brother (born 1971) who initially refused biochemical screening reconsidered and had biochemical tests

Table 1. Biochemical characteristics of the FHH3 family.

|                      | Reference values | Index patient Before surgery 1 | Before surgery 2 | Before surgery 3* | After surgery** | Brother Before surgery | After surgery | Father |
|----------------------|------------------|--------------------------------|-----------------|-----------------|-----------------|------------------------|--------------|--------|
| Calcium, mmol/L      | 1.18–1.32        | 1.48                           | 1.46            | 1.40            | 1.18            | 1.48                   | 1.43         | 1.38   |
| Calcium, total, mmol/L | 2.20–2.55       | 2.68                           | 2.60            | 2.40            | 2.36            | 2.71                   | 2.73         | 2.56   |
| PTH, pmol/L          | 1.6–6.9          | 13.5                           | 5.5             | 5.7             | 1.1             | 10.6                   | 7.0          | 7.1    |
| CCCR                 | 0.005            |                                |                 |                 |                 |                        |              |        |
| Magnesium, mmol/L    | 0.70–1.10        | 0.85                           | 0.80            | 0.73            | 1.00            | 1.00                   | 1.02         | 0.93   |
| Phosphate, mmol/L    | 0.71–1.53        | 0.74                           | 0.54            | 1.28            | 0.77            | 0.77                   | 0.81         |        |
| 25-hydroxyvitamin D, nmol/L | 50–160        | 48                             | 60              |                 | 53              | 53                     | 61           |        |
| Alkaline phosphatase, U/l | 35–105         | 45                             | 37              | 33              | 44              | 34                     | 76           |        |
| Creatinine, µmol/L   | 60–105           | 76                             | 83              | 80              | 93              | 99                     | 91           |        |

Means of the biochemical measurements of affected members in the FHH3 family. CCCR, calcium-creatinine clearance ratio. *treated with cinacalcet. **treated for hypoparathyroidism.
performing showing hyperparathyroid hypercalcemia (Table 1). He only had weak complaints of tiredness. Due to the occurrence of a MEN1-like syndrome in the family, he was tested for mutations similar to the index case and underwent colonoscopy without any pathological findings. Further examinations revealed a CCCR = 0.005, no renal calcifications, normal BMD (except for a hip T-score of −1.1) [14]. No parathyroid adenoma was identified on neck ultrasound or parathyroid scintigraphy. He was referred to PTX, during which he had 2.5 hyperplastic glands removed. The lower right gland was not identified, but as PTH fell from 17.0 to 3.7 pmol/L intraoperatively the surgery was considered successful. PTH increased, however, to supranormal levels within 10 days following surgery with persistently elevated ionized calcium levels (1.39–1.46 mmol/L).

During the course of the diseases, we had developed methods to test for FHH2 and FHH3. We therefore screened the two brothers for mutations in AP2S1 and GNA11 and found a c.44G>A substitution in AP2S1 resulting in p.Arg15His, a mutation previously reported to cause FHH3 [6, 10, 11]. Screening of the remaining family revealed that the brothers had inherited the mutation from their father, whereas the mother, a brother, and three children were mutation negative (Fig. 1). The affected father reported no symptoms of hypercalcemia.

**Histopathological examination**

The histopathological analysis of the glands from the index patients showed one gland with a focus of hyperplasia, two glands with diffuse hyperplasia, and an intrathyroidal process diagnosed as a parathyroid adenoma. The hyperplasia in one gland was characterized by dense fibrosis with hyperplasia of both chief and oxyphil cells and loss of lipid cells. In the other glands, hyperplasia was also present and characterized by loss of lipid cells and increased amount of chief cells. The adenoma showed uniform water-clear cells with solid growth pattern and few scattered isles of oxyphil cells. There was complete loss of lipid cells. The removed glands from the brother also showed hyperplasia, which in one gland was characterized by loss of lipid cells and hypercellularity of both chief cells and oxyphil cells, whereas the other two glands were characterized by a more normal fat distribution although still with hypercellularity of oxyphil and chief cells (Fig. 2).

**Discussion**

AP2S1 mutations causing FHH3 have recently been reported and only sparse data are available on patients with FHH3. We believe that the disease courses of the patients reported raise several clinical important issues. First of all, the index case represents the first reported association between a NET and FHH3. Secondly, our report supports the previously reported notion on some patients with FHH3 having symptomatic hypercalcemia. Finally, to avoid unnecessary surgery and risk of postsurgical hypoparathyroidism, our report calls for increased awareness of the presence of mutations in other genes than the CASR, which may cause FHH.

The co-occurrence of a NET and hyperparathyroid hypercalcemia in a relatively young patient leads to the suspicion of a MEN1 syndrome. MEN1 is autosomal dominant inherited and is characterized by PHPT, pancreatic tumors, pituitary tumors, and more seldom NETs typically located to the adrenal gland, or derived from the embryonic foregut [16, 17]. The clinical diagnosis is considered when more than one organ system is affected. Our index patient presented with assumed PHPT, which is typically the first manifestation of MEN1 and occurs in 95% of cases [18, 19]. In addition, he had a NET (formerly known as carcinoid), which have been reported to occur in 4% of MEN1 patients [16, 19, 20]. In retrospect, the clinical MEN1 diagnosis was not clear-cut, with several lines of evidence suggesting that our index patient was most likely a so-called phenocopy, that is, a phenotype resembling MEN1 without being it [21, 22]. The NET found in our index patient was located to the hindgut, which is very rare in MEN1. In addition, the patient was negative for all mutations known to cause MEN1, although about 5–15% of considered MEN1 cases are genetically unverified [17, 23, 24]. Furthermore, only the index patient had affection of more than one organ, and as the penetrance of MEN1 is close to 100% in patients older than 50 years, it is unlikely that the 71-year-old father should be unaffected [20, 25]. The low CCCR is

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**Figure 1.** Pedigree for the FHH3 family. Circles are female, and squares are male. Black indicates hypercalcemia, white normocalcemia, and gray is unknown. The arrow points at the index patient.
also not typical for PHPT, but can occur due to low vita-
m in D. According to Thakker et al., MEN1 phenocopies
are seen in up to 10% of cases with the causative muta-
tions found in other genes including CDC73 and CASR.
Consequently, the absence of mutations in the MEN1
gene should lead to analysis of these genes [26]. Our case
adds AP2S1 to the genes that need to be considered, when
examining a clinical, but genetically unverified MEN1
patient.

In addition to the biochemical findings, the presumed
diagnosis of PHPT was supported by symptoms, which
could be attributable to hypercalcemia. This is in contrast
to FHH1 patients who in general are asymptomatic [7].
Reports of coexisting FHH1 and PHPT have previously
been published [27–29]. It may therefore be considered
whether the index patient had developed PHPT superim-
posed on his inherited FHH3, which is supported by the
histopathological findings showing multiple gland affec-
tion with both adenoma and hyperplasia. No previous
reports on histopathological findings in parathyroid
glands from FHH3 patients report adenomas, but only
diffuse hyperplastic or normal glands [10, 11, 30]. How-
ever, the findings of similar serum calcium levels, PTH
levels, and low CCCR in the index patient and his
brother (Table 1) do not support that the index patient
had developed PHPT on top of his inherited FHH3.
Rather, our findings support previous reports on hyper-
calcemic symptoms in approximately 20% of FHH3
patients. AP2S1 mutations are associated with different
phenotypes, where p.Arg15His give cause to the mildest
phenotype, that is, the lowest degree of elevated calcium
levels, and also the least degree of hypocalciuria [10].
Interestingly, no correlations have been found between
genotype and symptoms, and it can be speculated
whether the reported symptoms are induced by iatrogenic
factors as proposed by Marx et al. who found a similar
level of hypercalcemic symptoms among FHH1 patients
in 1981 [31]. However, further characteristics of FHH3
patients need to be performed in order to clarify whether
FHH3 is indeed associated with a more severe course, as
well as whether there is any correlation to neuroen-
docrine tumors.

In conclusion, we have reported the first case with co-
ocurrence of FHH3 and a NET in the distal ilium.
Although our index case was most likely a phenocopy,
resembling an atypical MEN1-like syndrome, we believe
that further attention should be paid to whether FHH3
may be associated with other endocrine tumors. Further-
more, our cases underline the importance of considering
FHH3 in families with hyperparathyroid hypercalcemia,
including patients with a clinical suspicion of MEN1 who
are mutation-negative, as this may spare patients for the
worry of being diagnosed with a cancer susceptibility syn-
drome and PTX.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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