

## Carcinoma of Two Parathyroid Glands Caused by a Novel *MEN1* Gene Mutation – a Rare Feature of the MEN 1 Syndrome

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**Summary.** Multiple endocrine neoplasia type 1 (*MEN 1*) is a rare syndrome inherited in an autosomal dominant pattern, characterized by combinations of tumors of the parathyroid glands, pituitary gland, and pancreatic islet cells and more rare tumors of endocrine organs and nonendocrine tissues. Germline mutations in the *MEN1* gene are responsible for the *MEN 1* syndrome, leading to an inactive form of menin protein. Benign lesions of the parathyroid glands are characteristic in patients with the *MEN 1* syndrome; however, patients can develop parathyroid carcinomas very rarely. This report presents a clinical case of the *MEN 1* syndrome: a 39-year-old woman underwent surgery for carcinoma of two parathyroid glands as well as was treated for pituitary prolactinoma, which caused infertility, and malignant insulinoma; the patient had multiple subcutaneous lipomas as well. Genetic analysis revealed a novel germline mutation in the *MEN1* gene – a nucleotide insertion at codon 43 in exon 2 (c.129insA), which caused the occurrence of the *MEN1* syndrome. The clinical case of the *MEN 1* syndrome presented here is relevant in gathering the data on etiopathogenesis of not only *MEN 1* syndrome, but an extremely rare pathology – parathyroid carcinoma – as well.

### Introduction

Multiple endocrine neoplasia type 1 (*MEN 1*) is a rare syndrome inherited in an autosomal dominant pattern, characterized by combinations of tumors of the parathyroid glands, pituitary gland, and pancreatic islet cells (1). Some patients may also develop benign adrenocortical, thyroid tumors; gastric, bronchial, and thymic carcinoid tumors; subcutaneous and visceral lipomas, cutaneous leiomyomas, angiofibromas and collagenomas, ependymomas of the spinal cord (1). The parathyroid lesions associated with the *MEN 1* syndrome – hyperplasia and adenomas causing primary hyperparathyroidism – are usually benign, and carcinoma of the parathyroid glands is very rarely diagnosed in patients with the *MEN 1* syndrome and it is not included in diagnostic clinical criteria of the *MEN 1* syndrome. The *MEN 1* syndrome is caused by germline mutations in the *MEN1* tumor suppressor gene located in 11q13, which inactivate a protein, menin (2, 3). Mutations in this gene are determined in approximately 90% of patients with clinically diagnosed

*MEN 1* syndrome (4). In the literature, the association of the *MEN 1* syndrome with parathyroid carcinoma has been reported very rare (5, 6), and not always mutations in the *MEN1* gene are identified in patients (7).

### Case Report

Written informed consent was obtained from all the participants, and the study was approved by the Bioethics Committee of Lithuanian University of Health Sciences (former Kaunas University of Medicine). A 39-year-old woman was examined and treated for two enlarging masses palpable in the left and right lobes of the thyroid gland at the Clinic of Endocrinology, Hospital of Lithuanian University of Health Sciences. The patient complained of general weakness, fatigue, weight loss, and bilateral galactorrhoea as well. Lesions in the thyroid were followed-up for 4 years (from the age of 35 years). Repeat aspiration biopsy of the node in the left lobe of the thyroid gland showed atypical cells suspicious for malignancy, which was not seen during previous cytologic examination. The patient was found to have an increased plasma parathormone level (34.3 pmol/L; normal range, 1.18–8.43

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pmol/L) as well as calcium (3.34 mmol/L; normal range, 2.15–2.5 mmol/L;  $\text{Ca}^{2+}$  1.5 mmol/L; normal range, 0.98–1.13 mmol/L) and phosphorus (0.73 mmol/L; normal range, 0.9–1.53 mmol/L) concentrations. The patient had a 4-year history of galactorrhea and amenorrhea (from the age of 35 years), which were detected some months earlier than thyroid nodules; she was unable to conceive. Elevated plasma prolactin level ( $>3600$  mIU/L) and nuclear magnetic resonance imaging of the pituitary gland led to the diagnosis of pituitary microprolactinoma (0.9×0.8×0.7 cm in size). Treatment with bromocriptine (2.5–12.5 mg per day) resulted in a decrease in prolactin concentration to 76 mIU/L; the return of menses was documented. After one year of treatment with bromocriptine, the patient became pregnant (at the age of 36 years) and gave birth to a 2800-g full-term male infant. After birth, the infant was breastfed for 14 months.

Given that tumors in the thyroid were enlarging, atypical cells were found at biopsy, and elevated PTH level and symptoms of hyperparathyroidism were documented, the patient was suspected of harboring carcinoma of the left parathyroid gland as well as tumor of the right parathyroid gland (adenoma). The patient at the age of 39 years underwent thyroidectomy with parathyroidectomies and neck lymphadenectomy as well. After the surgery, developed hypocalcemia was corrected with calcium supplements, and thyroid hormone replacement therapy for hypothyroidism was administered. Histological examination revealed carcinomas of the two parathyroid glands: the right inferior parathyroid carcinoma was 2.5 cm in diameter and the left superior parathyroid carcinoma was 2.0 cm in diameter. Both the tumors grew penetrating the capsule of the parathyroid glands, invaded into the thyroid gland, and metastases of parathyroid carcinoma to the right lobe of the thyroid gland were observed. Fig. 1 shows photomicrographs of both parathyroid carcinomas.

Taking into account the combination of parathyroid carcinoma and pituitary prolactinoma in the patient, the MEN 1 syndrome was suspected. During the examination of the patient, multiple subcutaneous lipomas ( $n=15$ ) measuring between 1.5 and 4 cm in diameter were documented. Aiming to detect other tumors typical of the MEN 1 syndrome, computed tomography of the abdomen was performed. A computed tomography scan confirmed the presence of multiple tumors in the head and tail of the pancreas (between 1.1 and 3.2 cm in diameter) as well as a metastatic focus about 1.1 cm in diameter in the hepatic segment S7. A sonogram of the kidneys revealed calculi in the right kidney. A lesion measuring 1.2×1.5 cm in size was seen in the right adrenal gland. At biopsy of the pancreatic tu-

mor, malignant cells were found. Pancreatoduodenal resection as well as resection of the left adrenal gland was recommended for the patient; however, she refused further treatment. After 6 months, the patient experienced severe, recurrent hypoglycemic episodes; she developed multiple liver and pulmonary metastases and ascites. She died after 1.5 years following the first surgery (at the age of 41 years).

### Family History

Fig. 2 shows the scheme of possible inheritance of the MEN 1 syndrome. A family history revealed that the patient's father (I-1) died due to complications of refractory gastric ulcer (possibly undiagnosed gastrinoma) at the age of 44 years. The patient's mother (I-2) was long-lived, and the cause of her death was not related to oncological disease. The patient's sister (II-2) was healthy and had no clinical signs characteristic of the MEN 1 syndrome. No clinical signs typical of MEN 1 syndrome were noted in a 4-year-old patient's son (III-1) as well.

### Genetic Testing

For genetic testing, patient's DNA was extracted from blood leukocytes using the SORPO<sup>®</sup> DNA purification kit ("Thermo Fisher Scientific" UAB, Lithuania) according to the standard protocol presented together with the kit. From 2 to 10 exons of the *MEN1* gene were amplified (primers sequences can be found at <http://www.sciencemag.org> and <http://www.nhgri.nih.gov>). Their sequencing was performed using a capillary sequencer, 3130xI Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Analysis of DNA sequencing results showed a nucleotide insertion at codon 43 in exon 2 (c.129insA). Fig. 3 depicts DNA sequencing. Due to this nucleotide insertion, codon 115 of the *MEN1* gene becomes a premature stop codon, leading to inactivation by producing a truncated form of menin, composed of only 114 amino acids. A search for this mutation in the Human Gene Mutation Database performed on July 9, 2010, showed no analogous mutation in the *MEN1* gene. Therefore, we think that this is a novel mutation of the *MEN1* gene, causing the MEN 1 syndrome. The patient's son (III-1) and sister (II-1) underwent genetic testing as well: the son inherited an analogous mutation in the *MEN1* gene, and no mutation was found in the sister.

### Discussion

Parathyroid carcinoma is a very rare malignant tumor with an estimated incidence of 0.5%–1% among primary hyperparathyroidism cases except for Japan where this tumor is reported more frequently, in 5% of cases (8). Only a few cases of parathyroid carcinoma have been described in patients

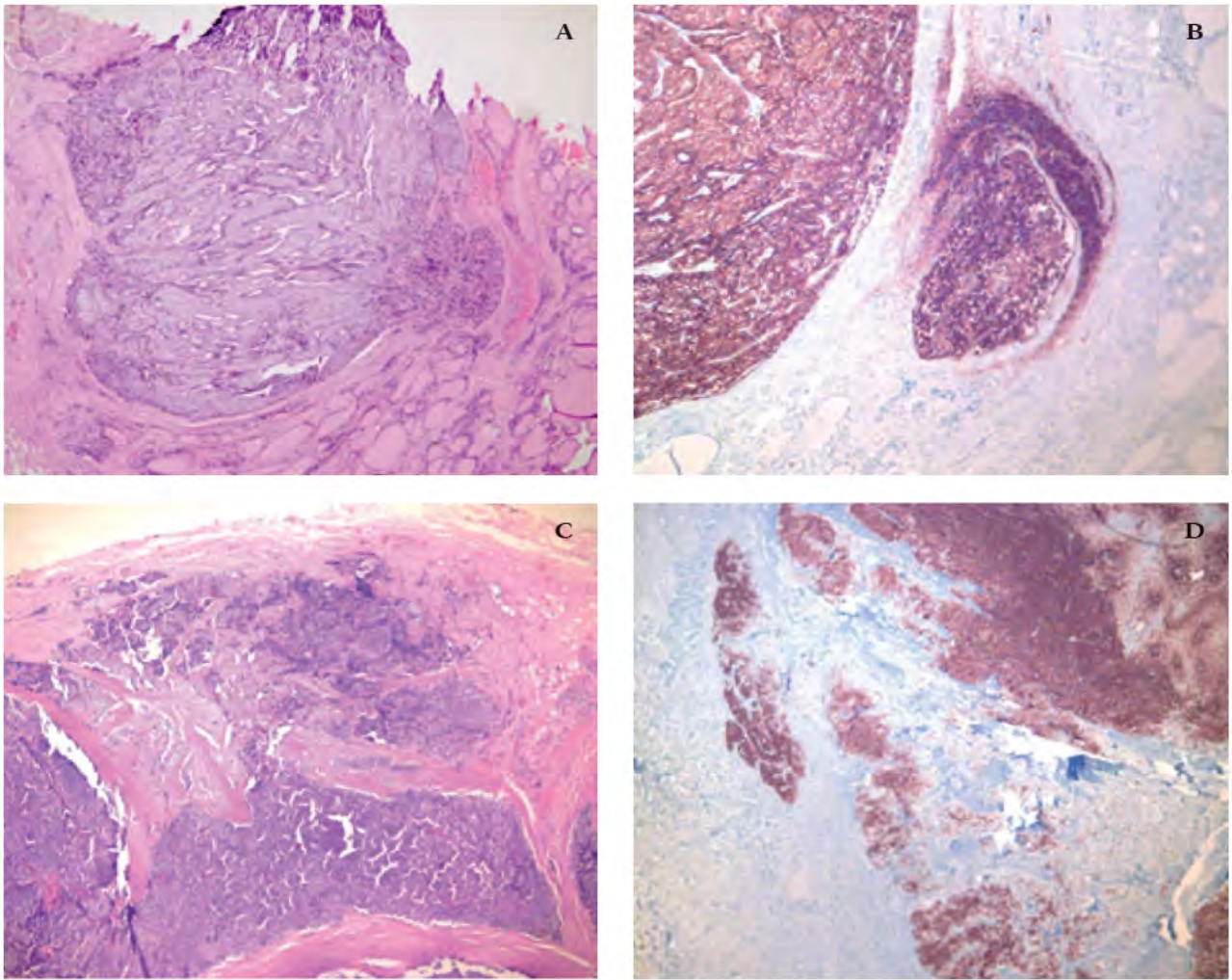


Fig. 1. Histological examination of the parathyroid glands

Carcinoma of the left parathyroid gland invading thyroid tissues: A) hematoxylin–eosin staining (×40); B) immunohistochemistry: positive reaction to parathormone (×100). Carcinoma of the right parathyroid gland: C) hematoxylin–eosin staining (×40); D) immunohistochemistry: positive reaction to parathormone (×100).

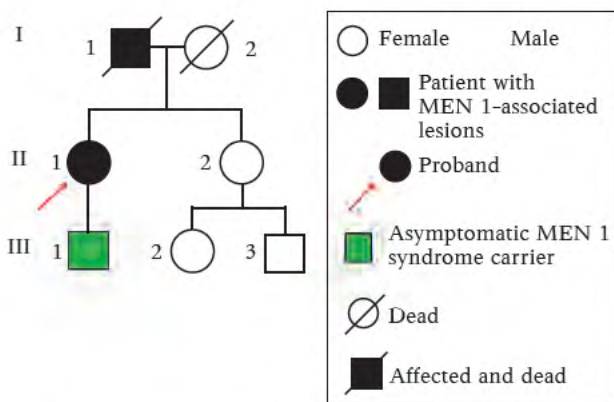


Fig. 2. The scheme of MEN 1 syndrome inheritance (clinical data of the patients are provided in the text)

II-1 and III-1 individuals are positive for *MEN1* gene mutation; II-2 individual is negative for *MEN1* gene mutation; I-1, 2; III-2, 3 genetic testing was not performed.

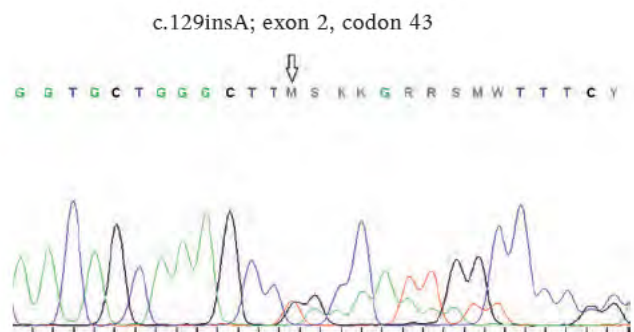


Fig. 3. Sequencing trace of the *MEN1* gene

with the MEN 1 syndrome (5–7, 9), and bilateral parathyroid carcinomas are even more rare. However, parathyroid carcinoma can be a typical feature of the hyperparathyroidism–jaw tumor syndrome (HPT-JT). The overwhelming majority of parathyroid carcinomas are functioning tumors – due to hyperparathyroidism, hypercalcemia and symptoms related to it, such as fatigue, weakness, weight loss, anorexia, can occur; psychiatric manifestations and impairments of the digestive system (nausea, vomiting, abdominal pain, etc.) are described, renal stones and osteoporosis may develop as well (8). Besides calcium metabolism-related disorders, patients can exhibit symptoms associated with growing tumor masses, such as a palpable mass and dysphagia. Even 70% of patients with parathyroid carcinoma present with a palpable mass in the neck at diagnosis (8). Our patient developed carcinomas of two parathyroid glands leading to hyperparathyroidism and hypercalcemia, complicated with renal stones. The establishment of diagnosis was worsened by the fact that in the presence of asymptomatic hyperparathyroidism and hypercalcemia, the patient refused surgical treatment, and repeat biopsies revealed atypical cells only after 4 years. Therefore, it is difficult to assess if parathyroid carcinomas were the primary lesions or they occurred due to hyperplasia of the parathyroid glands.

The second most common manifestation of the MEN 1 syndrome is tumors of the pancreatic islet cells (1, 10). These tumors are found in 41%–85% of patients with the MEN 1 syndrome (1). Gastrinomas are most common and account for 20%–60% of pancreatic islet cell tumors; insulinomas occur in about 10%–35% of patients; and glucagonomas (3%), VIPomas (1%–5%), and other hormone-secreting tumors are diagnosed very rarely (1). Multifocality is characteristic of MEN 1-related pancreatic islet cell tumors; one-third of these tumors may undergo malignant transformation (1). In this case presented here, a family history was notable for possible gastrinoma in the patient's father – due to gastrin hypersecretion, he developed recurrent gastric ulcer and died because of its complications at the age of 44 years. Our patient developed multiple pancreatic tumors. A biopsy of pancreatic tumors revealed malignant cells, and patient's refusal of further treatment led to the occurrence of severe, recurrent hypoglycemic episodes and multiple metastases to the liver and lungs. Therefore, we think that the patient could develop malignant insulinoma, which caused poor patient's prognosis. Malignant pancreatic islet cell tumors are the leading MEN 1-associated cause of death in patients with the MEN 1 syndrome (1, 10, 11).

About 21%–65% of patients with the MEN 1 syndrome develop pituitary tumors (1, 10). In about

24% of patients with MEN 1, pituitary tumors are the first manifestation of the MEN 1 syndrome (1, 10). These tumors can cause syndromes of hormone excess (prolactin, somatotropin, adrenocorticotrophic hormone, etc.) as well as local symptoms due to mass effect such as headache, visual field defects, and deficiency syndrome of all pituitary hormones, resulting from pituitary necrosis due to mechanical impact of the tumor (1). Prolactinomas are most common, accounting for 41%–76% of all pituitary tumors (1, 10). In our case, prolactinoma also was the first manifestation of the MEN 1 syndrome: prolactin hypersecretion caused galactorrhea and amenorrhea, and the patient was unable to conceive. Treatment with bromocriptine, suppressing prolactin hypersecretion, resulted in the decreased prolactin level, and after return of menses, the patient became pregnant and gave birth to a full-term male infant who inherited the MEN 1 syndrome.

Our patient developed the tumors of all three most common localizations, associated with the MEN 1 syndrome – tumors of the parathyroid glands, pancreatic islet cells, pituitary gland – as well as adrenal tumor and multiple cutaneous lipomas. A combination of tumors involving all three major localizations is not frequent in patients with the MEN 1 syndrome and it occurs in 15%–20% of patients (1). Our patient was first diagnosed with pituitary prolactinoma and later parathyroid tumors, and pancreatic islet cell tumors, which caused poor patient's prognosis. In our case, no lesions typical of HPT-JT syndrome – multiple ossifying fibromas of the mandible and maxilla, cystic and neoplastic renal abnormalities, benign and malignant uterine tumors – were detected (12).

More than 450 germline mutations of the *MEN1* gene, causing MEN 1 syndrome, have been described (13). Nucleotide insertions and deletions are most common in patients with the MEN 1 syndrome, leading to truncated and inactive forms of menin protein (such mutations are found in approximately 70% of MEN 1 cases) (13). Our patient as well as her son had the same germline mutation in the *MEN1* gene (c.129insA), a nucleotide insertion causing a premature stop codon terminating menin synthesis. Menin is predominantly a nuclear protein involved in transcriptional regulation, genome stability, cell division, and proliferation. A truncated and dysfunctional protein leads to altered signal transduction and cell cycle regulation, and malignant transformation takes place. No genotype-phenotype correlation in the setting of the MEN 1 syndrome has been established; therefore, it is complicated and difficult to predict tumor localization in asymptomatic MEN 1 carriers and aggressiveness of the syndrome course. As the estimated penetrance of *MEN1* gene mutations is high

(about 95%–100%) (1, 13, 14), genetic screening and monitoring of syndrome carriers is purposeful aiming at early recognition of MEN 1-related tumors, initiation of timely treatment, and improvement of patients' prognosis (1, 10).

### Concluding Remarks

Parathyroid carcinoma is an extremely rare tumor not only in patients with the MEN 1 syndrome, but also in cases arising sporadically. A clinical case of the MEN 1 syndrome presented here is of interest not only because of the rare occurrence of parathyroid pathology, but also because of a novel germline mutation identified in the *MEN1* gene,

which caused the MEN 1 syndrome, manifesting as a combination of two parathyroid carcinomas, multiple malignant pancreatic insulinomas, and pituitary prolactinoma. A novel mutation in the *MEN1* gene is relevant in gathering the data on etiopathogenesis of not only MEN 1 syndrome, but a very rare pathology – parathyroid carcinoma – as well.

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### Statement of Conflict of Interest

The authors state no conflict of interest.

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