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CASE REPORT

Co-existence of glucagonoma with recurrent insulinoma in a patient with multiple endocrine neoplasia-type 1 (MEN-1)

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Abstract Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by tumors of the parathyroid glands, the anterior pituitary, and the endocrine pancreas. Our patient was a 58-year-old man who manifested typical features of MEN-1 including primary hyperparathyroidism, lung carcinoid, and lipomas and insulinoma. He was admitted to our hospital because of recurrent hypoglycemia and a growth of pancreatic tumors. The first operation for insulinoma was performed when he was 20 years old. We found a germline mutation of the *MEN1* gene (E45G, exon 2) in this patient. According to these examinations and his clinical course, the patient was diagnosed as having a recurrence of insulinoma. He subsequently underwent surgery for the pancreatic tumors. The majority of these tumor cells were immunohistochemically positive for insulin and negative for glucagon. A few nodules showed immunohistochemical staining positivity for glucagon but they were negative for insulin. Although it is uncommon for patients with MEN1 to exhibit insulinoma and glucagonoma, this case suggests the need for careful analysis of pancreatic tumors in patients with MEN1.

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Introduction

Multiple endocrine neoplasia type 1 (MEN-1) is a hereditary syndrome characterized by a predisposition to hyperplastic and neoplastic disorders. It arises predominantly from endocrine organs such as parathyroids, the anterior pituitary, and the endocrine pancreas. More than 90% of the incidence of this disease is caused by heterozygous germline mutations of the *MEN1* gene, located at region 11q13 [1]. This is suggested to be a tumor suppressor gene that encodes a transcriptional regulator, menin [2].

Pancreatic endocrine tumors (PETs) are uncommon, comprising only 2–3% of all pancreatic neoplasms [3]. Nearly 25% of all PETs were found to be a component of MEN-1 [4]. Malignant pancreatic endocrine tumors are the most common MEN-1-related cause of death in MEN-1 kindreds [5]. Pathologic examination of the pancreas in patients with MEN-1 has demonstrated multiple neuroendocrine tumors [6]. Tumors producing pancreatic polypeptide are the most common pancreatic endocrine tumor in MEN-1 patients, occurring in 80–100% of these cases. Many patients develop functional pancreatic endocrine tumors, sometimes coincident with pancreatic polypeptide-producing tumors. Of these, most have gastrinoma, approximately 20% have insulinoma, 3% have glucagonoma, and 1% produce vasoactive intestinal peptide (VIPoma) [7].

While the insulin-producing tumor may be one of several islet cell tumors in the patient, the tumor that is making the insulin is usually solitary and is relatively large, on the order of 2–4 cm [8, 9]. Patients usually present with

symptoms of neuroglycopenia during fasting hypoglycemia. Glucagonomas occur in 3% of all MEN-1 patients. They are often large at presentation, 5–10 cm, and malignancy is common. Patients present with signs and symptoms of hyperglycemia and diabetes mellitus usually precedes the diagnosis of glucagonoma [10]. A previous report indicated that both insulinoma and glucagonoma were co-localized in the same patient by immunohistochemistry [11], but DNA analysis of the *MEN-1* gene could not be performed. Here, we report a case of MEN-1 with insulinoma and glucagonoma that was confirmed by DNA analysis.

Case report

The patient was a 58-year-old man who had a past history of an insulinoma in the pancreas, carcinoid tumors of the right lung, a parathyroid tumor in the left lower gland with primary hyperparathyroidism, and lipomas in the right leg at age 20, 50, 51, and 54 years, respectively, and the insulinoma and lung carcinoid tumors were treated with repeated surgeries. The first insulinoma was removed from our patient at age 20, and this was followed by a long period of remission. To genetically confirm the diagnosis of MEN-1, his germline *MEN-1* gene mutation was confirmed by PCR amplification and direct sequencing. Sequencing of genomic DNA extracted from the patient's blood showed a germline mutation of the *MEN1* gene (E45G, exon 2) (Fig. 1). This DNA variation has been reported previously [12].

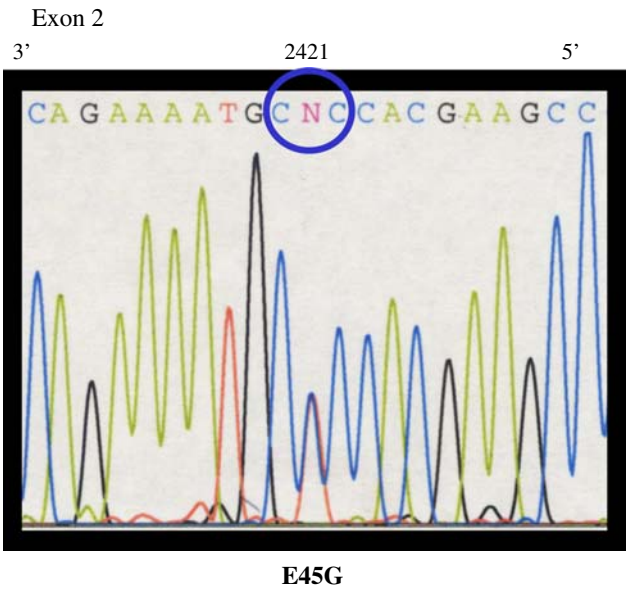

Fig. 1 Direct DNA sequence analysis shows one heterozygous mutation at codon 2421 in exon 2 (E45G) of the *MEN-1* gene. This mutation changed the 45th amino acid, glutamic acid (E), to glycine (G)

During this time, the patient was admitted to our hospital because of recurrent hypoglycemia and a growth of pancreatic tumors. Physical examination revealed a body temperature of 36.4°C, blood pressure of 130/80 mmHg, a heart rate of 61/min, body weight of 87 kg, and he was 175 cm tall. Results of initial laboratory examinations are listed in Table 1. Mild hypercalcemia with high intact-parathyroid hormone (intact-PTH) concentrations were observed. The results also revealed that the patient's serum insulin level was inappropriately elevated at 16.3 μU/ml, despite a simultaneous serum glucose level of 65 mg/dl. The demonstration of low plasma glucose plus elevated or normal insulin in the absence of ketonuria caused a suspicion of insulinoma. The levels of serum glucagon and of other pancreatic, adrenal, or pituitary hormones were almost within normal ranges. A dynamic abdominal computerized tomography (CT) showed two pancreatic masses, in the head and the body, measuring 1.0–1.5 cm, respectively. According to these examinations and the clinical course, the patient was diagnosed as having a recurrence of insulinoma. He subsequently underwent surgery for the pancreatic tumors, which required an enucleation of the tumor from the pancreatic body, and a distal resection. Grossly, there were three tumors in the pancreatic body and tail measuring 1–1.5 cm in diameter, which had a white solid cut surface and was well encapsulated.

Pathological examination of nodules in excised tumors after the operation revealed that well differentiated tumor cells that were almost same size and were arranged in cords

Table 1 Laboratory data of the patient

	Laboratory data	Normal range
Insulin	16.3	3–11 (μIU/ml)
Glucagon	122	40–140 (pg/ml)
Gastrin	179	30–159 (pg/ml)
ACTH	59	4–48 (pg/ml)
Prolactin	5.5	0–10 (ng/ml)
GH	6.0	0–5 (ng/ml)
Cortisol	11	5–17.9 (μg/dl)
Intact-PTH	105	10–65 (pg/ml)
Glucose	65	70–109 (mg/dl)
Calcium	10.5	8.2–10.2 (mg/dl)
C-peptide	3.02	0.43–2.35 (ng/ml)
White blood cell	4300	4700–8700 (/μl)
Red blood cell	434	400–540 ($\times 10^4$/μl)
Hemoglobin	13.3	13–17 (g/dl)
Urine 17-OHCS	7.9	4–12 (mg/day)
Urine sugar	Negative	
Urine ketone	Negative	

or in a rosette pattern had an eosinophilic cytoplasm and a large, round nucleus (Fig. 2a). The majority of these tumor cells were immunohistochemically positive for insulin and negative for glucagon (Fig. 2b). Examination of tissue from elsewhere around the nodular regions revealed that there were some discrete irregular small tumors varying in size from 0.5 to 5 mm (Fig. 2c). The majority of these small tumors were also immunohistochemically positive for insulin, but a few had positive immunohistochemical staining for glucagons and were negative for insulin (Fig. 2d). No other pancreatic endocrine hormones could be identified among the multiple sections examined. Therefore, these results revealed the co-existence of multiple insulinomas and glucagonomas in the same pancreas.

The patient experienced normalization of insulinoma syndrome after surgery, his condition improved, and he was discharged.

Discussion

MEN-1 syndrome is an autosomal dominant inherited disease that involves development of neoplasms of the parathyroid glands, hypophysis, pancreatic islets, and other neuroendocrine cells. More than 90% of this disease is caused by heterozygous germline mutations of the *MEN1* gene [1]. The *MEN1* gene consists of one non-coding exon and nine coding exons that encode a 610-amino acid protein, menin [2]. Many germline mutations (more than 80) have been reported in the *MEN1* gene [12]. The mutations include deletions, nonsense mutations, insertions, and missense mutations, of which deletions are the most common mutations. They are scattered along the entire coding region of the *MEN1* gene from exons 2 to 10, depending on the length of each exon. Exons 2 and 3 are the most common regions for the appearance of mutations, but no hot spots have been observed yet [13]. Bartsch et al.

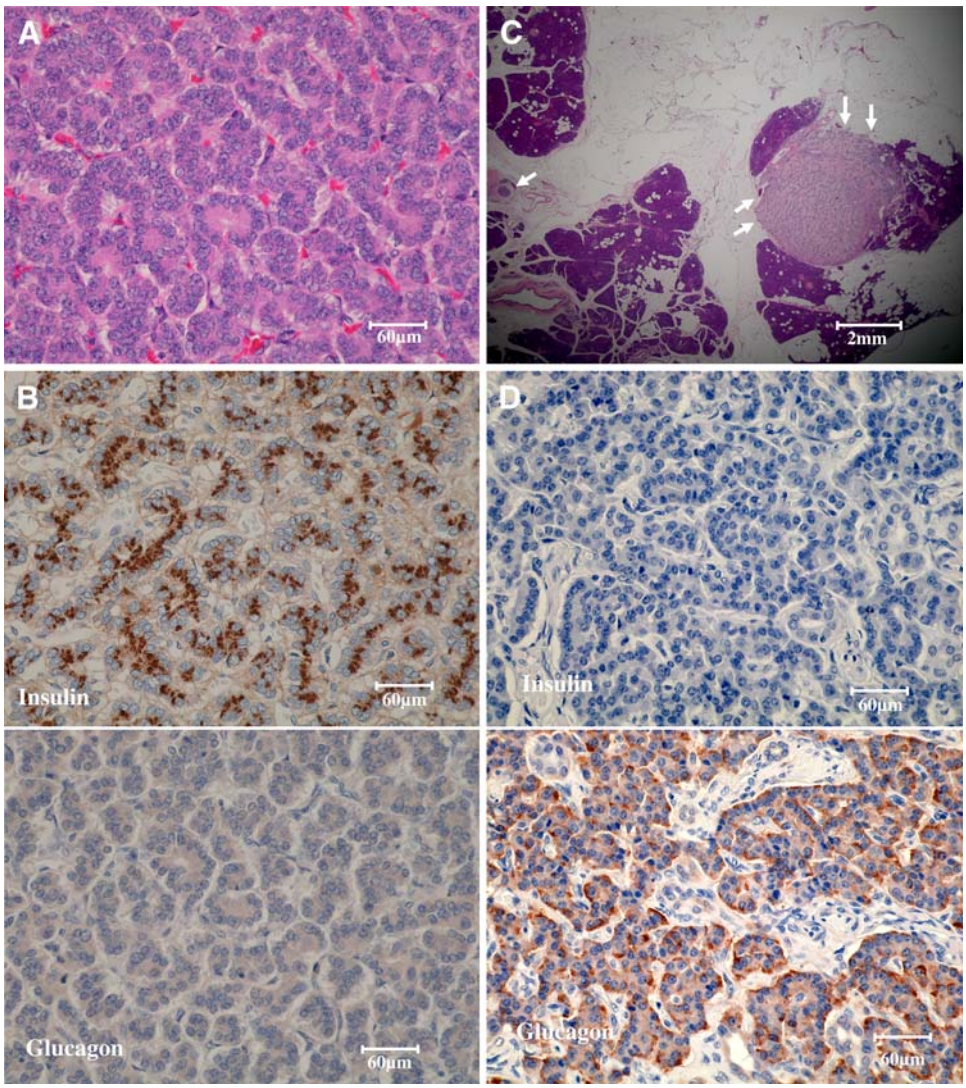

Fig. 2 **a** One of main tumors removed from body of the pancreas, stained with hematoxylin and eosin; ×200. **b** One of the main tumors removed from the body of the pancreas; immunocytochemical insulin staining is positive for the majority of the tumor cells, and no glucagon-positive cells were found in the same tissue ×200. Guinea pig anti-insulin antibody or rabbit anti-glucagon antibody was used as first layer in the peroxidase anti-peroxidase complex (PAP) method. The counterstain was hematoxylin staining. **c** Tissues removed from elsewhere around main tumors, stained with hematoxylin and eosin; ×40. It is revealed that there are some discrete irregular small tumors varying in size from 0.5 to 5 mm (*arrows*). **d** One of tissues removed from elsewhere around main tumors, immunocytochemical stain of glucagon is positive for the majority of the tumor cells, and no insulin-positive cells are shown in the same tissue ×200. Guinea pig anti-insulin antibody or rabbit anti-glucagon antibody was used as first layer in the peroxidase anti-peroxidase complex (PAP) method. The counterstain was hematoxylin staining

reported a genotype–phenotype relationship for MEN-1 PETs [13]. They found that patients with truncating nonsense and frameshift mutations in the C- or N-terminal regions of the menin gene have a significantly higher rate of malignancy (55% vs. 10%) and earlier recurrence (26 vs. 92 months) than patients with other mutations. Our case involved heterozygous missense mutations at codon 2421 in exon 2 (E45G) of the *MEN-1* gene. This mutation changed the 45th amino acid glutamic acid (E) to glycine (G). We previously identified this mutation (E45G) in two families. One proband from this family exhibits hyperparathyroidism, a carcinoid tumor, and lipoma, while another has an endocrine pancreas tumor (insulinoma) associated with hyperthyroidism. However, we cannot predict a significant effect of the missense mutation (E45G) on exon 2, because of the lack of information about the structure of menin and its function in the pancreatic tumor.

In MEN-1 cases, the most common form of pancreatic tumor is the pancreatic polypeptide-producing tumor (20–40%), followed by gastrinoma (20–60%), insulinoma (5–10%), glucagonoma (3%), and the VIPoma (1%) [14]. At this time, there are few reports of multi-hormonal mixed tumors as in our case. These cases are rarely presented as recurrent insulinoma syndrome complicated by the unexpected discovery of glucagonomas. Usually, glucagonomas associated with MEN-1 are asymptomatic, or they only become apparent in association with glucose metabolism disorder, or with other symptoms much less obvious such as necrolytic migratory erythema, weight loss, stomatitis, diarrhea, and anemia, although insulinomas manifest with symptoms of hypoglycemia. In this case, excess glucagon secretion was not detected, and it was probably masked by the presence of additional large insulinomas.

Insulinomas are the most frequent of all functioning PETs. About 83% of insulinoma are single occurrences, 12% represent multiple occurrences, and 5–10% are associated with MEN-1 [15]. Recurrent multiple insulinomas, as in our case, are rare, and most are also associated with MEN-1 [16]. Malignancy as shown by metastasis, probably the only reliable criterion, is also a rare complication, and constitutes about 8% of insulinomas [17].

The glucagonoma is a low incident tumor associated with 1.2% of all pancreatic neoplasms or 3–5% of the MEN-1 syndrome cases. These are commonly single tumor, although 10% are multiple [18, 19]. The malignant character of a glucagonoma is not based on morphologic criteria, but on its ability to metastasis [20]. About 70% of cases present metastasis in peri-pancreatic tissues such as the liver, spleen, lymph nodes [21]. Thus their behavior is usually malignant at diagnosis.

The relationship between insulinoma and glucagonoma is not clear in regard to the occurrence of mixed tumors. Our case appears to be one in which two functionally distinct tumors with different biological behaviors co-exist. One possibility is that prolonged hyperinsulinemia and hypoglycemia induced a secondary A-cell hyperplasia leading to neoplasia. However, there is no evidence of hyperplasia of A-cells alone, and the multiple small glucagons-producing tumors in this case are probably disseminating growths originating from a glucagonoma. In view of the postulated origin of pancreatic endocrine tumors from multipotent stem cells in the ductular epithelium, rather than from the islets [22], it would seem more likely that these tumors are different expressions of an increased tendency to endocrine cell neoplasia. Another possibility is that the transformation of insulinoma syndrome into a glucagonoma syndrome has been reported previously [6], although this earlier report described a malignant type of insulinoma. Our case is apparently due to an alteration in the predominant cell type in a mixed endocrine cell tumor, which had no obvious malignant features histologically. Therefore, the former possibility is more compatible with our case at this time.

In conclusion, we report an unusual case of pancreatic glucagonoma associated with recurrent insulinoma in a patient with typical MEN-1. In this case, the two tumor types appear to be distinct. Although the molecular mechanisms of tumorigenesis in our patient are as yet uncertain and further investigations are necessary, we must sufficiently address the possibility that insulinoma may co-exist with another PET, especially glucagonoma. There is thus a risk of malignant transformation in cases of recurrent insulinomas that occur after long-term remission, as in the case presented here. Therefore we must consider the necessity of continuing careful periodic screening of endocrine and systemic disorders for such patients, in addition to genetic screening for diagnosis, in the hope of convincingly identifying individuals at risk of developing the clinical features described in this report.

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