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An unusual phenotype of MEN1 syndrome with a SI-NEN associated with a deletion of the *MEN1* gene

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Summary

We report about a young female who developed an unusual and an aggressive phenotype of the MEN1 syndrome characterized by the development of a pHPT, malignant non-functioning pancreatic and duodenal neuroendocrine neoplasias, a pituitary adenoma, a non-functioning adrenal adenoma and also a malignant jejunal NET at the age of 37 years. Initial Sanger sequencing could not detect a germline mutation of the *MEN1* gene, but next generation sequencing and MPLA revealed a deletion of the *MEN1* gene ranging between 7.6 and 25.9 kb. Small intestine neuroendocrine neoplasias (SI-NENs) are currently not considered to be a part of the phenotype of the MEN1-syndrome. In our patient the SI-NENs were detected during follow-up imaging on Ga68-Dotatoc PET/CT and could be completely resected. Although SI-NENs are extremely rare, these tumors should also be considered in MEN1 patients. Whether an aggressive phenotype or the occurrence of SI-NENs in MEN1 are more likely associated with large deletions of the gene warrants further investigation.

Learning points:

- Our patient presents an extraordinary course of disease.
- Although SI-NENs are extremely rare, these tumors should also be considered in MEN1 patients, besides the typical MEN1 associated tumors.
- This case reports indicate that in some cases conventional mutation analysis of MEN1 patients should be supplemented by the search for larger gene deletions with modern techniques, if no germline mutation could be identified by Sanger sequencing.

Background

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant inherited tumor syndrome that is caused by germline mutations in the *Menin* suppressor gene on chromosome 11q13 (1). Its incidence is about 2–3 per 100 000/year and the penetrance is ~100% by the age of 50 years (2) (3) (4) (5) (6). MEN1 is typically characterized by the development of neuroendocrine

tumors in different endocrine organs, including the parathyroid glands, the pancreas and duodenum, the anterior pituitary gland and, less frequent, the adrenal glands, the thymus and the bronchii (5). The detection and adequate treatment of GEP-NETS is important, since pNENs are together with thymic carcinoid the most common cause of death in MEN1 (7) (8) (9) (10) (11). Here we report the rare case of a MEN1 patient with pHPT, malignant NF-pNENs, a non-functioning duodenal NEN, non-functioning pituitary adenoma and a malignant small intestine neuroendocrine neoplasia (SI-NEN) that was associated with a large germline deletion of the MEN1 gene. To the best of our knowledge, this is the fourth report of a SI-NEN in a MEN1 patient. Currently, SI-NENs are not considered to be a part of the phenotype of the MEN1-syndrome. In our patient the SI-NEN was detected during follow-up imaging on Ga68-Dotatoc PET/CT. Beside the development of an atypical tumor lesion in our patient, she also presented with a large germline deletion of the MEN1 gene. Initial conventional mutation analysis with Sanger sequencing did not reveal a germline mutation. Only years later, multiplex-ligation-dependent probe amplification (MLPA) was performed and was able to detect a large gene deletion. In conclusion this case report outlines that examiners should be aware of SI-NENs as a extremely rare organ manifestation and that conventional mutation analysis of MEN1 patients should be supplemented by the search for larger gene deletions with modern techniques, if no germline mutation could be identified by Sanger sequencing.

Case presentation

The first clinical manifestation of MEN1 in the presented female patient was pHPT at age 20 years. She presented with recurrent abdominal pain at her family doctor. An esophagogastroduodenoscopy was performed and did not reveal any gastric ulcers. The laboratory examination revealed hypercalcemia (>2.6 mmol/l) and an inappropriate serum parathyroid hormone level (>45 pg/nl). Initially an enlarged right lower parathyroid gland was resected. Because of persistent HPT a completion parathyroidectomy with autotransplantation of parathyroid tissue in the left forearm was performed at age 20. At this time, the possibility of MEN1 was not considered by her physicians. The family history, however, was also highly suggestive for MEN1, since her father had pHPT and had died of metastatic thymic carcinoid at age 52. At age 31 the patient presented self-motivated for the first time in our hospital to perform an endocrine work-up, since her family history was suggestive for MEN1. The clinical work-up 2007 showed normal plasma hormone for insulin-like growth factor (IGF1) 256 μg/l (115–307 μg/l), prolactin concentration 17.3 μg/l (2.8–25.0 μg/l), gastrin level 67.6 pg/ml (−125 pg/ml), c-petide 2.27 μl (0.8–6 μg/l), proinsulin 5.4 pmol/l (−11 pmol/l), chromogranin A 14.0 U/l (0–50 U/l) and parathyroid hormone 18 ng/l (11–65 ng/l), glucagon 69.3 pg/ml (59–177 pg/ml), plasma basal level of adrenocorticotrophic hormone (ACTH) 16.4 pg/ml (−60 pg/ml) and plasma basal lever of cortisol 67 μg/l (43–224 μg/l). The pancreatic polypeptide was slightly increased with 642 pg/ml (−400 pg/ml). The low-dose dexamethasone suppression test was also within the normal range, so that the visualized pituitary adenoma was classified as nonfunctional. The magnetic resonance imaging (MRI) of the pituitary gland showed a small adenoma with a maximum size of 5× 3 mm. A computer tomography scan (CT) of the lung revealed a thymic hyperplasia, but no evidence for thymic or bronchial carcinoids. Endoscopic ultrasound (EUS) of the pancreas showed six NF-pNENs with a maximum size of 15 mm in the pancreatic body and tail. A fine needle aspiration biopsy of the pancreatic nodules during EUS was not performed. Furthermore, an abdominal MRI was carried out in 2007, which revealed a homogeneous liver parenchyma. In addition, the MRI showed hyperintense pancreatic lesions, which were also visualized in EUS. In summary, the patient had a pHPT, a nonfunctioning microadenoma of the pituitary gland and multiple NF-pNENs, thus fulfilling the criteria for MEN1 (12). Therefore, the patient was included in our annual MEN1 screening program. Because all lesions were asymptomatic and non-functioning and the pNENs <2 cm, there was no need for a medical or surgical treatment at this time. At that time a mutation analysis of the *MEN1* and *CDKN27* genes with Sanger sequencing as previously reported by our group (13) revealed no germline mutation in either gene.

One year later, at age 32, a significant progression of pNENs was noted at routine screening. At this time EUS and MRI visualized eight tumors, and one lesion in the pancreatic body grew from 6.0×5.9 mm to 19.9×14.7 mm. Because of the rapid progression of these lesions we scheduled the patient for surgery. An IOUS-guided (intraoperative ultrasound-guided) distal pancreatectomy with lymphadenectomy to the level of the portal vein was performed to resect all pNENs >10 mm in size. Histopathologically five well differentiated neuroendocrine tumors (G1) were identified; the enlarged lymph node next to the pancreatic head was a lymph node metastasis. The immunohistochemical

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staining of the neuroendocrine pancreatic tumors were positive for chromogranin A and synaptophysin. The Ki 67 index was lower than 1%, the TNM stage was TXN1M0 according to the UICC stage III.

In the following 2 years a new lesion (7×4 mm) in the pancreatic head could be detected. In addition, there was also suspicion on a non-functioning adenoma in the left adrenal cortex. Due to her young age of 34 years and the consequences of completion pancreatectomy we decided to continue with close surveillance.

Until April 2012 the patient's situation remained stable with regard to the lesions in the pancreatic head and in other organs as the pituitary gland or the adrenal cortex. However, a new 14×12 mm sized lesion was detected in segment 6 of the liver and a new 15 mm sized nonfunctioning tumor in the first part of the duodenum. The CT-guided biopsy of the liver lesion demonstrated a metastasis of a well differentiated neuroendocrine tumor. The patient was scheduled for resection of the liver metastasis combined with enucleation of two pNENs (8 and 10 mm) out of the pancreatic head and resection of 10 mm sized duodenal NET via duodenotomy. The histopathological examination revealed a well-differentiated neuroendocrine tumor of the duodenum and pancreas which were negative for gastrin in immunohistochemistry. The tumor in the liver was a metastasis of a well differentiated neuroendocrine tumor. The Ki67 index of this tumor was <1%. The synaptophysin staining was positive.

One year later, at age 36, a completion pancreatectomy was performed because of a newly developed, rapidly progressive pNEN in the pancreatic head (max size in EUS 15 mm). Although close follow-up was recommended to the patient at this time, the patient insisted to undergo surgery. Only a part of the completion pancreatectomy specimen was histopathologically analyzed and revealed six G1 pNENs, all <20 mm in size. In February 2014, one year later, the patient had to undergo relaparotomy with adhesiolysis for a small bowel obstruction.

In December 2014 the patient suffered an acute upper gastrointestinal bleeding which required red blood cell transfusions. Esophagogastroduodenoscopy revealed an ulcer at the gastrojejunostomy and a high dose proton pump inhibitor therapy was initiated. Control esophagogastroduodenoscopy in February 2015 showed a persistent ulcer at the gastrojejunostomy anastomosis resulting in stenosis and recurrent bleeding. A normal secretine test could exclude Zollinger-Ellison syndrome. We also performed laboratory examination to rule out recurrence of pHPT, the calcium and parathyroid hormone levels were within the normal range. A Ga68-Dotatoc PET/CT revealed a solitary tracer accumulation in the small intestine ventral of the aortic bifurcation (Fig. 1). Initially this lesion was interpreted as a lymph node metastasis or another tumor of the small intestine. Based on the symptoms and the visualized solitary lesion another abdominal reexploration was performed. Exploration of the small bowel and the mesenteric root revealed a 10 mm sized tumor in the middle part of the jejunum. A small bowel resection with regional lymphadenectomy and a removal of the gastrojejunostomy were performed. Histopathological analysis revealed a NET of the jejunum (G2, pT3, pN1 (3/14); L1 V0 Pn0 R0) and chronic ulcerative, potentially ischemic lesions at the gastrojejunostomy. The immunohistopathological staining with synaptophysin was positive. The Ki67 index was <1% (Fig. 2). The postoperative course was uneventful and the condition of the patient improved dramatically. After a follow-up of 6 months now, the patient presented again with similar symptoms as in December 2014. An esophagogastroduodenoscopy was performed in August 2015 and revealed again an ulcer at the gastrojejunostomy. Besides the proton pump inhibitor therapy, a therapy with sucralfate is initiated. A control esophagogastroduodenoscopy is planned in a few months. Furthermore, a Ga68-Dotatoc PET/CT was performed and this time no pathologic

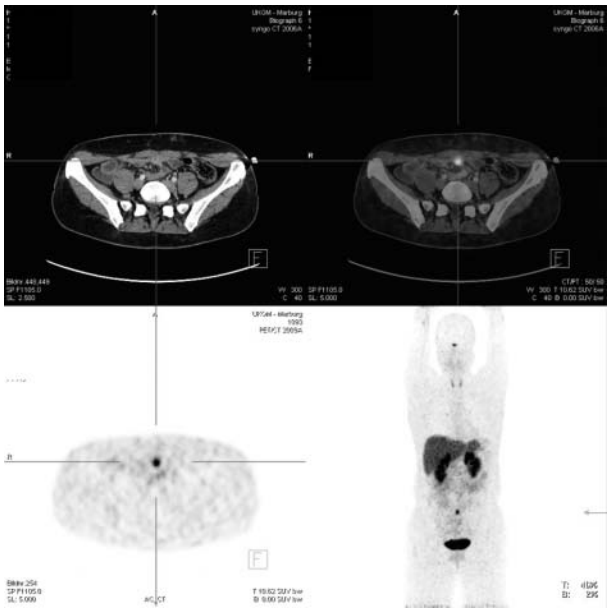

Figure 1
In February 2015 a Ga68- Dotatoc-PET/CT was performed, which revealed a solitary tracer accumulation in the small intestine ventral of the aortic bifurcation. Histopathologically this tumor showed up as a jejunum NET.

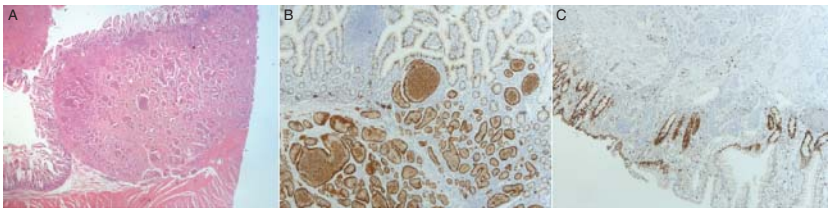

Figure 2
(A) well-differentiated tumor of the jejunum (hematoxylin-eosin), (B) Immunohistopathological staining with synaptophysin, (C) Ki67 Index <1% of the well-differentiated tumor of the jejunum.

tracer accumulation could be detected. A control examination was recommended in 6 months.

Investigation

Molecular genetic analysis

Initial mutation analysis of the *MEN1* and *CDKN1B* genes with Sanger sequencing in 2007 as previously reported by our group (13) revealed no germline mutation nor small deletion in either gene. Therefore, we decided because of the unusual phenotype to perform modern molecular analysis with target enrichment and next generation sequencing. For enrichment of cancer susceptibility genes, we used ~50 ng genomic DNA from peripheral blood and applied the TruSight Cancer Illumina kit (Illumina, San Diego, CA, USA), which targets the coding sequences of 94 genes associated with a predisposition towards cancer. All samples were processed according to the manufacturer's protocols. Sequencing was carried out on an Illumina MiSeq instrument as 150 bp paired-end runs with V2 chemistry. Data analysis was performed with the 'Biomedical Genomics Workbench' version 2.1.2 (Qiagen), using standard settings. Reads were aligned to the human reference genome (GRCh37/hg19); duplicate reads and reads that did not map unambiguously were omitted from variant calling. Copy number detection was carried out on a customized array CGH following the manufacturer's recommendations with the exception of inversely used fluorescent dyes. The array CGH design covered all coding regions and intermediate introns of the genes that are analyzed by Illumina's TruSight Cancer panel. The maximum probe density of the design is 1 probe per 200 bp. In addition, 500 kb of flanking regions of the genes are covered by a probe density of 1 probe per 5 kb. The design was realized on an 8×60k array CGH platform from Agilent (Agilent, Santa Clara, CA, USA). An Agilent DNA microarray scanner (G2505C) was used for signal detection and data normalization was carried out with standard settings of the Feature Extraction software version 10.7.3. Data analysis was performed with Agilent's Genomic Workbench 7.0.4.0. The ADM-2 algorithm was applied to calculate aberrations. A minimum of four consecutive probes had to be affected for an automated call. The threshold was set to 5.9. The results were additionally checked by visual control of all probes on the Genomic Workbench. All nucleotide positions refer to the Human Genome March 2009 Assembly (NCBI37/hg19). CGH analysis revealed a deletion of 7.6–25.9 kb in size encompassing the entire MEN1 gene (Fig. 3).

Independent verification of the *MEN1* deletion was obtained by using MLPA. The SALSA MLPA P017 probemix version C1 was used according to the manufacturer's instructions (MRC-Holland, Amsterdam, The Netherlands). The analysis (including negative controls) was repeated twice and confirmed the heterozygous deletion of MEN1 each time (Fig. 3).

Our patient does not have any children or paternal relatives, that is why we could not perform molecular diagnostic in other family members.

Treatment

A detailed description of the different treatments is provided in the part 'case presentation'.

Outcome and follow-up

Close follow-ups are performed in our patient, to make sure that new tumors are detected at an early stage. Currently, MRI of the abdomen and EUS are planned every 6 months to evaluate potential new adrenal, liver or other gastrointestinal lesions. Furthermore, control esophagogastroduodenoscopies are also performed in regular intervals of 6 months. Besides the imaging methods, closely controls are carried out in laboratory

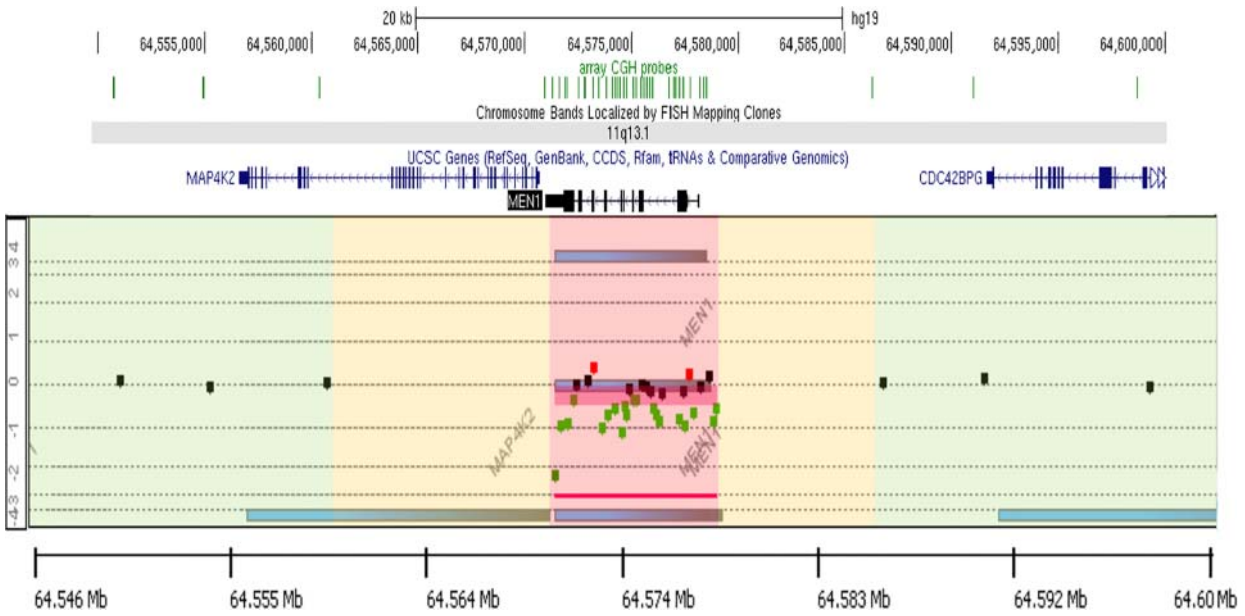

Figure 3

Deletion of MEN1 identified by array CGH. The upper part was taken from the UCSC genome browser and displays the scale, position of the array CGH probes, chromosomal band, and genes according to UCSC genes. The lower part was taken from Agilent's genomic workbench and visualizes the measured values of the probes. While some probes indicate a deletion (green dots) other probes show a normal copy number (black dots). The deletion was confirmed by MLPA (not shown). Red background displays the minimal size of the deletion, yellow background indicates the areas where the breakpoints reside, green areas indicate a normal copy number.

investigations. Currently, the MRI of the pituitary gland did not show an increased size of the known nodule. A control MRI of the pituitary gland is planned in a year.

Discussion

This case study reports a rare and an aggressive phenotype of MEN1 that is associated with a germline deletion of the *MEN1* gene. The organ manifestations of the reported young female patient included so far pHPT, malignant NF-pNENs, NF-duodenal NEN, pituitary adenoma, non-functioning adrenal adenoma and a malignant jejunal NET. To the best of our knowledge this is the fourth report of a SI-NEN in a MEN1 patient. Recently Agarwal *et al.* (14) reported a case of a 62 year old patient with MEN1 and an ileal tumor. In this patient the missense mutation S583P was detected in exon 10 of the *MEN1* gene. Initially the patient presented with pancreatic mass in the hospital. The patient was scheduled for an operation. The histopathological examination of the pancreatic mass revealed a well-differentiated pancreatic neuroendocrine carcinoma. Later the patient was diagnosed with pHPT. Three years after initial diagnosis of MEN1 the patient developed flushing, diarrhea and fatigue. Diagnostic work-up detected an ileal NET with several liver metastases. Our patient did not develop symptoms or liver metastases, but also had malignant SI-NEN with lymph node metastases. Dotzenrath *et al.* (15) also reported one MEN1 patient, when they analyzed the syndrome associated malignancies in 42 MEN1 patients. One patient presented besides a pHPT a NET of the ileum without metastasis. In this patient a typical mutation was not found and MEN1 was suspected due to neuroendocrine tumors in two distinct organs. In 1996 Ponssen *et al.* (16) reported about an uncommon case of a 50 years old female patient with a clinically non-functioning pituitary adenoma found in the maxillary sinus. Several years later this patient developed a NEN of the caecal valve. At this time mutational analysis was not performed because it was not available. The diagnosis of MEN1 was based on the development of neuroendocrine tumors in two different organs. The family history was negative for the MEN1 syndrome. Typically, the combined occurrence of tumors of the parathyroid glands, the pancreas, the anterior pituitary gland and less frequently of the adrenals, thymus and bronchii are characteristic for MEN1 patients (5) and germline mutations can be identified in the MEN1 gene on chromosome 11q13 in about 85–90% of patients (1) (2) (4). Nonetheless, there was no detectable mutation in the MEN1 gene in our patient. So far more than 1000

disease-causing mutations have been reported, mostly missense, nonsense mutations and small deletions/insertions (1) (4) (14) (17) (18) (19). However, in about 5–10% of clinically obvious MEN1 patients no such mutation can be identified (2) (4). This holds true for our patient in whom no MEN1 germline mutation in the coding regions or in the splice sites could be identified by Sanger sequencing in 2007. Mutation analysis of CDKN1B gene, which is associated with MEN4, revealed also no germline mutation in our patient. MEN4 is characterized by a MEN1-like phenotype (12) (20) (21) (22). It has been previously hypothesized that MEN1 patients without mutations in the coding region of the MEN1 gene may have gross or whole gene deletions (4) (12). One Portuguese study identified large deletions involving complete exons in about a third of MEN1 patients in whom no mutations in the coding regions could be detected (23). Seven of ten MEN1 patients with large deletions (del Exon 7–3′ UTR and del 5′ – exon 9) had either aggressive pancreatic NET (gastrinoma, glucagonoma), bronchial or thymic NET, but none had a SI-NEN. In the presented patient we detected a large 5.6 kb MEN1 gene deletion. This patient was also characterized by the development of aggressive pNENs and a malignant jejunal NET, both, with metastases that developed during short term follow-up of 2–5 years. Based on this very limited data one could hypothesize that MEN1 patients with large gene deletion might develop a more aggressive form of disease, which was also reported by other authors (24) (25) (26). On the other hand, there are also some studies revealing no special correlation between aggressive disease and mutation type (4) (19). These controversial statements show that further investigations are necessary to make a clear statement.

As shown in the presented case, conventional mutation analysis of MEN1 patients should be supplemented by the search for larger gene deletions with modern techniques, if no germline mutation could be identified by Sanger sequencing. MLPA is a quantitative highly sensitive and accurate multiplex PCR technique able to detect losses of whole exons, even the whole gene or other gross intra-genetic modifications as in the presented case (1).

Current clinical practice guidelines for MEN1 recommend that MEN1 patients and their families should be included into a screening program to reduce morbidity and mortality and to achieve an early detection of MEN1 associated tumors (5). Proposed imaging includes SRS scintigraphy or Ga68 PET/CT should be repeated in regular intervals. This imaging will potentially lead to the early detection of SI-NENs, if the examiners are aware of this extremely rare organ manifestation in MEN1.

Mostly results and findings of case reports are difficult to exert to other cases. Although, there are well documented guidelines concerning screening and molecular diagnostic in MEN1 patients (1) (5), it is still quite difficult for caretaking physicians of MEN1 patients to find a proper treatment concept. Further modern molecular diagnostic techniques should be considered if typical MEN1 mutations are not detectable. In our patient the diagnosis of MEN1 syndrome was already set in 2007, but it took 8 years to discover a mutation. Molecular diagnostic work does not effect the treatment of the affected patients, but it has an implication for other family members. We know that our study report is a very rare case, nevertheless, our case report should act as reminder that MEN1 patients should be kept under close surveillance to detect typical MEN1 lesions at an early stage. Furthermore, the caretaking physicians should be aware of atypical tumors. Although extremely rare, SI-NEN might be part of the phenotype of MEN1. Whether this and other malignant NETs are associated more likely with large deletions of the MEN1 gene awaits further investigation.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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Patient consent

Written informed consent has been obtained from the presented female patient for publication of the submitted article and accompanying images.

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