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ORIGINAL ARTICLE

A new mutation in the menin gene causes the multiple endocrine neoplasia type 1 syndrome with adrenocortical carcinoma

M. Haase · M. Anlauf · M. Schott · S. Schinner · E. Kaminsky · W. A. Scherbaum · Holger S. Willenberg

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Abstract Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant tumor syndrome that may be caused by mutations in the *MEN1* gene on 11q13. Loss of function of the tumor suppressor gene *MEN1* leads to synchronous or metachronous appearance of neuroendocrine tumors arising from neuroendocrine cells of the parathyroid and pituitary glands, the duodenum and pancreatic islets, and other endocrine organs such as the adrenal cortex. We here present a patient with MEN1 who developed hyperparathyroidism, multiple well differentiated functionally inactive neuroendocrine tumors of the pancreas and an adrenal carcinoma. We describe a new mutation at codon 443 in the coding region of exon 9 in the *MEN1* gene, where a cytosine residue was exchanged for adenosine (TCC > TAC) and, consequently, serine for tyrosine (p.Ser443Tyr; c.1327C > A). Also, we provide clinical data that may add to the genotype–phenotype discussion. We conclude that the novel mutation in the *MEN1* gene described herein was clinically relevant.

Keywords Neuroendocrine tumor · Adrenal carcinoma · Parathyroid gland · Hypercortisolism · Multiple endocrine neoplasia

M. Haase · M. Schott · S. Schinner · W. A. Scherbaum · H. S. Willenberg (✉)
Department of Endocrinology, Diabetes and Rheumatology, University Hospital Duesseldorf, Duesseldorf
Moorenstr. 5, D–40225, Germany
e-mail: Holger.Willenberg@uni-duesseldorf.de

M. Anlauf
Department of Pathology, University Hospital Duesseldorf, Duesseldorf, Germany

E. Kaminsky
Praxis für Humangenetik-Altona, Hamburg, Germany

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is a dominantly inherited familial tumor syndrome and develops as a consequence of germline mutations in the *MEN1* gene on 11q13 [1]. Germline mutations are heterozygous and somatic loss of the normal *MEN1* allele has been observed in MEN1 tumors. Nowadays, more than 500 mutations in the *MEN1* gene are described [2]. Recently, mutations in other genes have been found to be associated with the MEN1 syndrome, including cyclin-dependent kinase inhibitors *p15*, *p18*, *p21*, and *p27* [3]. This suggests that menin interacts with other factors for full tumor suppressor activity. Loss of function of menin leads to synchronous or metachronous occurrence of endocrine tumors arising from (neuro-)endocrine cells of the parathyroid and pituitary glands, the duodenum and pancreatic islets, and other endocrine organs such as the adrenal cortex. There are multiple works addressing the genotype–phenotype-correlation issue [2]. Here, we describe a novel mutation in the *MEN1* gene that caused the MEN1 syndrome with aggressive neoplasms of the endocrine pancreas and the adrenal.

Case report

In 1996, a 45-year-old female patient presented to an endocrinologist because of hypercalcemia. The diagnosis of primary hyperparathyroidism with hypercalciuria was made. However, there were also hints for hypercortisolism, and a 2-cm nodule in the right adrenal gland was found on computed tomography (laboratory data are given in Table 1). The patient was operated on an enlarged parathyroid gland at the inferior pole of the right thyroid lobe in

Table 1 This table shows endocrine function tests before the operation on the parathyroid gland (1997) and after operation on the right adrenal gland and the pancreas (2005, time of presentation in our clinic)

Hormones	1997	2005	Normal
Basal ACTH (pg/ml)	8.9	10.1	9.0–52.0
Basal cortisol (μg/dl)	13.8	22.8	6.0–19.0
Cortisol after 1 mg overnight dexamethasone (μg/dl)	3.8	4.3	<1.8
Diurnal secretion of free cortisol (μg/24 h)	51	–	20–90
Serum calcium (mmol/l)	2.8	2.58	2.15–2.55
Inorganic phosphorous (mmol/l)	0.66	0.85	0.87–1.45
Intact parathyroid hormone (pg/ml)	73.9	61.4	9.0–55.0
Diurnal calcium diuresis	12.5	–	3.8–8.3

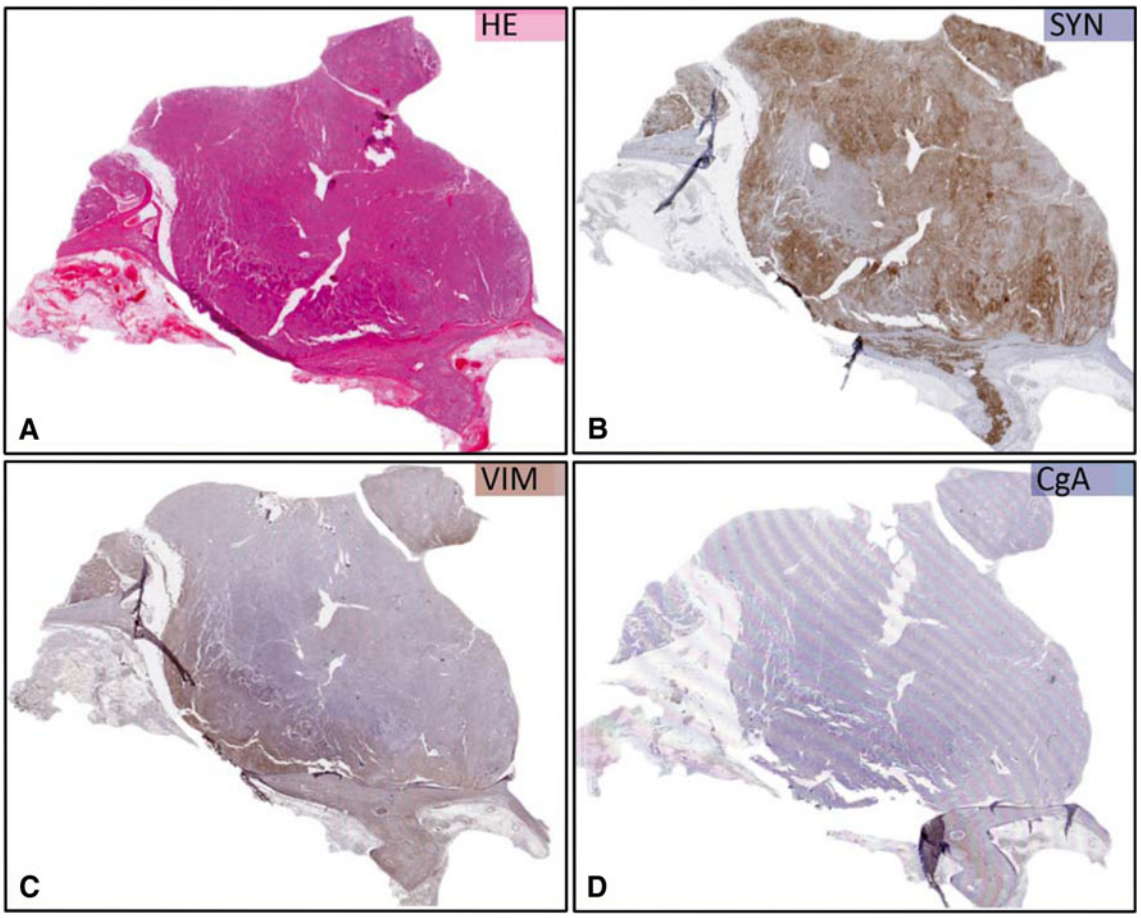

Fig. 1 Adrenal cortical carcinoma, operated in April 2005. **a–d** Whole mount scans demonstrating a sharply demarcated adrenal cortical tumor (hematoxylin and eosin *HE*; **a**), revealing moderate expression of synaptophysin (SYN; **b**), weak expression of vimentin (VIM; **c**) and the absence of chromogranin A (CgA; **d**)

1997. The histopathology showed nodular hyperplasia of the parathyroid and no signs of malignancy. Since her mother was also operated on a parathyroid gland 10 years earlier; it was suggested to the patient to have her followed up for other manifestations of the MEN1 syndrome.

However, the patient did not agree and she presented with abdominal pain 8 years later. Imaging studies revealed three lesions in the pancreas and a 12-cm mass in the right adrenal gland. Histopathology revealed an adrenal cortical carcinoma (ACC) with lymph- and haema-angio-invasion and capsular invasion. The diagnosis was confirmed by immunohistochemistry showing focal positivity for pancytokeratin, alpha-inhibin, and synaptophysin, but no expression of chromogranin A (Fig. 1). The ACC had

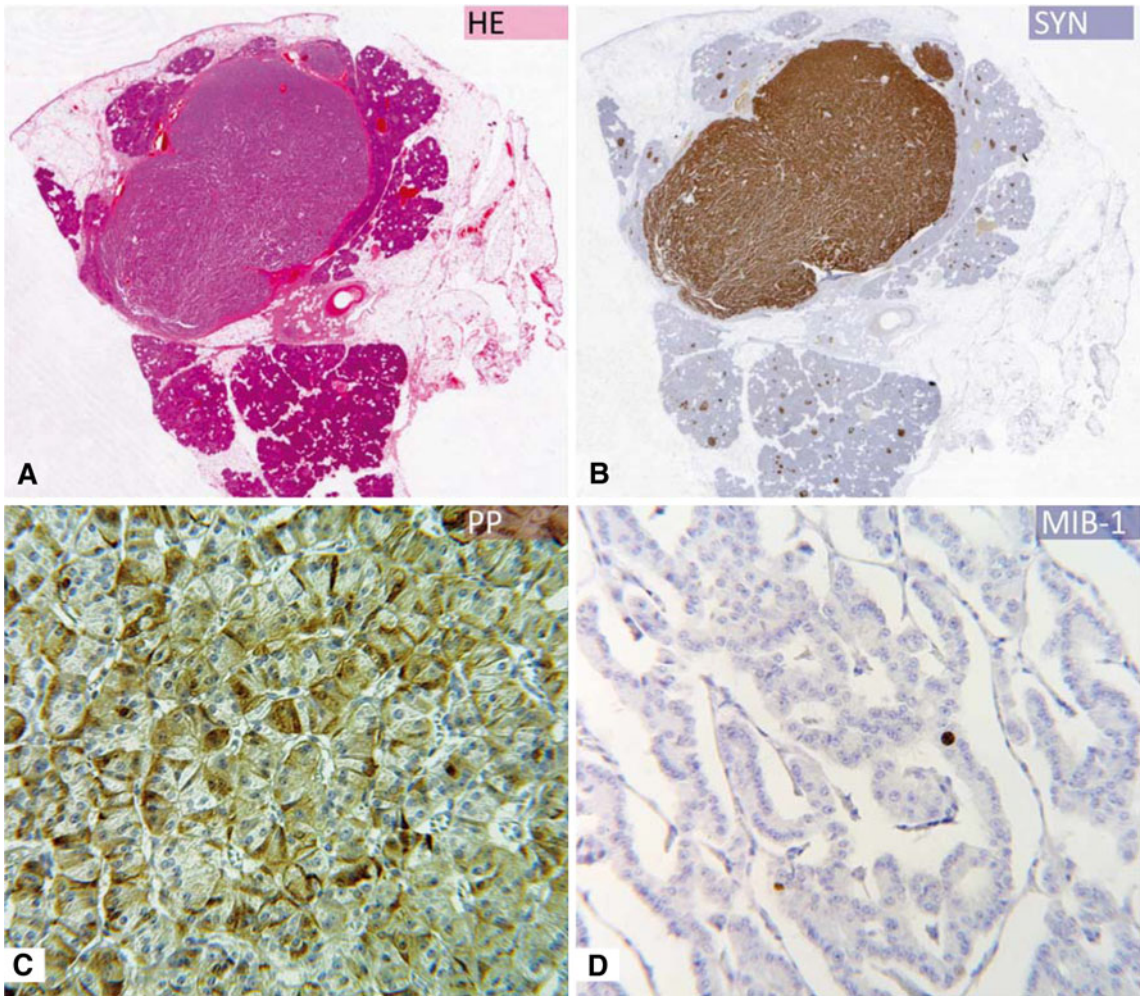

Fig. 2 MEN1-associated pancreatic neuroendocrine tumor. **a** and **b** whole mount photographs of an intrapancreatic sharply demarcated neuroendocrine neoplasm of less than 2 cm in size (hematoxylin and eosin *HE*; **a**), revealing strong immunoreactivity for synaptophysin (SYN; **b**). **c** Expression pf pancreatic polypeptide (PP) in the majority of tumor cells (high-power magnification). **d** High differentiated neuroendocrine architecture with trabecular growth pattern and less than 1% MIB-1 immunoreactive tumor cells (high-power magnification)

architectural and cytologic features that generally recapitulate the normal adrenal cortex. Necrosis was not present. Mitosis was less than 5 per 50-high power fields. The tumor showed a trabecular and nesting growth pattern and low nuclear atypia (Weiss score 3; on a scale from 0 to 9).

The pancreatectomy specimen showed two intrapancreatic neuroendocrine tumors with a diameter of 1.5 cm each and trabecular growth pattern, respectively (Figs. 2, 3). Both tumors showed less than 1% MIB-1-positive cells. Immunohistochemically, the majority of tumor cells stained positive for pancreatic polypeptide with some scattered glucagon cells (less than 5%). Further peptide hormones (i.e., insulin, somatostatin, and gastrin) were absent. In addition, five microadenomas (less than 0.5 cm in diameter) with expression of glucagon were present within the pancreatic parenchyma. No lymph node metastases were found. In summary, an ACC of the right adrenal gland (Weiss score 3), two MEN1-associated (functionally silent) well differentiated pancreatic neuroendocrine tumors with expression of pancreatic polypeptide and an MEN1-associated pancreatic microadenomatosis were diagnosed (pT1(m) pN0 pMx V0 L0 R0) (G1; less than 1% MIB-1 positive cells).

The ACC and the pancreatic neuroendocrine tumors were surgically removed without further endocrine work-up. The patient was transferred to our clinic afterwards.

We saw a 54-year-old adipose woman (height 160 cm, weight 87.6 kg). Her blood pressure was 140 over 80 mm mercury, heart rate 88/min, temperature 37.5°C. A review of her post-operative imaging studies and her laboratory

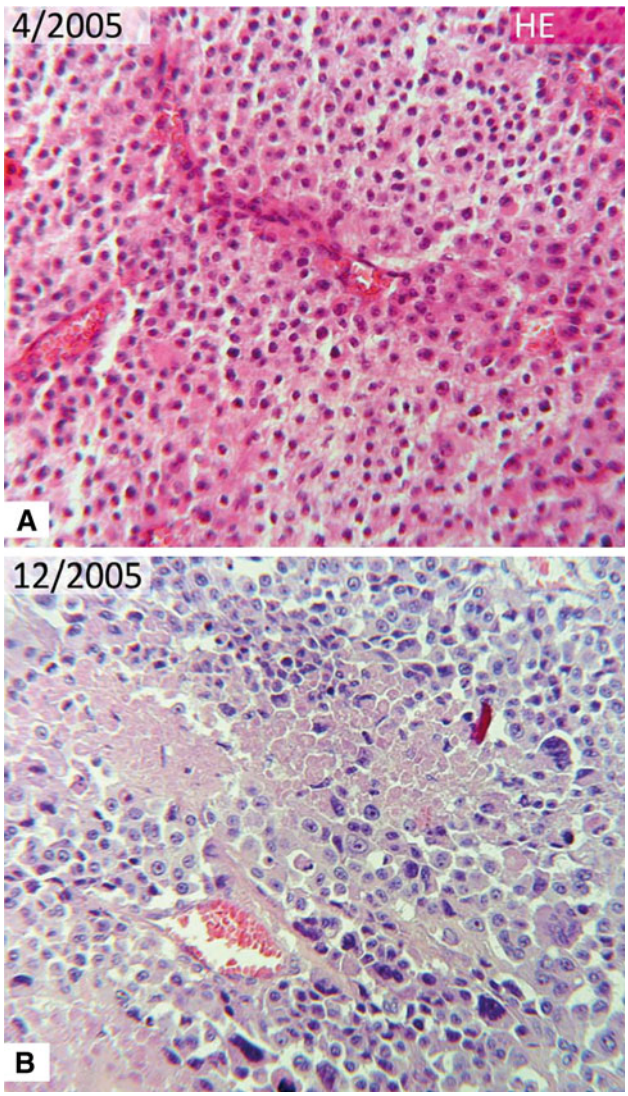

Fig. 3 Comparative morphology of adrenal cortical carcinoma (April 2005, Dec 2005). **a** The adrenal cortical carcinoma which was operated on in April 2005 reveals patternless sheets of high differentiated clear cells (hematoxylin and eosin *HE*; high-power magnification). **b** The recurrent adrenal cortical carcinoma (operated on in Dec 2010) shows focal necrosis, high nuclear atypia and giant tumor cells (high-power magnification)

data showed that the left adrenal gland was also affected by two tumors and that the patient had again hypercalcemia (Fig. 4). She was slightly hypercortisolemic, and hypercalcemia was due to primary hyperparathyroidism (Table 1). On ultrasound of the neck we saw 1.0 × 1.1 × 1.2 cm large hypoechogenic round lesion consistent with a proliferated parathyroid gland (Fig. 5). This time the patient agreed for genetic testing and was tested positive for a heterozygous mutation in the *MEN1* gene. She had a hitherto undescribed mutation at codon 443 in the coding region of exon 9 of the *MEN1* gene, where a cytosine residue was exchanged for adenosine (c.1327C > A) and serine for tyrosine (p.Ser443Tyr).

Because of her malignant disease and because the two lesions in the left adrenal gland had not been described previously, the patient was started on mitotane therapy. In addition, we performed an ultrasound of the upper abdomen and ordered a computed tomography study 3 months after the operation. Surprisingly, we found a mass in the region of the right adrenal gland, measuring 7.6 × 6.5 × 6.6 cm (Fig. 5), that was interpreted as recurrent disease of the ACC on computed tomography (Fig. 4). The adrenal tumors in the left adrenal gland had not increased in size. The patient was again operated. Histopathology revealed a highly pleomorphic tumor with diffuse architecture, extensive necrosis, high proliferative activity, and cellular atypia with gross invasion of the right kidney and the surrounding tissue (Weiss score: 9; on a scale from 0 to 9) conformable with a high malignant recurrence of the initially diagnosed ACC (Fig. 3). The patient died after surgery because of central thromboembolism before radiation of the tumor bed could be initiated.

Discussion

Here we describe a patient with the MEN1 syndrome who developed recurrent primary hyperparathyroidism, well-differentiated (functionally silent) neuroendocrine tumors of the pancreas and multiple adrenocortical tumors, one of high malignant behavior. This disorder was most likely due to a novel mutation in the *MEN1* gene. The point mutation (c.1327C > A, p.Ser443Tyr) was supposedly associated with loss of function. The patient's mother was already dead at the time of this study, and no original material, e.g., paraffin-embedded tissue, was available for DNA analysis. Therefore, we were not able to verify this mutation in affected relatives. However, this mutation in exon 9 could interfere with the binding of several proteins, including JunD, check point suppressor 1, activator of S-phase kinase, PRA2, and nm23β [2].

Since individuals who carry a heterozygous germline mutation in the *MEN1* gene develop tumors in neuroendocrine cells that lose heterozygosity, genotype–phenotype studies are problematic. However, it can be assumed that, once active, the p.Ser443Tyr mutation predisposes individuals to a rather aggressive tumor behavior in the pancreas and adrenals. Interestingly, it took the tumor in the right adrenal gland 8 years to grow from 2 to 12 cm in diameter, but only 3 months after complete resection to gain a size of 7 cm.

It is intriguing to speculate that some time has passed before a conversion step from a benign to a malignant adrenal lesion has occurred. Of note, the two tumors in

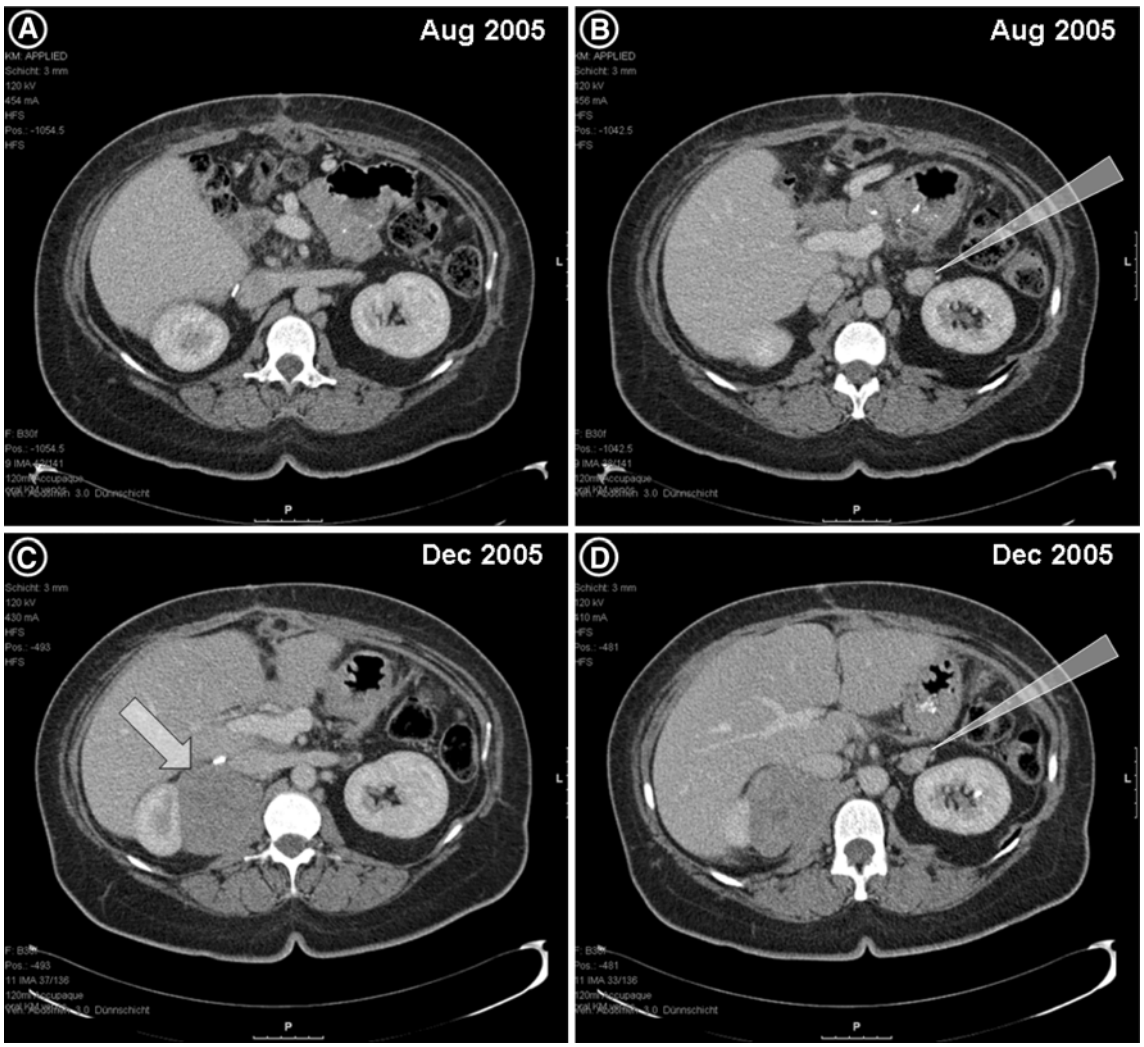

Fig. 4 Pictures obtained by computed tomography of the patient's abdomen in the venous phase after administration of contrast material, status after pancreatectomy and resection of the right adrenal gland. **a** No right adrenal remnant is seen, a X-ray-dense clip indicates the site of the former right adrenal tumor. **b** A second mass in the left adrenal gland is present (*arrowhead*) and located inferior to the clip seen in **a**. **c** Shows a recurrent mass (*arrow*) at the side of the former adrenal cortical tumor that was absent in **b**. **d** No significant increase in tumor size of the left adrenal gland tumor (*arrowhead*)

the left adrenal gland did not change in size within 3 months of observation. A benign behaviour of adrenal lesions occurs frequently in MEN-1 patients [4–6]. Interestingly, loss of heterozygosity in the menin gene or the menin gene locus 11q13 is not generally observed in MEN-1 adrenocortical adenomas. Also, sporadic adrenocortical adenomas do in general not display somatic mutations within the menin gene [7–12]. This led to the hypothesis that adrenal tumorigenesis is not directly associated with menin but rather an indirect phenomenon, probably due to trophic factors of extrapituitary origin: patients with the MEN-1 syndrome and adrenal tumors have pancreatic lesions also [4, 13, 14]. Of note, the presence of mutations in the menin gene or aberrations within the 11q13 locus is associated with malignancy of adrenal cortical neoplasms [4, 7, 8, 13, 14]. However, there are additional grounds to believe that other factors than the mutation type also influence the biology of MEN1 neoplasms [15, 16]. One such factor may be Wnt signaling. Components of the Wnt signaling pathway were reported to interact with menin [17, 18]. In addition, we and other groups showed that active Wnt signaling results in activation of steroidogenesis via steroidogenic factor-1 and stabilization of β-catenin is associated with the occurrence of adrenal tumors even in hereditary tumor syndromes [19, 20].

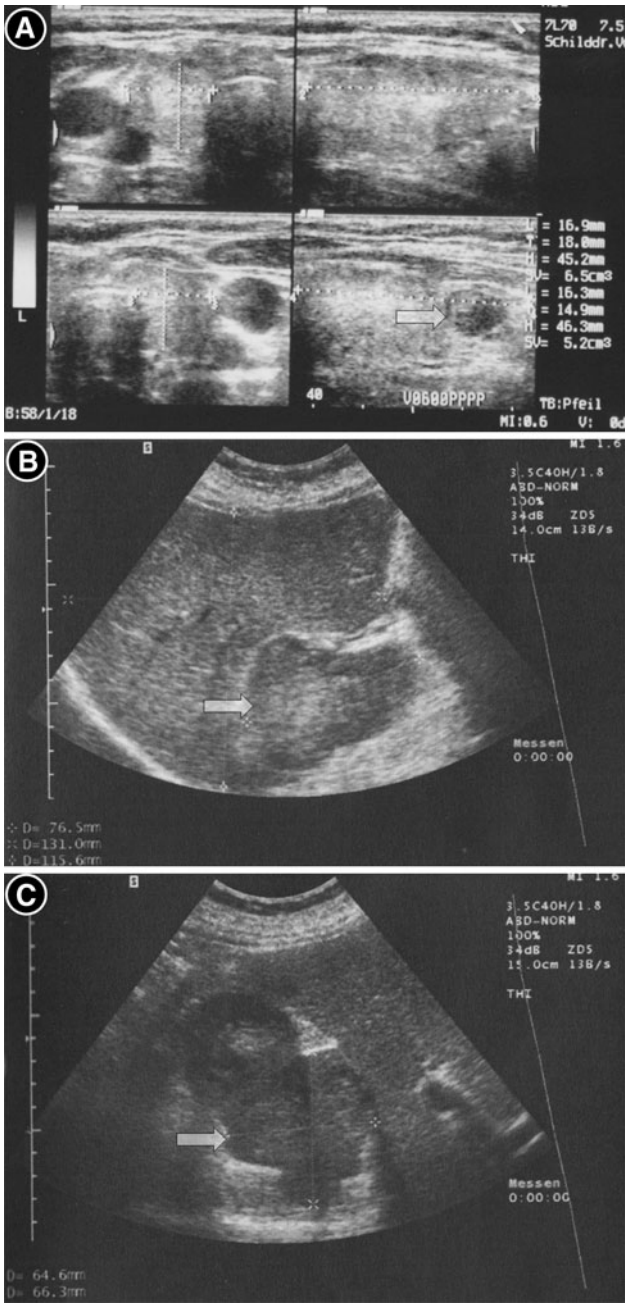

Fig. 5 Pictures obtained by ultrasound of the patient, B-mode. **a** Ultrasound (7.5 MHz sonohead) of the patient's neck and is composed of four pictures: *upper left* is a transversal view through the right thyroid lobe (volume: 6.5 ml), *upper right* is a sagittal view through the right thyroid lobe, *lower left* is a transversal view through the left thyroid lobe (volume: 5.2 ml), *lower left* is a sagittal view through the left thyroid lobe. In proximity to the inferior pole of the left thyroid lobe, a hypoechogenic mass is visible and corresponds to an enlarged parathyroid gland (*arrow*). **b** and **c** Ultrasound (3.5 MHz sonohead) of the patient's abdomen and transversal views from ventral **b** and dorsal **c** to the right adrenal mass that became apparent in Dec 2005 (*arrows*)

In conclusion, this case tells us that the p.Ser443Tyr mutation in the *MEN1* gene was clinically relevant and that additional factors drive adrenal tumorigenesis in the MEN1 syndrome.

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