RESEARCH

Adrenocortical carcinoma in patients with MEN1: a kindred report and review of the literature

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Abstract

Objective: Up to 40% of multiple endocrine neoplasia type 1 (MEN1) patients may have adrenal cortical tumors. However, adrenocortical carcinoma (ACC) is rare. The clinical manifestations, prevalence, inheritance and prognosis of ACC associated with MEN1 remain unclear. Here we report the clinical manifestations and prevalence of ACC in patients with MEN1.

Design and methods: A retrospective analysis of ACC associated with MEN1 patients at a single tertiary care center from December 2001 to June 2017. Genetic analysis of *MEN1* and other ACC associated genes, loss of heterozygosity (LOH) of *MEN1* locus, immunohistochemistry staining of menin, P53 and β -catenin in ACC tissue were performed.

Results: Two related patients had ACC associated with MEN1. The father had ENSAT stage IV tumor with excessive production of cortisol; the daughter had nonfunctional ENSAT stage I tumor. Both patients carried novel germline heterozygous mutation (c.400_401insC) of *MEN1*. The wild-type *MEN1* allele was lost in the resected ACC tissue from the daughter with no menin staining. The ACC tissue had nuclear β -catenin staining, with heterozygous *CTNNB1* mutation of 357del24 and P53 staining in only 20% cells.

Conclusions: ACC associated with MEN1 is rare and may occur in familial aggregates.

Key Words

- ▶ adrenocortical carcinoma (ACC)
- ▶ multiple endocrine neoplasia type 1 (MEN1)
- ▶ TP53
- ▶ CTNNB1

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Introduction

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disease characterized by the presence of endocrine tumors mainly affecting parathyroid, pituitary and pancreatic islet. Adrenal lesions occur in 20–55% of MEN1 cases and the majority is adrenocortical adenomas or hyperplasia. A small fraction of MEN1

patients developed adrenocortical carcinoma (ACC) (1, 2, 3, 4, 5, 6, 7, 8, 9). In fact, ACC in general is a rare malignancy with high mortality. Its incidence was 0.7–2.0 cases per million population per year and the overall 5-year survival was only 20–59% (10, 11). However, most previous reports about ACC associated with MEN1

were case descriptions. The clinical features, prevalence, inheritance and prognosis of ACC associated with MEN1 remain unclear.

The molecular pathogenesis of ACC has been associated with the tumor suppressor gene *TP53*. An unusually high incidence of ACC has been found in children in southern Brazil and a founder germline mutation in *TP53* has been found in 78–97% of these children (12, 13). In addition, ACC occurred in 3–7% of adults and 50–80% of children patients of Li–Fraumeni syndrome, which was caused by germline *TP53* mutation (14). WNT/ β -catenin signaling pathway also plays an important role in sporadic adrenocortical tumorigenesis. Activating point mutations of *CTNNB1* have been identified in over 25% ACCs (15, 16, 17, 18). *ENC1* and other β -catenin target genes were overexpressed in ACCs (19). Coincident β -catenin activation and *TP53* inactivation predicted poor outcome (20). In the recently published TCGA, profiles of ACC, *TP53* and *CTNNB1* mutations were the most common mutations in ACC, and *MEN1* was one of the five significantly mutated genes in ACC. Seven percent of tumors harbored inactivating *MEN1* mutations (11), consistent with a prior study identifying recurrent somatic *MEN1* mutation in ACC (21). However, the role of *MEN1* in ACC tumorigenesis remains to be clarified.

In the current study, we described a MEN1 family with ACC in a cohort of MEN1 patients. Furthermore, we explored the preliminary roles of *MEN1* in ACC tumorigenesis and performed a literature review of previously reported cases.

Subjects and methods

Patient

We performed a retrospective analysis of a prospectively collected database of MEN1 patients in our institution (Ruijin Hospital, Shanghai Jiaotong University, School of Medicine). This database included consecutive patients diagnosed with MEN1 according to current clinical practice guidelines recommendations (22) from December 2001 to June 2017. Patients were followed up every three months as part of our institutional protocol. The diagnosis of ACC was based on histological samples evaluated by two independent pathologists. We also performed a comprehensive literature search of PubMed, Ovid MEDLINE and Ovid EMBASE for the terms of adrenocortical carcinoma, adrenal lesion, adrenal and multiple endocrine neoplasia type 1 limited

to English publication. The last search in this study was updated in June 2017. This study was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University, School of Medicine. Consent has been obtained from each patient after full explanation of the purpose and nature of all procedures used.

DNA isolation and sequencing

Genomic DNA was isolated from peripheral blood sample by using the QIAamp DNA Blood Mini Kit (QIAGEN) and paraffin-embedded tissue by using the QIAamp DNA FFPE Tissue Kit (QIAGEN). Exon 2–10 of the *MEN1* gene, coding region of *TP53* and exon 3 of *CTNNB1* were amplified and analyzed by Sanger sequencing. The primer sequences of *MEN1* were previously reported (23), while the primer sequences of coding sequence (CDS) of *TP53* and exon 3 of *CTNNB1* are listed in Supplementary Table 1 (see section on [supplementary data](#) given at the end of this article).

Loss of heterozygosity assay

Loss of heterozygosity (LOH) analysis was performed using tumor DNA and matched leucocyte DNA. Three short tandem repeat (STR) markers (D11S4946, D11S1983 and D11S4940) were used to determine the region of LOH. *MEN1* gene is located between D11S4946 (intragenic in 5' untranslated region, about 500bp from *MEN1* transcription start site) and D11S1983, transcribing from telomere to centromere. D11S4940 is located approximately 93kb 5' of *MEN1* gene. The STR markers were amplified by fluorescent carboxyfluorescein (FAM)-labeled primers. The PCR products were resolved on an ABI 3730xl sequencer together with GeneScan 500 LIZ as size marker and quantified with GeneMapper v4.0 (Applied Biosystems). Markers were considered informative if two alleles were detected in normal tissue (peripheral blood leucocytes). LOH is defined as positive if the allele peak ratio is >1.5.

Immunohistochemistry

Formalin-fixed, paraffin wax-embedded tissue was cut into 4 μ m-thick sections. Menin protein was stained by the anti-menin antibody from Bethyl Laboratories (A300-105A) at appropriate dilution (1:600), as previously described (24). β -catenin was stained by β -catenin antibody from Cell Signaling Technology (9562) at dilution of 1:400. Normal parathyroid tissue was employed as positive control,

while negative controls were performed by pancreatic neuroendocrine tumors (PNETs) of patient IV:5 (self-control) with known loss expression of *menin* (somatic sequencing revealed homozygous *MEN1* mutation and IHC staining showed absence of *menin*).

Results

Patient description

A total of 121 patients (68 families) were diagnosed as *MEN1* from 2001 to 2017 and 33.9% had adrenal lesion. From those with adrenal lesions, 65.1% were nonfunctional adenomas, 25.6% were adenomas with hypercortisolism, 2.3% were adenomas with aldosteronism, 2.3% were pheochromocytomas and ACCs account for 4.7%. The median age at the diagnosis of adrenal tumors was 44.5 years (34.3–50.8 years) and a nearly equal sex distribution (male: female=1.05:1). Two cases of ACC in the same kindred were found, providing a frequency prevalence of 1.5% in the overall *MEN1* group (the number of *MEN1* families with ACC divided by the number of *MEN1* families).

The index case was a 51-year-old man (patient III:4) complaining of hypertension and muscle weakness. His hypertension (150/90 mmHg) started 8 months ago. Five months later, he started to have edema and weakness of the lower extremities. Hypokalemia (2.4–3.81 mmol/L) and diabetes (fasting plasma glucose, 10.1 mmol/L and HbA1C, 7.7%) was diagnosed by local community hospital. He was prescribed with antihypertensive drugs, potassium supplement and antidiabetic drugs. He had 3 kg loss during the past eight months. Hypertension and hypokalemia indicated adrenal disease. Physical examination found no sign of Cushing's syndrome. Evaluation of adrenal hormone revealed loss of cortisol rhythm (8:00–16:00–24:00 25.81–25.77–23.82 µg/dL) and elevated urinary free cortisol (UFC) (1287 µg/24h, normal 21–111 µg/24h). Overnight dexamethasone suppression test (1 mg ODST) failed to suppress morning cortisol levels (28.5 µg/dL, normal <5 µg/dL). The ODST, in concordance with lower ACTH levels (8.91 pg/mL, normal 12.00–78.00 pg/mL), indicated adrenal Cushing's syndrome. His aldosterone vertical supine position test was normal. Ultrasound revealed a 14 cm mass in the left adrenal gland and renal compression. To exclude malignant diseases, we performed abdominal CT, bone scan and ¹⁸F-fluorodeoxyglucose positron emission tomography-computed tomography (FDG-PET/CT). Abdominal CT

(Fig. 1A) showed a left adrenal gland mass (the Hounsfield units was 28 on plain scan and 40 on enhanced CT scan) and multiple pancreatic tumors located in the uncinate process of pancreas and pancreatic duct dilation. Bone scan showed lesions in right third and fifth anterior rib, and left inferior segment of femur. PET/CT scan showed an enormous and irregular left adrenal mass and metastasis to left supraclavicular and mediastinal lymph nodes, bilateral lung and uncinate process of pancreas. In addition, he had incidental finding of hypercalcemia (2.71–2.79 mmol/L, normal range 2.00–2.75 mmol/L) with hypophosphatemia (0.45–0.58 mmol/L, normal range 0.80–1.60 mmol/L) in the presence of normal serum albumin (35 g/L). His 24-h urinary calcium was high (8.00 mmol/24h). Serum PTH level was elevated (270.1 pg/mL, normal range 15.0–68.3 pg/mL) while vitamin D [²⁵(OH)₂D₃] was normal (69.54 nmol/L, normal range >50 nmol/L). Both ultrasonography and technetium sestamibi (MIBI) scan (Fig. 1B) revealed bilateral parathyroid adenoma.

Adrenal carcinoma, bilateral parathyroid adenoma and suspected pancreatic tumors suggested *MEN1*. MRI of pituitary disclosed microadenoma (Fig. 1C) with normal prolactin, growth hormone, thyroid-stimulating hormone, luteinizing hormone, follicle-stimulating hormone, insulin-like growth factor and insulin-like growth factor-binding protein 3. Genetic testing found a hitherto undescribed heterozygous mutation at codon 97 of *MEN1* gene, c.400_401insC (Fig. 2A).

He was clinically and genetically diagnosed as *MEN1* with hyperparathyroidism, ACC and nonfunctional pituitary adenoma. The staging of ACC according to the European Network for the Study of Adrenal Tumors (ENSAT) classification was stage IV and the 5-year survival was only 18% (25, 26). He received mitotane and succumbed to progressive disease 7 months after diagnosis.

We screened *MEN1* c.400_401insC mutation in his kindred and found eight carriers (Fig. 1E). Three family members had been dead of PNETs before genetic screening. The tumor components of his family members are listed in Table 1. The proband is the offspring of a consanguineous marriage. Four of the eight mutation carriers were evaluated for *MEN1*-related tumors and three members were affected by adrenal tumors. Two of which had adrenocortical adenomas (ACAs) and the proband's daughter was affected with ACC as well (Fig. 1E).

The daughter (patient IV:5) was 27 years old with no complaints. Her blood pressure was 120/70 mmHg and had no symptoms or signs of hypercortisolism.

Figure 1

Left adrenal tumor on CT scan (A), bilateral parathyroid adenoma on MIBI (B), pituitary microadenoma on MRI (C) of the proband (III:4). Right adrenal tumor on CT scan of patient IV:5 (D). Pedigree tree (E).

The preoperative imaging investigation included the following: pituitary microadenoma, hyperplasia of the parathyroid glands, a mass in the junction of body and tail of the pancreas (2.2×3.0 cm) and a mass in the right adrenal gland ($4 \text{ cm} \times 3.7$ cm). ACC was considered because of necrosis, hemorrhage and cyst formation on the CT scan (the Hounsfield units was 34.5 on plain scan and 59.1 on enhanced scan, Fig. 1D). Both the cortical and the medulla hormone profiles were normal. Neuron-specific enolase was elevated (26.29 ng/mL , normal $<17.00 \text{ ng/mL}$). Biochemical measurements revealed elevated serum calcium (2.72 mmol/L) and PTH levels (210 pg/mL). Pituitary hormones, gastrin, glucose and insulin levels were normal. She was diagnosed as MEN1 syndrome with primary hyperparathyroidism, nonfunctional pituitary adenoma, nonfunctional PNET and adrenal tumors, possibly malignant. She received

laparoscopic right adrenalectomy. The histological findings included the following: round to oval cells, with scant eosinophilic cytoplasm and moderate to marked nuclear pleomorphism, arranged in a loose growth pattern (Fig. 2B); abnormal karyokinesis ($>5/50\text{HPF}$), necrosis and capsular invasion; positive staining of Melan-A, inhibin, CD56, P53 ($20\%+$) and focal cytokeratins (AE1/AE3). Ki-67 proliferation index was 10%. Melan-A and inhibin are the markers of the adrenocortical origin. The positive staining of these two proteins excluded renal cell carcinoma and other histologically similar tumors (27). The tumor was defined as an adrenocortical carcinoma according to Weiss's criteria with the Weiss score of 7 (on a scale from 0 to 9): high nuclear grade, mitotic rate greater than 5 per 50 high-power fields, atypical mitoses, clear cells comprising 25% or less of the tumor, diffuse architecture (greater than one-third of the tumor), necrosis and invasion of

Figure 2

Direct and subclone sequencing of *MEN1* with germline DNA from the proband and direct sequencing of *MEN1* with somatic DNA from patient IV:5 (A). Hematoxylin and eosin staining of ACC from patient IV:5 (B). Menin absence of ACC tumor from patient IV:5 with menin antibody from Bethyl Laboratories (C) and β -catenin antibody (D). The amplification is 200 $\times$ and 400 $\times$, respectively. LOH assay of *MEN1* locus (E) in ACC tumor cell from patient IV:5. STR markers D11S4946, D11S1983 and D11S4940 flanking *MEN1* gene were displayed respectively. PBMC, peripheral blood mononuclear cells. The arrows indicated allele loss.

capsule of tumor. The presence of three or more criteria correlates with subsequent malignant behavior (10, 28, 29). Six months later, she received robot-assisted resection

of pancreatic body and tail. The tumor was diagnosed as well-differentiated neuroendocrine tumor of the pancreas and grade G1 in histology according to WHO classification

Table 1 Twelve MEN1 patients in the kindred of MEN1.

ID	Gender	Age of onset ^a	Age of MEN1 diagnosis ^a	Age of death ^a	Tumor component	Histological grade	Stage	Hormone production
III:4 The proband	Male	50	51	51	PHPT, PA PNET	NA		No hormone excess
III:2	Female	49	54	Alive	ACC PHPT, PA PNET ACA	NA NA NA	ENSAT stage: IV TNM stage: IA	No hormone excess No hormone excess No hormone excess
IV:3	Male	26	28	Alive	PHPT, PA PNET ACA	G2 Weiss score: 0	TNM stage: IB	No hormone excess No hormone excess
IV:1	Male	30	30	Alive	PHPT PNET	NA	TNM stage: IA	No hormone excess
IV:5	Female	27	27	Alive	PHPT PNET ACC	G1 Weiss score: 7	TNM stage: IB ENSAT stage: I	No hormone excess No hormone excess
IV:7	Female	No onset	21	Alive			NA	
IV:8	Male	No onset	20	Alive			NA	
V:3	Male	No onset	7	Alive			NA	
V:4	Female	No onset	4	Alive			NA	
II:3	Male	ND	Dead before diagnosis	40	Malignant PNET		NA	
II:1	Female	ND	Dead before diagnosis	51	Insulinoma? (hypoglycemia)		NA	Died of hypoglycemia, so insulinoma was suspected
III:6	Male	40	Dead before diagnosis	40	PNET		NAs	

^aYear.

NA, not available; PA, pituitary adenoma.

in 2010 (30), and the TNM stage was IB according to the American Joint Cancer Committee (AJCC) Cancer Staging (31). Currently, patient IV:5 has been followed up for 1 year with no evidence of recurrent or metastatic disease.

The proband's son (patient IV:3) had an ACA in the left adrenal gland and was resected with PNET. The Weiss score of ACA was 0 and PNET was classified as G2 and the TNM stage was IB. Both ACA and PNET were considered as nonfunctional without hormone excess. He was also affected by nonfunctional pituitary microadenoma and primary hyperparathyroidism. The serum calcium was 2.79 mmol/L (normal range 2.00–2.75 mmol/L) and he did not receive surgery for pituitary and parathyroid. The proband's elder sister (patient III:2) had nonfunctional multiple nodular hyperplasia in bilateral adrenal glands. Pancreatic MRI revealed a PNET (1.5 × 1.3 cm) in her uncinate process of pancreas (TNM stage: IA) and pituitary MRI showed a microadenoma. She did not receive surgery for the adrenal glands, pancreas and pituitary. Besides, she was diagnosed as primary hyperparathyroidism and received subtotal parathyroidectomy + thymectomy with the pathology of parathyroid hyperplasia (Table 1).

Analysis of MEN1 and other candidate genes in ACC tissue

ACC tissue was obtained from patient IV:5 during surgery. The resected ACC tissue harbored homogenous frame-shift mutation of c.400_401insC in *MEN1* gene (Fig. 2A). The resected ACC also showed LOH of chromosome 11q13, where *MEN1* gene was located (Fig. 2E) and menin was negatively stained (Fig. 2C).

We also analyzed the expression and mutation of *TP53* and *CTNNB1*. We found positive β-catenin staining in the nucleus (Fig. 2D) and P53 staining in only 20% cells. DNA sequencing revealed a somatic 357del24 mutation of *CTNNB1* and no mutation in *TP53*.

Discussion

In this study, we found ACC can exhibit familial aggregation in MEN1 kindred with novel *MEN1* germline mutation. Both alleles of *MEN1* gene were damaged in the ACC tissue.

By a thorough literature review, we found additional 19 cases of ACC associated with MEN1 from nine reports (Supplementary Table 2) (1, 2, 3, 4, 5, 6, 7, 8, 9). None of the 19 cases were related. To our knowledge, this is the first time a kindred of ACC associated with MEN1 has been reported, proving that ACC can exhibit familial aggregation in MEN1 patients. The pooled prevalence of ACC in MEN1 was 18/1187 (three MEN1-associated ACCs were case reports). The mean and the median age at diagnosis of ACC was 37 and 33 years respectively, younger than sporadic ACC (46–55 years) (32, 33). Nonfunctional ACC was found in 7/18 (three cases were not available) of the total cases, similar with sporadic ACC (40%) (34). Functional ACCs can cause feminization (2/11), virilization (4/11), hypercortisolism (4/11) or both virilization and hypercortisolism (1/11). The median tumor size was 7.5 cm (2.8–15.0 cm). Three out of five cases had metastasis. Fourteen patients had germline mutation testing for *MEN1* gene and all were positive; 6/14 of the mutation located in exon 2 (Supplementary Table 2). Thirteen patients had follow-up data but two of them were unavailable for the ENSAT stage. All patients with stage I and II diseases survived more than 5 years. All stage IV patients died ($n=3$) and there were no patients with stage III. Worse prognosis was associated with higher ENSAT stage. Furthermore, worse prognosis was also associated with tumor size larger than 7.5 cm and diagnostic age older than 33 years. Both groups of patients had median survival time as 0.25 (95% CI: 0.25 to 3.00) years, while it had not been reached for those ≤ 7.5 cm or those ≤ 33 years ($P=0.0025$). The current guidelines do not recommend regular monitoring of the adrenal glands. We therefore recommended annual evaluation of those with preexisting adrenal lesions, especially for patients younger than 33 years old.

The novel germline frame-shift mutation (c.400_401insC) of *MEN1* gene was pathogenic because it segregated with MEN1 phenotype: 8/12 mutation carriers were affected by MEN1-related lesions (primary hyperparathyroidism, PNET or pituitary adenoma), the other four mutation carriers refused evaluation due to young age (IV:7, IV:8, V:3, V:4 were 21, 20, 7, 4 years old, respectively) or lack of symptoms; those without *MEN1* mutation were free of MEN1 tumor. The affected ninety-seventh amino acid is located in N-terminal domain (NTD), which is highly conserved in evolution. Menin acts as a scaffold protein, interacting with LEDGF (Lens Epithelium-Derived Growth Factor) and MLL1 on the surface of NTD. Mutations located in NTD would decrease

LEDGF-Menin interaction, therefore diminishing Hoxc8 expression and leading to tumor formation (35, 36).

In the current study, we found homozygous mutant *MEN1* allele in ACC associated with MEN1 with negative menin protein staining, indicating a possible role of loss function of menin in ACC tumorigenesis. Two major pathways are altered in ACC: β -catenin pathway and p53/Rb signaling. *CTNNB1* (16%) and *TP53* (16%) were the most common mutant genes (21). About 30% of β -catenin-activated transgenic mice developed malignant adrenal tumors by 17–18 months. ACCs were absent from the tumor profile of p53-deficient or mutant mice, possibly due to the different physiology between humans and mice (37). Deletion of exons 8 and 9 of *TP53* and a Ser⁴⁵ of *CTNNB1* mutation in H295R cells and H193Y mutation of *TP53* in SW13 cells has previously been described (15, 38, 39). Consistently, we found the MEN1-associated ACC tissue had nuclear β -catenin staining, with heterozygous *CTNNB1* mutation of 357del24 and P53 staining in 20% cells. Based upon these findings, we suggested that additional events such as altered β -catenin pathway and p53/Rb signaling may be required for malignant transformation.

In summary, ACC occurs rarely but can exhibit familial aggregation in MEN1 patients. Regular screening strategies should be recommended for MEN1 patients with a family history of ACC, especially for those younger than 33 years and with adrenal lesion.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/EC-18-0526>.

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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References

- 1 Skogseid B, Larsson C, Lindgren PG, Kvanta E, Rastad J, Theodorsson E, Wide L, Wilander E & Oberg K. Clinical and genetic features of adrenocortical lesions in multiple endocrine neoplasia type 1. *Journal of Clinical Endocrinology and Metabolism* 1992 **75** 76–81. (<https://doi.org/10.1210/jcem.75.1.1352309>)
- 2 Kharb S, Pandit A, Gundurthi A, Garg MK, Brar KS, Kannan N & Bharwaj R. Hidden diagnosis of multiple endocrine neoplasia-1

- unrevealed during workup of virilization caused by adrenocortical carcinoma. *Indian Journal of Endocrinology and Metabolism* 2013 **17** 514–518. (<https://doi.org/10.4103/2230-8210.111672>)
- 3 Griniatsos JE, Dimitriou N, Zilos A, Sakellariou S, Evangelou K, Kamakari S, Korkolopoulou P & Kaltsas G. Bilateral adrenocortical carcinoma in a patient with multiple endocrine neoplasia type 1 (MEN1) and a novel mutation in the MEN1 gene. *World Journal of Surgical Oncology* 2011 **9** 6. (<https://doi.org/10.1186/1477-7819-9-6>)
 - 4 Skogseid B, Rastad J, Gobl A, Larsson C, Backlin K, Juhlin C, Akerstrom G & Oberg K. Adrenal lesion in multiple endocrine neoplasia type 1. *Surgery* 1995 **118** 1077–1082. ([https://doi.org/10.1016/S0039-6060\(05\)80117-5](https://doi.org/10.1016/S0039-6060(05)80117-5))
 - 5 Waldmann J, Bartsch DK, Kann PH, Fendrich V, Rothmund M & Langer P. Adrenal involvement in multiple endocrine neoplasia type 1: results of 7 years prospective screening. *Langenbeck's Archives of Surgery* 2007 **392** 437–443. (<https://doi.org/10.1007/s00423-006-0124-7>)
 - 6 Langer P, Cupisti K, Bartsch DK, Nies C, Goretzki PE, Rothmund M & Roher HD. Adrenal involvement in multiple endocrine neoplasia type 1. *World Journal of Surgery* 2002 **26** 891–896. (<https://doi.org/10.1007/s00268-002-6492-4>)
 - 7 Gatta-Cherifi B, Chabre O, Murat A, Niccoli P, Cardot-Bauters C, Rohmer V, Young J, Delemer B, Du Boullay H, Verger MF, *et al.* Adrenal involvement in MEN1. Analysis of 715 cases from the Groupe d'étude des Tumeurs Endocrines database. *European Journal of Endocrinology* 2012 **166** 269–279. (<https://doi.org/10.1530/EJE-11-0679>)
 - 8 Haase M, Anlauf M, Schott M, Schinner S, Kaminsky E, Scherbaum WA & Willenberg HS. A new mutation in the menin gene causes the multiple endocrine neoplasia type 1 syndrome with adrenocortical carcinoma. *Endocrine* 2011 **39** 153–159. (<https://doi.org/10.1007/s12020-010-9424-3>)
 - 9 Goudet P, Dalac A, Le Bras M, Cardot-Bauters C, Niccoli P, Levy-Bohbot N, du Boullay H, Bertagna X, Ruszniewski P, Borson-Chazot F, *et al.* MEN1 disease occurring before 21 years old: a 160-patient cohort study from the Groupe d'étude des Tumeurs Endocrines. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 1568–1577. (<https://doi.org/10.1210/jc.2014-3659>)
 - 10 Fassnacht M, Libe R, Kroiss M & Allolio B. Adrenocortical carcinoma: a clinician's update. *Nature Reviews Endocrinology* 2011 **7** 323–335. (<https://doi.org/10.1038/nrendo.2010.235>)
 - 11 Zheng S, Cherniack AD, Dewal N, Moffitt RA, Danilova L, Murray BA, Lerario AM, Else T, Knijnenburg TA, Ciriello G, *et al.* Comprehensive pan-genomic characterization of adrenocortical carcinoma. *Cancer Cell* 2016 **30** 363. (<https://doi.org/10.1016/j.ccell.2016.07.013>)
 - 12 Ribeiro RC, Sandrini F, Figueiredo B, Zambetti GP, Michalkiewicz E, Lafferty AR, DeLacerda L, Rabin M, Cadwell C, Sampaio G, *et al.* An inherited p53 mutation that contributes in a tissue-specific manner to pediatric adrenal cortical carcinoma. *PNAS* 2001 **98** 9330–9335. (<https://doi.org/10.1073/pnas.161479898>)
 - 13 Pinto EM, Billerbeck AEC, Villares MCBF, Domenice S, Mendonca BB & Latronico AC. Founder effect for the highly prevalent R337H mutation of tumor suppressor p53 in Brazilian patients with adrenocortical tumors. *Arquivos Brasileiros de Endocrinologia and Metabologia* 2004 **48** 647–650. (<https://doi.org/10.1590/S0004-27302004000500009>)
 - 14 Else T, Kim AC, Sabolch A, Raymond VM, Kandathil A, Caoili EM, Jolly S, Miller BS, Giordano TJ & Hammer GD. Adrenocortical carcinoma. *Endocrine Reviews* 2014 **35** 282–326. (<https://doi.org/10.1210/er.2013-1029>)
 - 15 Tissier F, Cavard C, Groussin L, Perlemonne K, Fumey G, Hagnere AM, Rene-Corail F, Jullian E, Gicquel C, Bertagna X, *et al.* Mutations of beta-catenin in adrenocortical tumors: activation of the Wnt signaling pathway is a frequent event in both benign and malignant adrenocortical tumors. *Cancer Research* 2005 **65** 7622–7627. (<https://doi.org/10.1158/0008-5472.CAN-05-0593>)
 - 16 Gaujoux S, Tissier F, Groussin L, Libe R, Ragazzon B, Launay P, Audebourg A, Dousset B, Bertagna X & Bertherat J. Wnt/beta-catenin and 3',5'-cyclic adenosine 5'-monophosphate/protein kinase A signaling pathways alterations and somatic beta-catenin gene mutations in the progression of adrenocortical tumors. *Journal of Clinical Endocrinology and Metabolism* 2008 **93** 4135–4140. (<https://doi.org/10.1210/jc.2008-0631>)
 - 17 Tadjine M, Lampron A, Ouadi L, Horvath A, Stratakis CA & Bourdeau I. Detection of somatic beta-catenin mutations in primary pigmented nodular adrenocortical disease (PPNAD). *Clinical Endocrinology* 2008 **69** 367–373. (<https://doi.org/10.1111/j.1365-2265.2008.03273.x>)
 - 18 Tadjine M, Lampron A, Ouadi L & Bourdeau I. Frequent mutations of beta-catenin gene in sporadic secreting adrenocortical adenomas. *Clinical Endocrinology* 2008 **68** 264–270. (<https://doi.org/10.1111/j.1365-2265.2007.03033.x>)
 - 19 Giordano TJ, Thomas DG, Kuick R, Lizyness M, Misk DE, Smith AL, Sanders D, Aljundi RT, Gauger PG, Thompson NW, *et al.* Distinct transcriptional profiles of adrenocortical tumors uncovered by DNA microarray analysis. *American Journal of Pathology* 2003 **162** 521–531. ([https://doi.org/10.1016/S0002-9440\(10\)63846-1](https://doi.org/10.1016/S0002-9440(10)63846-1))
 - 20 Ragazzon B, Libe R, Gaujoux S, Assie G, Frattucci A, Launay P, Clauser E, Bertagna X, Tissier F, de Reynies A, *et al.* Transcriptome analysis reveals that p53 and [beta]-catenin alterations occur in a group of aggressive adrenocortical cancers. *Cancer Research* 2010 **70** 8276–8281. (<https://doi.org/10.1158/0008-5472.CAN-10-2014>)
 - 21 Assie G, Letouze E, Fassnacht M, Jouinot A, Luscip W, Barreau O, Omeiri H, Rodriguez S, Perlemonne K, Rene-Corail F, *et al.* Integrated genomic characterization of adrenocortical carcinoma. *Nature Genetics* 2014 **46** 607–612. (<https://doi.org/10.1038/ng.2953>)
 - 22 Thakker RV, Newey PJ, Walls GV, Bilezikian J, Dralle H, Ebeling PR, Melmed S, Sakurai A, Tonelli F, Brandi ML, *et al.* Clinical practice guidelines for multiple endocrine neoplasia type 1 (MEN1). *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 2990–3011. (<https://doi.org/10.1210/jc.2012-1230>)
 - 23 Ye L, Wang W, Ospina NS, Jiang L, Christakis I, Lu J, Zhou Y, Zhu W, Cao Y, Wang S, *et al.* Clinical features and prognosis of thymic neuroendocrine tumours associated with multiple endocrine neoplasia type 1: a single-centre study, systematic review and meta-analysis. *Clinical Endocrinology* 2017 **87** 706–716. (<https://doi.org/10.1111/cen.13480>)
 - 24 Jiang XH, Lu JL, Cui B, Zhao YJ, Wang WQ, Liu JM, Fang WQ, Cao YN, Ge Y, Zhang CX, *et al.* MEN1 mutation analysis in Chinese patients with multiple endocrine neoplasia type 1. *Endocrine-Related Cancer* 2007 **14** 1073–1079. (<https://doi.org/10.1677/ERC-07-0015>)
 - 25 Fassnacht M, Wittekind C & Allolio B. Current TNM classification systems for adrenocortical carcinoma. *Pathologia Europaea* 2010 **31** 374–378. (<https://doi.org/10.1007/s00292-010-1306-1>)
 - 26 Lughezzani G, Sun M, Perrotte P, Jeldres C, Alasker A, Isbarn H, Budaus L, Shariat SF, Guazzoni G, Montorsi F, *et al.* The European Network for the Study of Adrenal Tumors staging system is prognostically superior to the international union against cancer-staging system: a North American validation. *European Journal of Cancer* 2010 **46** 713–719. (<https://doi.org/10.1016/j.ejca.2009.12.007>)
 - 27 Mondal SK, Dasgupta S, Jain P, Mandal PK & Sinha SK. Histopathological study of adrenocortical carcinoma with special reference to the Weiss system and TNM staging and the role of immunohistochemistry to differentiate it from renal cell carcinoma. *Journal of Cancer Research and Therapeutics* 2013 **9** 436–441. (<https://doi.org/10.4103/0973-1482.119329>)
 - 28 Lau SK & Weiss LM. The Weiss system for evaluating adrenocortical neoplasms: 25 years later. *Human Pathology* 2009 **40** 757–768. (<https://doi.org/10.1016/j.humpath.2009.03.010>)
 - 29 Fassnacht M, Kroiss M & Allolio B. Update in adrenocortical carcinoma. *Journal of Clinical Endocrinology and Metabolism* 2013 **98** 4551–4564. (<https://doi.org/10.1210/jc.2013-3020>)

- 30 Rindi G, Arnold R, Bosman F & Capella C. Nomenclature and classification of digestive neuroendocrine tumours. In *WHO Classification of Tumours, Pathology and Genetics of Tumours of the Digestive System*, 4th ed, pp. 10–12. Eds FT Bosman, F Carneiro, R Hruban, *et al.* Lyon, France: International Agency for Research on Cancer (IARC) Press, 2010.
- 31 Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR & Winchester DP. The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. *CA: A cancer journal for clinicians* 2017 **67** 93–99. (<https://doi.org/10.3322/caac.21388>)
- 32 Luton JP, Cerdas S, Billaud L, Thomas G, Guilhaume B, Bertagna X, Laudat MH, Louvel A, Chapuis Y, Blondeau P, *et al.* Clinical features of adrenocortical carcinoma, prognostic factors, and the effect of mitotane therapy. *New England Journal of Medicine* 1990 **322** 1195–1201. (<https://doi.org/10.1056/NEJM199004263221705>)
- 33 Bilimoria KY, Shen WT, Elaraj D, Bentrem DJ, Winchester DJ, Kebebew E & Sturgeon C. Adrenocortical carcinoma in the United States: treatment utilization and prognostic factors. *Cancer* 2008 **113** 3130–3136. (<https://doi.org/10.1002/cncr.23886>)
- 34 Allolio B & Fassnacht M. Clinical review: adrenocortical carcinoma: clinical update. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 2027–2037. (<https://doi.org/10.1210/jc.2005-2639>)
- 35 Hughes CM, Rozenblatt-Rosen O, Milne TA, Copeland TD, Levine SS, Lee JC, Hayes DN, Shanmugam KS, Bhattacharjee A, Biondi CA, *et al.* Menin associates with a trithorax family histone methyltransferase complex and with the *hoxc8* locus. *Molecular Cell* 2004 **13** 587–597. ([https://doi.org/10.1016/S1097-2765\(04\)00081-4](https://doi.org/10.1016/S1097-2765(04)00081-4))
- 36 Matkar S, Thiel A & Hua X. Menin: a scaffold protein that controls gene expression and cell signaling. *Trends in Biochemical Sciences* 2013 **38** 394–402. (<https://doi.org/10.1016/j.tibs.2013.05.005>)
- 37 Wasserman JD, Zambetti GP & Malkin D. Towards an understanding of the role of p53 in adrenocortical carcinogenesis. *Molecular and Cellular Endocrinology* 2012 **351** 101–110. (<https://doi.org/10.1016/j.mce.2011.09.010>)
- 38 Sampaoli C, Cerquetti L, Gawhary RE, Bucci B, Amendola D, Marchese R, Misiti S, Novelli G, Toscano V & Stigliano A. p53 stabilization induces cell growth inhibition and affects IGF2 pathway in response to radiotherapy in adrenocortical cancer cells. *PLoS One* 2012 **7** e45129. (<https://doi.org/10.1371/journal.pone.0045129>)
- 39 Reincke M, Karl M, Travis WH, Mastorakos G, Allolio B, Linehan HM & Chrousos GP. p53 mutations in human adrenocortical neoplasms: immunohistochemical and molecular studies. *Journal of Clinical Endocrinology and Metabolism* 1994 **78** 790–794. (<https://doi.org/10.1210/jcem.78.3.8126158>)

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