Appendiceal NET in an 18-year-old woman, the youngest case of MEN4 with neuroendocrine manifestation: case report and review of the current literature

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ABSTRACT

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominantly inherited syndrome. It is caused by loss-of-function mutation of the MEN1 gene, and characterized by variable association of primary hyperparathyroidism, pituitary adenomas and neuroendocrine tumours (NETs). Up to 3% of MEN1-like syndromes present a loss-of-function mutation in the tumour-suppressor gene CDKN1B, and therefore constitute MEN4 syndrome. Data on MEN4 clinical behaviour, penetrance and associated manifestations are still incomplete.

We report the case of a young woman diagnosed with a rare NET G1 of the appendix at the age of 18 years. Genetic analysis revealed a germline missense mutation (c.397C>A), present in heterozygosity, of codon 133 in the CDKN1B gene. To date only 26 mutations of CDKN1B have been described in association with a MEN4 phenotype. Subsequently, the patient’s sister, father and paternal uncle were found to be carriers of the same mutation but showed no clinical or biochemical signs of disease.

This is currently the youngest case of MEN4 with a gastrointestinal tract NET reported in the literature, and the first with appendiceal involvement. Despite the absence of disease within the proband’s family, ongoing screening would seem to be warranted, along the lines of that described by other authors for MEN1 patients.

KEYWORDS

MEN4, CDKN1B mutations, youngest case of NET in MEN4, appendiceal NET, MEN4 review.

Introduction

Multiple endocrine neoplasia (MEN) is a group of autosomal dominantly inherited syndromes characterized by the variable association of tumours in multiple endocrine, and sometimes nonendocrine, organs 1]. Four MEN syndrome types have been described to date [2,3]. Patients with MEN1 may develop parathyroid, pituitary and gastro-entero-pancreatic (GEP) neuroendocrine tumours (NETs), as well as other rare neoplasm [2,4]. The incidence of MEN1 has been estimated from random postmortem studies to be 0.25%. It is inherited with a high degree of penetrance.

The MEN1 gene is located on chromosome 11q13, which codes for menin, a protein that regulates transcription, genome stability, cell division and proliferation. Between 5% and 25% of patients with clinically suspected MEN1 do not have a pathogenic variant within the MEN1 coding region. Most of these cases are supposed to be due to phenocopies (5-10%), point mutations or deletions in non-coding regions [3]. Loss-of-function mutations in the cyclin-dependent kinase inhibitor 1B gene (CDKN1B) have an estimated frequency of around 3% [4], and lead to the diagnosis of MEN4 syndrome.

CDKN1B is a tumour suppressor gene with two exons located on chromosome 12 (12p13.1) that encodes the nuclear protein p27kip1, a cyclin-dependent kinase inhibitor that limits cell-cycle progression from the G1 to the S-phase by binding to and regulating the activity of cyclin-dependent kinases [5,6].

Data on MEN4 clinical behaviour, penetrance and associated manifestations are still incomplete.

To date, 26 different germline CDKN1B mutations have been reported to be associated with MEN4 phenotypes (Table 1). Primary hyperparathyroidism seems to occur in most cases and it is often the first manifestation of MEN4 [7,8]. Even though literature reports of MEN4 are increasing, the clinical phenotype remains uncertain [8]. In the present article we aim to describe and expand on current phenotypic and genetic knowledge of MEN4 syndrome by presenting the clinical and genetic features of a patient and her kindred in whom a CDKN1B germline variant was detected.
Patients and methods

Clinical presentation
A young woman was referred to our clinic due to an incidental diagnosis of appendiceal NET following an appendectomy at 18 years of age. The histological examination revealed a 2 cm tumour with extension to subserous tissue. Ki67 expression was <3%, mitosis number was <2 x 10 HPF (High Power Fields). The immunohistochemistry pattern was CgA+, synaptophysin+, ENS+, CD56+ and CAM5.2+, resulting in a diagnosis of NET G1. A negative total body PET/CT with 68Ga-DOTATOC was performed, excluding distant disease foci. The tumour staging, according to the 8th AJCC TNM classification (American Joint Committee on Cancer), was pT3

<table>
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<th>CDKN1B MUTATION</th>
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<tr>
<td>c.692G&gt;A</td>
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<td>c.597dup19</td>
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<td>c.595T&gt;C</td>
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<td>c.678C&gt;T</td>
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<td>c.281C&gt;T</td>
<td>Missense</td>
<td>PHPT, hyperprolactinemia (without any pituitary tumour at MRI), breast cancer, ovarian cystoadenoma and papillary thyroid carcinoma</td>
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<td>PHPT, NFPA, adrenal single adenoma (PACS)</td>
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<td>c.121_122 delTT</td>
<td>Frameshift</td>
<td>PHPT and ACTH-secreting PitNET (x2), PHPT and NF pan-NET, PHPT and NFPA (3 patients), PHPT and breast cancer</td>
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<td>Missense</td>
<td>NF pan-NET, ovarian dermoid cyst</td>
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<td>c.280_281delinsG</td>
<td>Frameshift</td>
<td>PHPT (APA)</td>
<td>Mazarico Altisent et al.[26]</td>
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<tr>
<td>c.169C&gt;T</td>
<td>Nonsense</td>
<td>PHPT (multiple adenomas, one APA)</td>
<td>Mazarico Altisent et al.[26]</td>
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<tr>
<td>c.397C&gt;A</td>
<td>Missense</td>
<td>PHPT, papillary thyroid carcinoma, Hürthle cell thyroid adenoma, uterine leiomyoma, grade II meningioma, PHPT (multiple glandular hyperplasia), fibrocystic breast disease</td>
<td>Costa-Guda et al.[26]</td>
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</table>

**PHPT:** primary hyperparathyroidism; **APA:** atypical parathyroid adenoma; **NFPA:** non-functioning pituitary adenoma; **PitNET:** pituitary neuroendocrine tumour; **NET:** neuroendocrine tumour; **GH:** growth hormone; **ACTH:** adrenocorticotropic hormone; **NF:** non-functioning; **PRL:** prolactin; **PACS:** possible autonomous cortisol secretion.
Nx M0. Her previous medical history was unremarkable. Given the patient’s young age and the rare clinical presentation she underwent genetic analysis.

**Genetic testing of the CDKN1B gene and results**

Genetic testing for mutations in the *MEN1*, *CDKN1B*, *VHL*, *AIP* and *CDC73* genes was performed using PCR-based Sanger direct sequencing of genomic DNA from peripheral blood leukocytes. The purified sequences obtained were compared to the wild-type reference sequence for the human *CDKN1B* gene (NG_016341): a germline missense mutation (c.397C>A), present in heterozygosity, of codon 133 (p.Pro133Thr), was identified. Notably, the analysis of the patient’s DNA also showed the presence, in heterozygosity, of the polymorphism c.1254C>T (Asp418Asp) in exon 9 of *MEN1*, already described in the literature as a single nucleotide non-pathological variant [9].

The same *CDKN1B* mutation was found in the proband’s 28-year-old sister, 62-year-old father and 55-year-old uncle (Fig.1). Investigation of the family history revealed previous cases of colorectal cancer (the patient’s grandmother and her brother), but we were unable to perform any genetic testing. The patient and the identified family carriers were then offered a screening analysis for MEN4-related disease features. This included bloodwork to assess levels of glycemia, insulin, gastrin, VIP (vaso-intestinal peptide), glucagon, PP (pancreatic polypeptide), chromogranin A, NSE (neuron-specific enolase), parathyroid and pituitary function. Multiple 24-hour urinary samples were collected for assessment of cortisol levels and the patient underwent MRI of the sella turcica. None of the examinations gave significant findings.

**Discussion**

To date, 26 *CDKN1B* mutations have been described, a total of 63 carriers has been reported and only 46 individuals have shown at least one phenotypical manifestation of MEN4 (Table 1). The mutation discovered in our patient has already been described in three other papers. Costa-Guda et al. described isolated primary hyperparathyroidism (PHPT) in two patients, in one case due to a germline mutation and in the other a somatic mutation discovered in the parathyroid tissue specimens. Notably, in both specimens a somatic biallelic inactivation was documented on *MEN1* [10]. It is thought that part of the tumour suppressive function of menin is mediated through its effects on p27 expression; interestingly, however, inactivation of both menin and p27 in rats has been shown not to cooperate with tumour development [10,11]. Unfortunately, the tissue samples from our patient are unavailable for analogue genomic analysis but a heterozygous single nucleotide polymorphism (SNP) in the *MEN1* gene was also found in her genome. Borsari et al. reported a case of PHPT caused by a multiple glandular hyperplasia associated with breast fibrocystic disease. In this case, too, a germline heterozygous MEN1 variant was described [12]. Bugalho and Domingues reported PHPT caused by a single adenoma associated with multifocal papillary thyroid carcinoma, Hürthle cell adenoma, uterine leiomyoma and a grade II meningioma [13].

Our patient currently seems to be the only reported case of NET in association with the *CDKN1B* gene mutation c.397C>A at exon 1, and the first reported MEN4 syndrome case with involvement of the appendix. Appendiceal NETs (aNETs) are a rare clinical entity. They account for more than 50% of all primary tumours of the appendix and usually progress indolently [14]. In adults the majority are detected between the third and the fifth decade of life [15,16]. Most are diagnosed incidentally in 0.5-1% of appendectomy specimens [14]. A recent epidemiological study based on more than 30 million people from the US confirmed the low clinical incidence of new cases of aNETs, finding it to stand at around 0.4 per 100,000 individuals. In that study aNETs accounted for 0.2% of all NETs. The same study demonstrated that patients with aNETs were more likely to be

![Figure 1 Transmission of CDKN1B mutation (c.397C>A) through the proband’s family.](image-url)
female, Caucasian and to have a family history of primary gastrointestinal malignancy or a diagnosis of a genetic syndrome such as MEN1 [18]. Regrettably, there is a lack of epidemiological data on the real incidence of aNETs in MEN syndromes. PHPT has been widely reported to occur in more than 90% of MEN4 cases, and it is often the first manifestation of the syndrome, appearing around the fifth decade of life, with the youngest patient diagnosed at 14 years old [11,17].

In MEN1 patients PHPT usually occurs between the third and the fourth decade with the youngest case presenting at 8 years old [18]. Anterior pituitary adenomas generally occur in about 40% of MEN4 patients, a rate that is quite similar to what is observed in MEN1 patients, even though there are many differences in terms of the pituitary tumour histotypes [19,20]. Only one case of prolactinoma has been reported among CDKN1B-mutated patients and ACTH-secreting tumours account for 25–40% of pituitary tumours in this setting [21]. The mean age at diagnosis is between 30 and 35 years, although the youngest case reported was a 5-year-old girl [9,22]. In MEN4 patients, occurring in less than 20%, fewer than in MEN1 where they account for 55-70% of cases [6,20]. In MEN4 they are usually diagnosed between the fifth and the sixth decades [9,22]. With the exception of our case, a 34-year-old man with hepatic metastasis is the youngest patient reported in the literature [8]. Moreover, non-endocrine neoplasms such as breast cancer, prostate cancer, colorectal cancer, meningioma and adrenal adenoma have also been described in MEN1 patients [2,3,6,22]. MEN1 is reported to have a high penetrance, indeed after 50 years of age more than 95% of patients develop at least one of the clinical manifestations [2]. The clinical behaviour of MEN4 is still not completely known due to the paucity of data. The penetrance is thought to be incomplete and our patient is currently the only member of her family to have developed the disease. With the aim of identifying the reasons for this low penetrance, a genome-wide sequencing analysis (WGS) is ongoing in our patient and her family. Unfortunately, the results are still very preliminary and it was not possible to publish them in this article.

Conclusions

MEN4 is a relatively novel clinical entity. Due to the paucity of reports and cases, we are not able to provide solid epidemiological data about this disease and its related mutations in CDKN1B. Given the severe comorbidity associated with PHPT, pituitary adenomas and aNETs, and the lack of conclusive data about the real clinical features and their incidence in MEN4 patients, we suggest that all CDKN1B mutation carriers should be screened for the related comorbidities in the same way as the guidelines for MEN1 suggest, focusing in particular on screening for aNETs and Cushing’s disease [12-4].

It is to be noted that CDKN1B mutation is well recognized as a potential driver in the development of breast, prostate and colorectal cancer in MEN4 [20], strongly suggesting the value of a personalized screening pathway for these neoplasms in this population.

References


Conflict of Interest: The authors declare that there are no conflicts of interest.