Novel mutation c.1224_1225insGTCC (p.Cys409Valfs*41) of MEN1 gene in a multiple endocrine neoplasia type 1 case with insulinoma and primary hyperparathyroidism: first report of a Costa Rican case

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Abstract

Abstract Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder with tumor predisposition in the parathyroid gland, anterior hypophysis and pancreatic islet cells. Here we describe the first Costa Rican MEN1 case with a novel MEN1 mutation in a 37-year-old male with history of nephrolithiasis and recurrent hypoglycemia

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Introduction

Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant disorder with inter- and intrafamilial variability, without a good known genotype-phenotype correlation, characterized by a predisposition for tumors in the parathyroid gland, anterior pituitary and pancreatic islet cells (Falchetti, 2017; Falchetti et al., 2009; Jensen & Norton, 2017; Lemos & Thakker, 2008; Marini, Falchetti, Luzi, & Maria Luisa, 2009; Thakker et al., 2012). Non-classic endocrine and non-endocrine tumors may also develop such as adrenocortical tumors, thyroid tumors, lipomas, collagenomas, angiofibromas, meningiomas and carcinoid tumors (Falchetti, 2017; Lemos & Thakker, 2008; Marini et al., 2009; Thakker, 2014; Thakker et al., 2012). MEN1 syndrome is highly penetrant with half of patients developing signs and symptoms by 20 years of age (Marini et al., 2009).

The gene that causes the disorder, *MEN1* , was first identified in 1997 and since then more than 1800 mutations have been characterized (Falchetti, 2017). However, up to 10% of MEN1 patients will not have mutations in the coding region or adjacent splice sites of *MEN1* (Falchetti, 2017; Falchetti et al., 2009). Other genes such as *AIP, CDKN1B, CDKN2B, CDKN2C,* and *CDKN1A* have been related to MEN1 (Arnold, 2019).

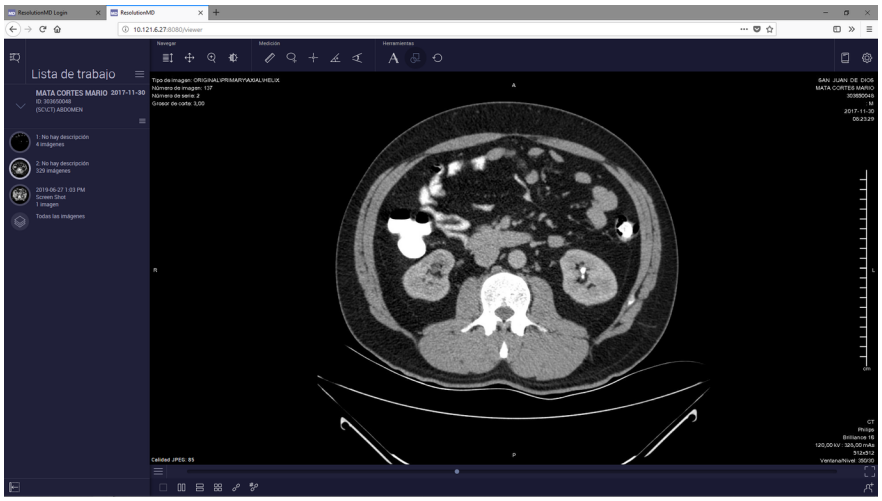
The diagnosis of MEN1 is made on the presence of two or more primary MEN1 tumor types (parathyroid, pancreatic, and pituitary adenomas); the occurrence of one of the MEN1-associated tumors in family members of a patient with a diagnosis of MEN1; or if a positive germline mutation in the *MEN1* gene is present (Thakker, 2014; Thakker et al., 2012). Here, we describe the first Costa Rican MEN1 case, molecularly confirmed, with a novel *MEN1* mutation, in a male patient with primary hyperparathyroidism (PHPT) and insulinoma.

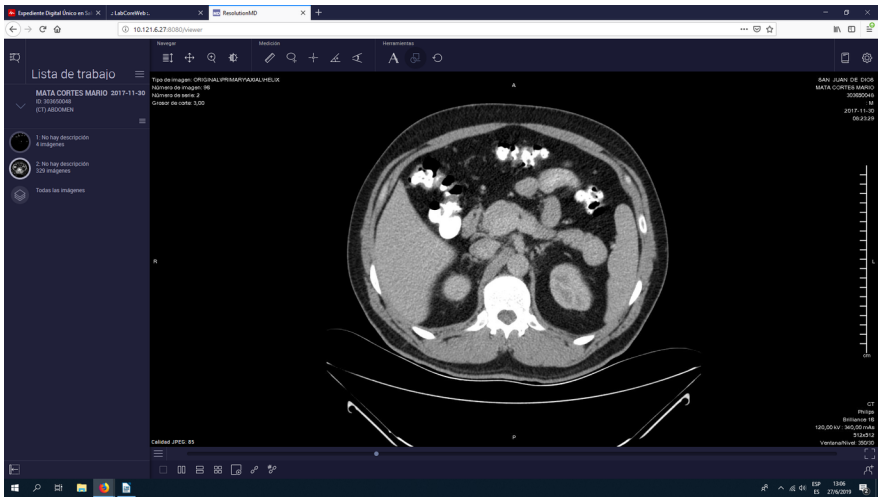
Case Report

In November 2017, a 37-year-old male with medical history of recurrent nephrolithiasis was referred to the endocrinology department at San Juan de Dios Hospital, C.C.S.S., in San José, Costa Rica. The patient had a 1-year history of recurrent episodes of diaphoresis and distal tremor in fasting states that resolved with eating. Over time these episodes became more frequent and symptomatic associating difficulty in waking up, depressive symptoms, and paresthesia in extremities.

On physical examination we describe a male with a body mass index of 31.4 Kg/m^2. He had symmetrical neck without palpable masses or adenopathies. His abdomen was soft and non-tender and there were no palpable masses. In the dorsal region, the patient presented two lipomas of approximately 2x2 cm and 1x1 cm. Neurologic examination was normal.

Initial laboratories revealed a fasting serum glucose of 38 mg/dL, serum calcium level of 11.3 mg/dL, and a serum intact parathyroid hormone (PTH) level of 69.5 pg/mL (normal range, 11-67 pg/mL) consistent with hypoglycemia and PHPT. All serum hypophysiary hormones levels were within the normal range. Gastrin could not be measured as it was not available at the hospital's laboratory. A fasting test was done. At three hours we documented a serum glucose of 47 mg/dL, serum insulin of 243 pmol/L (normal range, 43.3-170.4 pmol/L) and serum C- peptide of 6.58 ng/mL (normal range, 0.90-7.10 ng/mL) compatible with the diagnosis of insulinoma. Medical treatment with diazoxide and frequent feedings was initiated.





An abdominal CT scan showed one slightly hyperdense solid focal lesion of 28x27 mm localized in the pancreatic tail and bilateral nephrolithiasis (see figure 1).

B)

Figure 1. CT scan of abdomen. A) Solid lesion located in pancreatic tail (red arrowhead). B) Left nephrolithiasis.

Surgery was attempted and intraoperative direct pancreatic ultrasound revealed a single homogeneous mass of 25x20 mm in the pancreatic tail. A distal pancreatectomy plus splenectomy due to bleeding of the splenic vessels was done. The microscopic study of the specimens showed multiple nodules. Some of them revealed positivity for insulin and some for glucagon. Immunohistochemical studies, revealed positivity for cytokeratin 8-18, synaptophysin, chromogranin, E-cadherin, and a Ki-67 proliferation index <1%. Beta-catenin was negative in the nuclei and positive in membranes and cytokeratin 7 was negative. Those findings were consistent with multifocal neuroendocrine tumors.

Approximately 36 hours after the surgery, the patient presented an episode of hypoglycemia of 39 mg/dL. For this reason, medical treatment with frequent feedings and diazoxide was continued. The patient presented post-surgical complications with a pancreatic fistula, ascites, bilateral pleural effusion and acute renal insufficiency. Once he was stabilized the patient was discharged with medical treatment. However, a subsequent hospitalization was required for persistent hypoglycemic symptoms. During the two-month follow up CT scans and an endoscopic US (EUS) were done. Since no residual neuroendocrine tumor could be demonstrated it was decided, in conjunction with the patient, to continue with medical management and not perform surgical reintervention due to the high morbidity and mortality it represented.

Genomic DNA sequence analysis and deletion/duplication testing from peripheral blood leukocytes was done in a clinical commercial laboratory as part of a patient initiative. Direct sequence analysis of *MEN1* revealed heterozygosity for a novel pathogenic 4-bp insertion: c.1224_1225insGTCC(p.Cys409Valfs*41)

Molecular analysis of the variant showed that while this is not anticipated to result in nonsense mediated mRNA decay, it is expected to disrupt the last 202 amino acids of the MEN1 protein which result in truncation of the protein and its Nuclear Localization Signal (NLS) domains (see figure 2). This variant is not present in population databases (ExAC no frequency) and has not been reported in the literature in individuals with MEN1-related disease. ClinVar database contains an entry for this variant (Variation ID: 428066). Literature analysis showed that several different truncations located downstream of this variant (p.Arg516Profs*15, p.Arg516Glyfs*43 and p.Gln554*) have been determined to be pathogenic (Cardinal et al., 2005; Langer et al., 2001; Lemmens et al., 1997; Lemos & Thakker, 2008). This suggests that deletion

of this region of the MEN1 protein is causative of the disease.

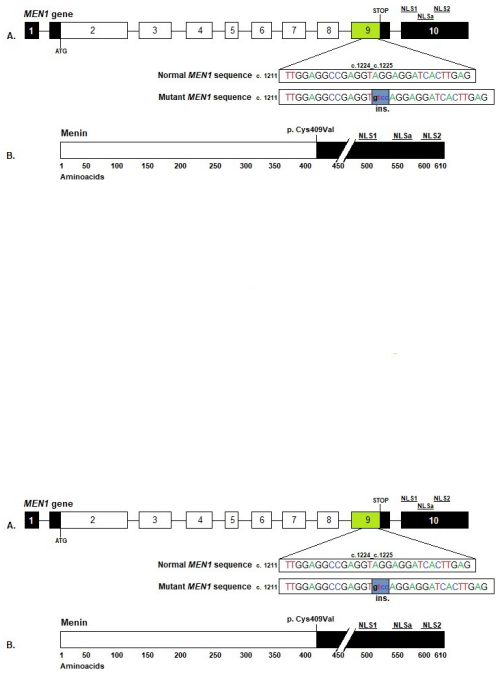


Figure 2. **A)** Schematic representation of the *MEN1* gene mutation c.1224_1225insGTCC. White and green boxes are translated exons. Dark boxes are untranslated regions which include NLS1, NLSa, NLS2 domains. **B)** Schematic representation of the mutant menin with the p.Cys409Valfs*41 variation. White box represents the amino acids of the menin protein that are transcribed. Dark boxes represent amino acids that are disrupted. Diagonal lines represent the stop signal at position 450 which results in truncation of the protein including the NLS1, NLSa, and NLS2 domains. Ins: insertion. NLS: Nuclear localization signal. Modified from: (Marini et al., 2009)

Discussion

The estimated prevalence of MEN 1 is between 1-10 per 100,000 (Jensen & Norton, 2017). MEN 1 syndrome was first described in 1903 by Erdheim in a report of an autopsy of a patient with acromegaly and enlarged parathyroid glands (Marini et al., 2009). In 1988, the *MEN1* locus was mapped to chromosome 11q13, and by 1997 *MEN1* mutations were confirmed to cause MEN1 (Chandrasekharappa et al., 1997; Jensen, Berna, Bingham, & Norton, 2008; Lemos & Thakker, 2008; Marini et al., 2009). To our knowledge this is the first confirmed case of a Costa Rican patient with a novel pathogenic *MEN1* mutation.

MEN1 is located on chromosome 11q13 and consists of 10 exons which contain a coding region of 1.83 kb organized into nine exons that encode a 610-amino acid protein called menin (Falchetti, 2017; Jensen et al., 2008; Jensen & Norton, 2017; Lemos & Thakker, 2008; Marini et al., 2009; Norton, Krampitz, & Jensen, 2015; Thakker, 2014; Thakker et al., 2012). Menin is an ubiquitous protein that has at least three nuclear

localization signals (NLS1, NLSa, and NLS2) at its C terminus, at amino acids 479-497, 546-572, and 588-608, respectively (Falchetti, 2017; Falchetti et al., 2009; Jensen et al., 2008; Lemos & Thakker, 2008; Marini et al., 2009; Thakker, 2014). These regions, rich in positively charged residues, have been reported to directly bind to double-stranded DNA which is thought to be necessary to target menin into the nucleus (Falchetti et al., 2009; Lemos & Thakker, 2008; Thakker, 2014). Menin interacts with several proteins involved in DNA transcriptional regulation, genome stability, cell division, cell proliferation, and epigenetic regulation (Jensen et al., 2008; Lemos & Thakker, 2008; Norton et al., 2015; Thakker, 2014).

Inheritance of a germline *MEN1* mutation (familial cases) or a*MEN1* mutation developed in an early embryonic stage (sporadic cases) predisposes an individual to develop tumors (Marini et al., 2009). *MEN1* is a tumor suppressor gene that follows the Knudson´s two hit hypothesis (Falchetti et al., 2009; Marini et al., 2009; Thakker et al., 2012).

MEN1 mutations can be identified in 70-95% of MEN1 patients (Lemos & Thakker, 2008; Marini et al., 2009). Of these mutations, 41% are frameshift insertions or deletions, 23% are nonsense mutations, 20% are missense mutations, 6% are in-frame insertions or deletions, 9% are splice site mutations and 1% large deletions[3]. Most frameshift and nonsense mutations are predicted to result either in a truncated protein or in loss of the translated protein because of nonsense-mediated mRNA decay (Lemos & Thakker, 2008). Our *MEN1* mutation causes a premature translational stop signal downstream to the 450 codon level and disrupts the last 202 amino acids which results in truncation of the functionally conserved NLS domains of menin 1 gene.

Skin and subcutaneous tumors may arise in 90% of MEN1 patients (Jensen & Norton, 2017). Approximately 88% of MEN1 patients present angiofibromas, 72% collagenomas, and 34% lipomas (Norton et al., 2015). The presence of cutaneous tumors may be helpful in the clinical presymptomatic diagnosis of MEN1 patients, as often they appear before any clinical manifestations of MEN1-associated hormone-secreting tumors (Marini et al., 2009). Our patient on clinical examination had two palpable lipomas.

PHPT affects 95% of patients and presents as the first endocrine MEN1 manifestation in 90-100% of cases(Marini et al., 2009; Norton et al., 2015; Thakker, 2014). PHPT presents typically between 20 and 25 years of age with 100% of penetrance by the age of 50 (Falchetti et al., 2009; Marini et al., 2009; Norton et al., 2015; Thakker, 2014). This is consistent with our case given that he presented PHPT as his first manifestation.

Insulinomas arise in about 10-30% of MEN1 patients and are the second most frequent functioning pancreatic islet tumor after gastrinomas (Marini et al., 2009; Norton et al., 2015; Thakker et al., 2012). Insulinomas usually occur in the third decade of life (Jensen et al., 2008; Marini et al., 2009; Norton et al., 2015; Thakker et al., 2012). The most reliable test for the diagnosis is a supervised 72h fast, during which an increased concentration of plasma insulin in association with hypoglycemia is demonstrated, along with an elevated C-peptide and proinsulin concentrations (Marini et al., 2009; Norton et al., 2015; Thakker et al., 2012). In our case, the diagnosis was made with a fasting test positive at 3 hours when the patient was 37 years old.

Insulinomas usually present as multiple lesions which are generally small (<2cm), benign, and distributed uniformly throughout the whole pancreas (Marini et al., 2009; Norton et al., 2015). The multiplicity makes it difficult to define which tumor is secreting the excessive insulin, nonetheless the majority of MEN1 patients typically have a dominant insulinoma predominantly found in the body or tail of the pancreas (Norton et al., 2015). In our case, the patient's recurrence of hypoglycemic episodes after surgery and the multiplicity of the nodules in histological studies suggests the presence of residual lesions in the remaining pancreas.

Insulinomas in MEN1 patients are almost invariably treated surgically (Jensen et al., 2008; Marini et al., 2009; Norton et al., 2015; Thakker et al., 2012). Diazoxide combined with frequent feedings is can also be used to control the hypoglycemia (Jensen et al., 2008; Norton et al., 2015). However, like in our case, approximately 40-50% of the patients do not respond adequately to this treatment

(Jensen et al., 2008). Long-acting somatostatin analogues such as octreotide or lanreotide are an alternative

treatment (Jensen et al., 2008). Nevertheless, they are effective in only 40-50% of patients(Jensen et al., 2008).

Anterior pituitary tumors occur in 15-90% of MEN1 patients (Marini et al., 2009; Thakker et al., 2012). Approximately 60% secrete prolactin, fewer than 25% secrete GH, 5% secrete ACTH, and the remainder appear to be non-functioning(Marini et al., 2009). Our patient presented serum hypophysiary hormones within the normal range.

Individuals in which there is a high suspicion of clinical MEN1 or those with familial MEN1, should be offered genetic counseling and *MEN1* mutation testing as early genetic diagnosis of *MEN1* is likely to reduce the morbidity and mortality related to the syndrome (Falchetti, 2017; Falchetti et al., 2009; Marini et al., 2009; Thakker et al., 2012). The identification of a germline *MEN1* mutation should prompt entry into a periodic clinical, biochemical, and radiological screening program (Thakker et al., 2012).

To our knowledge this is the first report of a molecularly confirmed case of MEN1 in our country and is the first report of the c.1224_1225insGTCC mutation related to a clinically affected patient.

Author contribution

All authors had equal contribution in conceptualization, writing, reviewing, and editing the document.

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This paper is used on an preprint server:

https://www.authorea.com/users/323618/articles/452222-novel-mutation-c-1224_1225insgtcc-p-cys409valfs-41-of-men1-gene-in-a-multiple-endocrine-neoplasia-type-1-case-with-insulinoma-and-primary-hyperparathyroidism-first-report-of-a-costa-rican-case?commit=bc88a3c18f7cca19219d1defc27e5fd535658736

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