

# Impact of a novel 14 bp *MEN1* deletion in a patient with hyperparathyroidism and gastrinoma

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## Summary

Multiple endocrine neoplasia type 1 (MEN-1) is a rare autosomal-dominant disease characterized by tumors in endocrine and/or non endocrine organs due to mutations in *MEN1* encoding a nuclear scaffold protein 'menin' involved in regulation of different cellular activities. We report a novel 14 bp *MEN1* deletion mutation in a 35-year-old female with history of recurrent epigastric pain, vomiting, loose stools and weight loss. On evaluation she was diagnosed to have multifocal gastro-duodenal gastrinoma with paraduodenal lymph nodes and solitary liver metastasis. She was also found to have primary hyperparathyroidism with bilateral inferior parathyroid adenoma. Pancreatico-duodenectomy with truncalvagotomy was performed. Four months later, radiofrequency ablation (RFA) of segment 4 of the liver was done followed by three and a half parathyroidectomy. *MEN1* screening was carried out for the patient and her family members. *MEN1* sequencing in the patient revealed a heterozygous 14 bp exon 8 deletion. Evaluation for pathogenicity and protein structure prediction showed that the mutation led to a frameshift thereby causing premature termination resulting in a truncated protein. To conclude, a novel pathogenic *MEN1* deletion mutation affecting its function was identified in a patient with hyperparathyroidism and gastrinoma. The report highlights the clinical consequences of the novel mutation and its impact on the structure and function of the protein. It also provides evidence for co-existence of pancreatic and duodenal gastrinomas in MEN1 syndrome. *MEN1* testing provides important clues regarding etiology and therefore should be essentially undertaken in asymptomatic first degree relatives who could be potential carriers of the disease.

## Learning points:

- Identification of a novel pathogenic *MEN1* deletion mutation.
- *MEN1* mutation screening in patients with pituitary, parathyroid and pancreatic tumors, and their first degree relatives gives important clues about the etiology.
- Pancreatic and duodenal gastrinomas may co-exist simultaneously in MEN1 syndrome.

## Background

Multiple endocrine neoplasia type 1 (MEN-1) is a rare autosomal-dominant disease characterized by the combined manifestations of tumors in the pancreas, parathyroid and pituitary glands. In addition, other

tumors like facial angiofibromas, adrenal cortical, carcinoid, collagenomas and lipomas have been reported in patients with MEN1 (1)(2). Hyperparathyroidism occurs as the first and foremost manifestation in 90% of the cases,

followed by gastroenteropancreatic (GEP) tumors including gastrinomas and insulinomas. Gastrinomas, involving over secretion of gastrin, are the most frequent type of GEP tumors occurring in ~40% of the cases (3)(4).

They arise sporadically or in association with MEN-1 syndrome caused by *MEN1* mutations which is a tumor suppressor gene encoding a nuclear protein Menin (4).

We report here a novel 14 bp *MEN1* deletion mutation in a female patient with gastro-duodenal gastrinoma with liver metastasis and primary hyperparathyroidism.

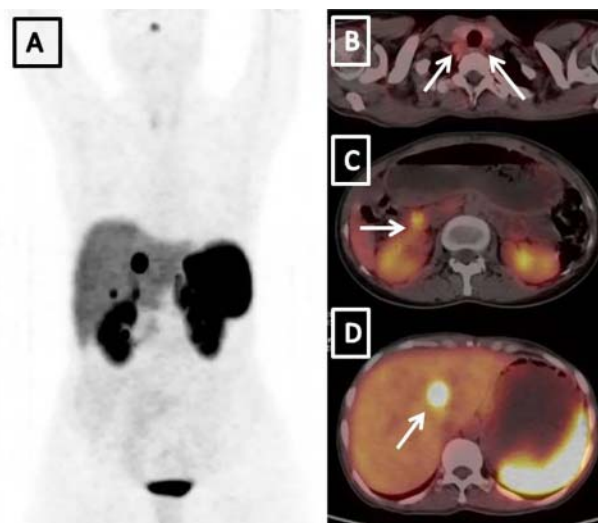
## Case presentation

A 35-year-old female presented with a 5-year history of recurrent epigastric pain, vomiting for the past 6 months, loose stools and weight loss for the past 3 months. She had been taking pantoprazole and ranitidine for 5 years but there was no improvement in her epigastric pain. At this stage, she was referred to our hospital for further assessment.

Her family history was negative. Physical examination was unremarkable and on investigation, she was found to have normal renal and hepatic functions except for hypercalcemia (tested twice 10.8 and 9.2 mg/dl (normal range=8.1–10.4 mg/dl) and hypophosphatemia (2 and 2.1 mg/dl (normal range=2.5–4.5 mg/dl)). Gastrointestinal endoscopy revealed thickened fundus and body of the stomach with edematous folds and multiple ulcers in the D1 and D2 segment of the duodenum. Computerized tomography (CT) scan showed mild diffuse mural thickening along posterior wall of the body and antrum of stomach. Further evaluations were carried out in view of raised calcium levels and ulcers extending into the second part of the duodenum. These investigations revealed elevated levels of serum gastrin –3043 pg/ml (normal range=0–100 pg/ml), 24 h urine calcium –330 mg/dl (normal range ≤200 mg/dl for her body weight) and serum intact parathyroid hormone (PTH) –630 pg/ml (normal range=15–65 pg/ml).

Endoscopic ultrasound revealed presence of two hyperechoic lesions in the pancreatic head measuring 15×15 and 6×6 mm respectively. <sup>68</sup>Ga-DOTANOC PET-CT was done for functional assessment of the lesions which showed multiple primary malignant somatostatin receptors expressing lesions in the gastric antrum, second part of duodenum, paraduodenal lymph node and solitary liver metastasis to segment 4 (Fig. 1).

Ultrasound examination of the neck revealed well defined hypoechoic masses indicative of parathyroid



**Figure 1**

Ga-68 Dotanoc PET/CT (A) maximum intensity projection images (B) transaxial fused PET/CT image at the level of thyroid showing mild tracer accumulation in bilateral parathyroid adenomas (arrow) (C) transaxial fused PET/CT image showing focal accumulation in the second part of duodenum (arrow) and (D) transaxial fused PET/CT images with focal tracer accumulation corresponding to the hepatic metastasis (arrow).

adenoma in the inferior pole of both right and left lobes of the thyroid gland.

In view of increased levels of PTH, parathyroid imaging was done using technetium-99m sestamibi (MIBI) scan which showed bilateral inferior parathyroid adenoma.

Targeted evaluation of hyperprolactinemia revealed that she had a history of galactorrhea but the serum prolactin levels, MRI Sella and visual field charting were normal.

She was screened for other components of MEN 1 (lipomas, angiofibromas, collagenomas) and the final diagnosis was multifocal gastro-duodenal gastrinoma with paraduodenal lymph node involvement with solitary liver metastasis. She also proved to have primary hyperparathyroidism with bilateral inferior parathyroid adenoma.

Based on the previous diagnosis, Whipple pancreaticoduodenectomy with truncalvagotomy was performed in which a tumor measuring 2 cm was removed from head of the pancreas, multiple lymph nodes were removed from mesocolon and from near pancreatic head.

Histopathological examination of multiple sections from grey white nodules of pancreas and duodenum revealed the cells to be positive for chromogranin and synaptophysin, showing features compatible with neuroendocrine tumors. Sections examined from the rest of the pancreas also showed foci of similar tumors whereas

Normal cDNA sequence	AGCTTGCTGGAGGCgggcgaggagcggcCGGGGGAGCAAAGCCAGGGCACCCAGAGCCAA
	14 bp deletion
Mutant cDNA sequence	AGCTTGCTGGAGGC*****CGGGGGAGCAAAGCCAGGGCACCCAGAGCCAA

**Figure 2**

Heterozygous deletion of 14 bp in exon 8 disrupting codon 385, leading to frameshift downstream and creating STOP codon at 403 position.

sections from proximal, distal resected ends and from gall bladder were free of tumors. On serial sectioning, three lymph nodes were identified, two of which showed tumor metastasis.

### Genetic screening

Detailed family history and pedigree information was collected. Peripheral blood sample was drawn in EDTA for molecular investigations after taking informed consent from the patient and available family members which included her two children, mother and sister. Genomic DNA was extracted using standard salting out protocol and subjected to PCR amplification of the *MEN-1* gene using 100 ng DNA, 2.5 mM MgCl<sub>2</sub>, 0.30 mM of each of the dNTPs (Invitrogen), 20 pM of each primer and 0.4 units of Taq Polymerase (Invitrogen) in a 25 µl volume mixture using thermocycler ABI 9700 (Applied Biosystems).

### Sequencing and mutation analysis

All the amplified products were purified using Qiagen Kits (Qiagen, GmbH), sequenced using BigDye Terminator Mix version 3.1 (Applied Biosystems) and analyzed on an ABI-3100 Genetic Analyzer (Applied Biosystems, Foster City,

CA, USA). Nucleotide sequences were compared with the published cDNA sequences of *MEN-1* (GenBank accession number ENSG00000133895) gene.

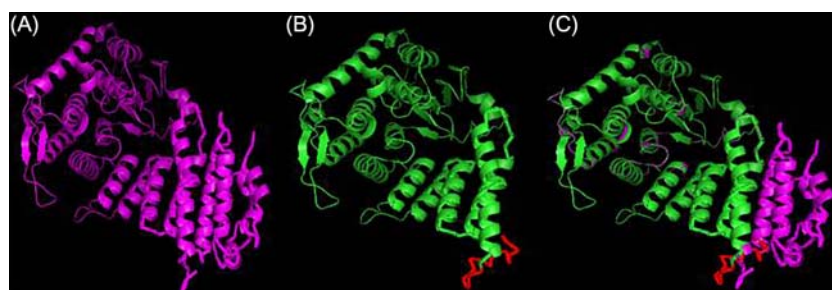
### Investigation

Direct sequencing of the *MEN-1* gene in the patient revealed a heterozygous 14 bp deletion mutation in exon 8 (Fig. 2). Evaluation for pathogenicity using MutationT@ster was in concordance with the structural analysis (5) which showed that the mutation leads to frameshift thereby causing premature termination of mRNA resulting into a truncated protein product (Fig. 3). Screening the family members revealed absence of the mutation in them.

### Treatment

The patient underwent Whipple pancreatico-duodenectomy with lymph node dissection. Four months later she underwent radiofrequency ablation (RFA) of segment 4 of liver and three, and a half parathyroidectomy after another 4 months.

Post surgery she developed severe hypocalcemia and was managed on high calcium and vitamin D. She was also



**Figure 3**

Showing structural analysis of the novel 14 bp deletion *MEN1* mutation. (A) Normal *MEN1* protein. (B) Mutant *MEN1* protein with premature truncation. The deletion causing frameshift leading to altered amino acid

sequence (shown in red C). The superimposed structural analysis of the normal and the truncated protein.

screened for any recurrent liver metastasis or hepatobiliary obstruction which was normal and the <sup>68</sup>Ga-DOTANOC PET-CT scan was also normal.

### Outcome and follow-up

To date she is on regular follow up, is on calcium and vitamin D replacement and asymptomatic.

### Discussion

We report here a novel 14 bp deletion *MEN1* mutation in a patient with primary hyperparathyroidism and gastroduodenal gastrinoma with liver metastasis.

Diagnosis of MEN1 syndrome is made if a patient has two or more MEN1-associated tumors, or if an individual has one MEN1-associated tumor and is a first-degree relative of a patient with MEN1. A diagnosis of MEN1 is also made when an individual has a germline *MEN1* mutation but is asymptomatic i.e. has not yet developed the clinical or biochemical manifestations of MEN1 tumors (6)(7).

*MEN1* (OMIM# 613733) located on chromosome 11q13.1 encodes a nuclear scaffold protein known as menin which plays a crucial role in many regulatory mechanisms involved in different cellular activities (8).

Menin protein, at the carboxyterminal region, has three main nuclear localization signals (NLSs) which are rich in positively charged amino acids. This highly charged region mediates the binding of menin protein to the DNA of interest and regulates the downstream physiological activities. Thus presence of NLS regions is necessary for the menin protein to interact and control transcription of various genes like induction of caspase 8 expression, down regulation of insulin-like growth factor binding protein-2 (IGFBP-2) gene, directing the menin protein into the nucleus, etc. (2)(10)(11).

Several missense, nonsense, splice site, deletions or regulatory region *MEN1* mutations have been reported to date for causing MEN1 syndrome but no significant correlation has been established between genotype and phenotype (9).

The heterozygous 14 bp exon 8 *MEN1* deletion mutation identified in the present study has not been described previously. The deletion leads to a frameshift downstream from amino acid position 385 and creates a premature stop codon at amino acid position 403. This results in a truncated menin protein lacking the three important NLS regions (Figs 2 and 3). To corroborate the findings from genetic screening and bioinformatics analysis, 100 chromosomes from healthy individuals

**Table 1** Clinical details of the patient.

S. no.	Clinical characteristics	Values
1	Age at presentation/sex	35 years/female
2	Family history	Negative
3	Duration of disease (years)	5 years
4	Serum gastrin (pg/ml)	3043
5	Tumour location	Pancreas and duodenum
6	Surgery performed	Pancreaticoduodenectomy
7	Number of tumors	Multiple
8	Metastasis	Lymph nodes and Liver

were screened to check for the presence of the same 14 bp deletion mutation. The change was not identified in any of them eliminating its possibility of being a polymorphism. This is in concordance with the results of the *in-silico* analysis which predicted the sequence change to be pathogenic resulting in the disease phenotype.

Hyperplasia of parathyroid gland leading to hypercalcemia has been consistently reported as the first manifestation of MEN1 syndrome but at the same time it has been documented that no obvious symptoms are experienced by some patients until later in life when left untreated. Similarly in the present study, the patient did not complain of any symptoms due to hyperparathyroidism. The chief complaint at presentation was epigastric pain which on evaluation was confirmed to be due to presence of gastrinoma. The duration between the onset of disease symptoms and clinical diagnosis was 5 years.

Gastrinomas are a heterogenous group of tumors with a difference in their primary location as they can be present at duodenum, pancreas and other sites; also they show variations in their clinical presentation as some tumors might be more aggressive and may metastasize to the regional lymph nodes and liver (12)(13)(14).

Differences in the findings have been observed regarding location of gastrinoma as some reports document that gastrinoma in patients with MEN1 syndrome is located mostly in duodenum whereas other studies report that pancreatic gastrinoma may exist simultaneously along with duodenal gastrinoma (15)(16) (Table 1).

The clinical features of MEN1 syndrome seen in our patient were consistent with the defined phenotype except for the presence of duodenal gastrinoma co-existing with pancreatic gastrinoma.

### Conclusion

In conclusion, we identified a novel pathogenic 14 bp deletion *MEN1* mutation in a patient with hyperparathyroidism and gastrinoma. The deletion was predicted to

result in a major defect at the C-terminal end of the protein affecting its normal functioning. The report highlights the clinical consequences due to presence of the novel deletion mutation and its structural-functional impact on the menin protein.

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**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 patient.

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**Author contribution statement**

S Birla carried out molecular genetic studies, literature search, data analysis and manuscript preparation. V P Jyotsna was involved in clinical management, manuscript preparation and editing. R Singla and M Tripathi were involved in clinical diagnosis and management. A Sharma supervised the genetic studies and was involved in data analysis, manuscript preparation and editing.

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