Case Series

An Indian family of multiple endocrine neoplasia type 1 (MEN1): molecular diagnosis, treatment and follow up

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Background/Objective: Multiple endocrine neoplasia type 1 (MEN1) is an autosomal, dominant syndrome, characterized mainly by the combination of tumors involving the parathyroid, pancreatic and pituitary glands. Genetic sequencing leading to early treatment of family members has not yet been reported in Indian patients.

Methods: We performed molecular analysis of the MEN1 gene to identify mutations in an Indian family with MEN1 syndrome. The proband was identified with multiple peptic ulcers because of multifocal recurrent gastrinomas, as well as parathyroid and pituitary adenomas. All the 10 exons of the MEN1 gene were amplified using the polymerase chain reaction (PCR). The MEN1 gene was then screened by direct DNA sequencing.

Results: The proband is asymptomatic 3 years after total pancreatectomy and removal of parathyroid adenomas. DNA sequencing revealed the presence of a heterozygous Y227X mutation in exon 4 of the MEN1 gene in the proband. Four of the seven mutant-carrying family members are at present asymptomatic. Following screening, one asymptomatic child has been identified with and treated for insulinoma and parathyroid adenoma.

Conclusion: Detection of the MEN1 gene mutation enables selection of family members for screening and long-term follow up.

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal, dominant hereditary cancer syndrome characterized by varying combinations of tumors involving the parathyroid, pancreatic and pituitary glands.1 The disease has a 95% degree of penetrance, indicating 95% probability of the mutant gene exerting its phenotypic effects in the offspring.2 MEN1 is a rare condition occurring in 1 in every 10,000 individuals.3 However, the incidence of MEN1 in the Asian population is unknown.4 To date, no genetic studies have been reported in family studies of MEN1 in the Indian population, though we have earlier reported a rare mutation of the MEN1 gene in a patient with multiple gastrinomas.5

In the present study, we performed MEN1 gene analysis in a MEN1 family of Indian origin.

Methods

Index patient
The proband (II2) consisted of a 35-year-old man admitted with a large duodenal ulcer (size 3 cm), and multiple ulcers in both the duodenum and jejunum. He had been symptomatic for 8 months but improved while on proton pump inhibitors; relapse occurred when treatment was withdrawn. His serum gastrin was >1000 pg/mL, serum calcium 11.2 mg/dL (normal 10.8 mg/dL) and serum parathormone 172 pg/mL (normal 12–72 pg/mL). Contrast-enhanced CT scan showed a lesion in the neck of the pancreas. MRI of the brain showed a small pituitary microadenoma with elevated prolactin levels. The patient underwent a parathyroidectomy and enucleation of the gastrinoma. He was asymptomatic, with normal gastrin levels for 6 months and thereafter presented with elevated gastrin levels and was detected to have multifocal gastrinomas in the head, body and tail of the pancreas with lymph node metastasis. After counseling, he underwent total pancreatectoduodenectomy. He is well on a 3-year follow-up, and is on insulin and pancreatic enzyme supplementation.

Family history
The patient’s elder brother (II1) died of a pituitary adenoma, which was operated elsewhere. His sister (II3) has...
been diagnosed with renal calculi with hypercalcemia, and has undergone a parathyroid adenoma excision.

His parents, his three children, the eldest of whom is 13 years old, and his sister’s two children were asymptomatic at the time of his surgery (Figure 1). A fourth child was born to him a year after his total pancreatectomy.

Controls
The controls for this study comprised 10 healthy individuals >40 years of age with normal serum calcium levels and no family history of cancer.

Mutation analysis
Blood was collected from family members after careful genetic counseling. The Hospital Ethics Committee approved this project. DNA was extracted from blood leucocytes using the modified procedure described by Miller et al. All the 10 exons, as well as the intronic regions at the exon–intron boundaries of the MEN1 gene, were amplified by the polymerase chain reaction (PCR), using genomic DNA, 200 mm dNTPs, 10 mm Tris, 50 mm KCl, 1.5 mm MgCl₂, 1.5 units of hot star Taq polymerase and 40 pmoles of the primer in a total volume of 50 mL. Thirty-five cycles of PCR with denaturation at 94 °C for 1 min, annealing for 50 sec and extension at 72 °C for 50 sec were carried out in the Eppendorf thermal cycler. The sequences of the primers and annealing temperatures used for various exons are given in the Table.

The PCR products of each exon of the MEN1 gene were subjected to bidirectional automated DNA sequencing. The sequences obtained were matched with the published MEN1 gene (Genbank accession No. U93237).

Results
Analysis of MEN1 gene in patient I
Sequence analysis revealed the presence of a heterozygote Y227X mutation in exon 4, leading to a change in the amino acid tyrosine to stop codon in menin protein (Figure 2b).

Family screen
The DNA sequencing of exon 4 of the patient’s family members showed the presence of the same mutation in 7 of 8 cases examined. The father did not carry the mutation; the mother was the carrier. Apart from the proband, the daughter, and 5 of 6 grandchildren were affected. Our results were confirmed by re-analyzing the EDTA blood samples of the proband and other family members, by direct DNA sequencing of exon 4.

Following these results, serum calcium levels were tested yearly from the gene carriers. The eldest child of the patient was detected to have raised serum calcium and insulin levels. Imaging revealed a 2-cm lesion in the tail of

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**Table. Primer sequences, annealing temperatures and PCR product sizes of various exons of MEN1 gene**

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pancreas. After enucleation of this lesion and removal of the parathyroid adenoma, his serum calcium and insulin levels were normal.

**Discussion**

The incidence of MEN1 in the Indian population is unknown and no published reports are available in the literature. The MEN1 gene sequence analysis revealed the presence of the Y227X mutation in exon 4. This mutation degrades the menin protein by ubiquitination, leading to non-functional truncated menin protein, which is consistent with the tumor suppressor mechanism of menin protein.7

Genetic testing of the patient’s family members at risk of MEN1 syndrome was crucial for diagnosing and identifying individuals carrying mutant alleles. With our screening strategy we were able to establish the presence of MEN1 at an early stage of disease. Individuals identified as mutant carriers are to be monitored clinically and biochemically and, if required, radiologically for the development of tumors. This will help in detecting the tumor at a preclinical stage so that early medical or surgical treatment can be initiated, thus reducing the rate of morbidity and mortality, as was the case with the patient’s son. On the other hand, DNA test showing absence of the mutation in the MEN1 gene will exclude the family member from further screening investigations, thus saving costs and offering psychological relief as there is certainty that the MEN1 syndrome will not develop.8

In conclusion, the present study shows the importance and usefulness of genetic diagnosis in MEN1 syndrome. Testing was shown to improve the quality of diagnosis and treatment in the proband and his family members.

**References**


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**Figure 2:** Sequence analysis of the Y227X mutation. The DNA sequence analysis shows the presence of a variation from TAC to TAG at codon 227 (arrow), leading to a change in the amino acid tyrosine to stop codon in the menin protein.