MEN1 gene mutations in 12 MEN1 families and their associated tumors

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Abstract

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant inherited tumor syndrome characterized by the development of multiple endocrine tumors. The gene responsible for the disease, termed MEN1 gene, has recently been isolated and germline mutations have been described in affected MEN1 individuals. Twelve unrelated German MEN1 families and their associated tumors (5 parathyroid tumors, 1 vipoma, 1 gastrinoma, 1 insulinoma) were characterized for MEN1 gene mutations by single-strand conformational variant (SSCV) analysis and DNA sequence analysis as well as for loss of heterozygosity on chromosome 11q13. We identified nine different heterozygous germline mutations (6 frameshift, 2 missense, 1 nonsense), eight of them were novel. Four of five informative MEN1-associated tumors revealed deletion of the second MEN1 allele, supporting the concept of a tumor suppressor gene. Furthermore, SSCV analysis proved an effective and sensitive method for the detection of menin mutations providing a reliable genetic screening approach supporting genetic counseling and clinical management of MEN1 family members.

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Introduction

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by the occurrence of tumors of the parathyroid glands, the pancreatic islet cells, and the anterior pituitary gland. Other manifestations include thyroid and adrenal adenomas, carcinoids of the foregut, lipomas and ependymomas of the spinal cord (1). Genetic linkage analysis and recombination studies have assigned the MEN1 gene to a minimal interval on chromosome 11q13 (2, 3) and very recently, the responsible gene, termed MEN1, was identified by positional cloning (4). Combined loss of heterozygosity (LOH) and pedigree studies support a tumor suppressor function of the gene. The MEN1 gene consists of 10 exons and encodes the 610 amino acid protein, menin, primarily localized to the nucleus (5). However, so far the sequence offered no clue to the function of the protein, and the precise role of menin remains to be understood. Heterozygous inactivating mutations of the MEN1 gene are scattered throughout the coding region in approximately 85% of investigated MEN1 families (4, 6–9). Thus far, no correlation between genotype and phenotype could be established.

Therefore, we characterized the spectrum of MEN1 gene mutations and of LOH of 11q13 in 19 MEN1 patients and 8 associated tumors from 12 unrelated families to evaluate the correlation between phenotype and genotype, as well as to support the hypothesis of a tumor suppressor gene. Furthermore, single-strand conformational variant (SSCV) analysis prior to DNA sequence analysis was evaluated for detection of MEN1 gene mutations to facilitate the predictive diagnosis of MEN1 individuals.

Patients and methods

Peripheral blood from 19 affected patients of 12 unrelated MEN1 kindreds was obtained after informed consent. MEN1 was defined as endocrine tumor in two of the three principal MEN1-related tissues; familial MEN1 was defined as MEN1 plus at least one first degree relative with a biochemical and/or histologically proven MEN1 endocrinopathy. Additionally, 8 tumors (5 parathyroid tumors, 1 vipoma, 1 gastrinoma, 1 insulinoma) of 6 MEN1 patients were available for LOH analysis of 11q13 sequences.

Genomic DNA from peripheral blood and tumor tissues was extracted with a commercial kit (QiaAmp, Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Blood and tumor DNA were evaluated for MEN1 gene mutations by SSCV analysis and direct
sequencing of the variants (10). In brief, primers encompassing the entire coding region in 15 separate PCR amplifications were used (7). Thirty cycles of 94°C for 30 s, annealing at 55–60°C for 30 s and extension at 72°C for 30 s were performed in a thermal cycler. PCR products were labeled by incorporation of [33P-α]dCTP. Amplification products were electrophoresed through 0.5 MDE gels (Serdogel, Boehringer Ingelheim, Heidelberg, Germany) with and without 5% glycerol at room temperature for 12 h at 6 W. Variant bands were excised from the gel, reamplified, and directly sequenced manually using the SequiTherm kit (Perkin Elmer, Norwalk, CT, USA). Samples exhibiting band shifts were reassayed with separate PCR products. All sequencing results were confirmed by repeat PCR amplification and sequencing on both strands. Mutations of families F2, F5, F8, F10, and F14 were also confirmed by restriction digestion analysis with HpaII, SmaI, BalI, EAgI and AvaII respectively.

DNA from peripheral blood and tumor samples was screened for LOH using three polymorphic markers on 11q13 as described previously (11). The markers included the intragenic microsatellite marker D11S4946 as well as PYGM and D11S4945 located proximal of the MEN1 gene. PCR products were electrophoresed through 6% polyacrylamide gels and visualized by autoradiography. LOH was defined as visible absence of an allele or a >50% reduction in the signal for one allele in the tumor compared with matching blood DNA.

Results

We identified 9 germline mutations in the MEN1 gene in 12 MEN1 families by SSCV analysis and direct sequencing of the variants. SSCV analysis revealed specific migration shifts in 10 of 12 families. Representative autoradiographs showing SSC variants indicative for particular base changes are presented in Fig. 1 (A,C,E,G). Sequence analysis of the conformational variants identified 6 frameshift, 2 missense, 1 nonsense mutations localized in exons 2, 3, 4, 5, 9 and 10. All mutations were heterozygous, and 8 mutations have not been described so far. Both missense mutations, L168P in family F5 and E26K in family F6, cosegregated with the disease and were not identified in 100 normal controls indicating predisposing mutations rather than rare polymorphisms. Additionally, two presumably benign polymorphisms, R171Q (CGG/CAG) and D418D (GAC/GAT) were encountered. The identified mutations and polymorphisms are summarized in Table 1. Representative examples of sequence alterations are presented in Fig. 1 (B,D,F,H). All 8 tumors (5 parathyroid adenomas, 1 vipoma, 1 gastrinoma and 1 insulinoma)
investigated demonstrated the identical mutation as detected in the corresponding germline DNA in one MEN1 allele. None of the tumors carried additional sequence changes. However, 4 of 5 informative tumors showed reduced intensity of at least one locus on 11q13 indicating inactivation of the second allele through LOH (Fig. 2). No significant correlation between genotype and phenotype could be identified (see Table 1).

### Discussion

This study confirms and extends the finding of the high prevalence of MEN1 germline mutations in patients with MEN1 (4, 6–9). We identified 9 different heterozygous mutations in 12 unrelated MEN1 families encompassing 3 deletions, 3 insertions, 2 missense and 1 nonsense mutations. Eight of these mutations have

![Figure 2](image)

**Figure 2** Examples of LOH of 11q13 sequences in MEN1-associated tumors. (A) The upper allele of marker D11S4945 (arrow) is lost in the parathyroid tumor of patient F6III2. (B) The intensity of the lower allele (arrow) of marker D11S4946 is significantly reduced in the parathyroid tumor (TH) and only slightly reduced in the gastrinoma (TG) as compared with the blood DNA of patient F2II1. Clear LOH in the gastrinoma might be masked by contamination of the tumor tissue with non-neoplastic tissue. (C) The upper allele (arrow) of the marker PYGM is highly reduced in the vipoma of patient F5II1. N, blood DNA; T, tumor DNA.

<table>
<thead>
<tr>
<th>Family identification number</th>
<th>Exon</th>
<th>Codon*</th>
<th>Base change</th>
<th>Restriction enzyme†</th>
<th>Family associated tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>F2</td>
<td>2</td>
<td>55 fs 64aaX</td>
<td>CCT → CCGG</td>
<td>Hpall</td>
<td>HPT, P, G</td>
</tr>
<tr>
<td>F6</td>
<td>2</td>
<td>E26K</td>
<td>GAG → AAG</td>
<td>–</td>
<td>HPT, P, NFP</td>
</tr>
<tr>
<td>F15</td>
<td>2</td>
<td>66 fs 50aaX</td>
<td>AGG → AAC</td>
<td>–</td>
<td>HPT, P, C of lung, NFP</td>
</tr>
<tr>
<td>F4</td>
<td>3</td>
<td>R171Q#</td>
<td>CCG → CAG</td>
<td>–</td>
<td>HPT, P</td>
</tr>
<tr>
<td>F5</td>
<td>3</td>
<td>L168P</td>
<td>CTG → CCG</td>
<td>Smal</td>
<td>HPT, P, Vip, G</td>
</tr>
<tr>
<td>F13</td>
<td>4</td>
<td>236 fs 12aaX</td>
<td>1 bp ins, GTG → GTTG</td>
<td>–</td>
<td>HPT, P, I</td>
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<tr>
<td>F14</td>
<td>5</td>
<td>T268X</td>
<td>TAT → TAG</td>
<td>Avall</td>
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</tr>
<tr>
<td>F8</td>
<td>9</td>
<td>437 fs 15aaX</td>
<td>1 bp del, GCC → CC</td>
<td>Ball</td>
<td>HPT, P, G, Glu, C of thymus</td>
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<tr>
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<td>10</td>
<td>510 fs 19aaX</td>
<td>2 bp del, GCA → G</td>
<td>–</td>
<td>HPT, P, I, C of thymus</td>
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<tr>
<td>F10</td>
<td>10</td>
<td>493 fs 65aaX</td>
<td>CGG → GG</td>
<td>EAGI</td>
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<tr>
<td>F3</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>F7</td>
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<td>–</td>
<td>–</td>
<td>–</td>
<td>HPT, P, I</td>
</tr>
</tbody>
</table>

* Codon designation according to GenBank U93236; fs, frameshift, which is followed by the number of amino acids (aa) and the stop codon X; † confirmed by restriction enzyme analysis; †, all exons were sequenced directly in addition to SSCV; ‡, polymorphism; polymorphism D418D is not listed, since it occurred in 42% (8/19) of tested probands; HPT, primary hyperparathyroidism; P, prolactinoma; G, gastrinoma; I, insulinoma; C, carcinoid; Vip, vipoma; Glu, glucagonoma; NFP, non-functioning endocrine pancreatic tumor; del. deletion; ins, insertion.
MEN1 gene mutations

not been described previously (Table 1). The mutations were distributed throughout the translated exons 2, 3, 4, 5, 9 and 10, and did not correlate with the phenotypes observed in the MEN1 patients. We cannot confirm the hypothesis of exon 2 being a mutational hot spot carrying more than 50% of identified mutations, since in our series only 30% of mutations were localized in this region (4, 6–9). The majority of mutations (7/9) were nonsense mutations, frameshift deletions or insertions, which are likely to result in a functional loss of the menin protein and are thus in keeping with the proposed role of the MEN1 gene as a tumor suppressor. According to the two-hit model of Knudson (12) it is believed that inherited loss of the menin protein or its function and somatic loss of the remaining allele are the key events of endocrine tumorigenesis. The data presented here provide further evidence for this theory, since 4 of 5 informative tumors of MEN1 patients revealed mutation of one allele and LOH of the MEN1 gene locus in the second allele. Furthermore, it has been shown by others that a significant proportion of sporadic MEN1-related tumors such as parathyroid adenomas, endocrine pancreatic tumors and pituitary tumors carry somatic deletions of one copy of the MEN1 gene and a specific mutation of the remaining allele (13–16). Definitive confirmation could not identify MEN1 mutations in MEN1 families direct DNA sequence analysis of the coding region did not detect any mutation. One possible explanation may be that these families are not linked to the MEN1 locus 11q13, as was described in one kindred by Stock and coworkers (17). More likely, these MEN1 patients may harbor mutations in the 5′ and 3′ untranslated regions, introns, or promoter regions of the MEN1 gene, which escaped our screening procedure. The methods used here also might miss a large germline deletion of more than one exon in extent, as only the normal copy of the respective MEN1 exon would be amplified by PCR.

Although the function of the menin protein is still unknown, the MEN1 gene now provides access to the molecular–genetic basis for the clinical management of MEN1 patients and their families. Previous genetic testing for preclinical MEN1 was based on variable numbers of short tandem repeats (VNTRs and STRs) flanking the MEN1 gene locus (18). In contrast to indirect linkage analyses, direct mutation analysis of the MEN1 gene can now be performed in single affected individuals. After genetic counseling family members can be screened for the particular mutation by SSCV, ASO (allele specific oligohybridization) or restriction enzyme analysis. Exclusion of family members from carrying the mutated MEN1 gene can lead to relief from their anxiety of having inherited the disease and exclusion from unnecessary clinical screening tests. On the other hand, attention and resources can be focused on the mutant gene carriers who have a very high risk for developing the disease. These patients should be encountered in a clinical screening program that will enable earlier detection, and effective treatment of MEN1-associated tumors.

Acknowledgements

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References


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