Menin mutations in patients with multiple endocrine neoplasia type 1

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Abstract
Multiple endocrine neoplasia type 1 (MEN-1) is a familial cancer syndrome with parathyroid, pituitary and enteropancreatic tumors. The disease phenotype segregates with markers on chromosome 11q13. Very recently a new gene was cloned from this region and was found to carry mutations in 14 of 15 unrelated MEN-1 patients. The gene was termed menin and is predicted to code for a tumor suppressor protein of 610 amino acids, but its precise function is totally unknown. To confirm this finding we used PCR from genomic DNA and direct sequencing to analyze exons 2 through 10 of the menin gene in eight patients from four pedigrees with MEN-1 syndrome or an affected relative. We identified four different heterozygous mutations, three of them are novel: one nonsense mutation, one large deletion of 32 bp and two insertions, all of them located in exon 2. Our results confirm that patients with MEN-1 carry mutations in the menin gene.

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Introduction
Multiple endocrine neoplasia type I (MEN-1) is an autosomal dominant disease with familial segregation of tumors arising from the parathyroid, pituitary or endocrine pancreas and duodenum. Other manifestations include thyroid and adrenal adenomas, angiomyolipomas, lipomas, carcinoids of the foregut and ependymomas of the spinal cord. The responsible gene has been assigned to a small interval on chromosome 11q13 (1) and the interval has been narrowed and physically mapped by several groups (2–5). Very recently a new gene termed menin has been cloned from this region and was found to carry missense and nonsense mutations as well as deletions in 14 of 15 unrelated MEN-1 families (6). Most pedigrees of MEN-1 families showed cosegregation with markers on 11q13 (7–11) but there is evidence that the locus at 11q13 may not be the only one involved in families with MEN-1 syndrome (12). Therefore additional data confirming the results of Chandrasekharappa et al. (6) that MEN-1 patients have menin mutations are required.

The function of the predicted menin protein is unknown but it is thought to be a tumor suppressor. The predicted amino acid sequence has no homologies to other proteins and sequence analysis failed to identify probable functional domains. Analysis of mutation types and localization with respect to phenotype might provide information about functional domains of the menin protein.

We analyzed seven patients from three families and one sporadic-disease patient with MEN-1 syndrome for mutations in the menin gene and describe their disease phenotype.

Patients and methods
Peripheral blood specimens of seven patients from three kindreds and one additional patient with a clinical MEN-1 syndrome were obtained (Table 1). Total DNA was extracted from 1 ml whole blood with a commercial kit (QiaAmp, Qiagen, Hilden, Germany) and was used for amplification of exons 2 through 10 of the human menin gene (6). Thirty cycles of 95 °C for 30 s, annealing for 15 s and extension at 72 °C were performed in a thermal cycler; initial denaturing and final extension lasted 5 min. Exons 3 and 4, 5 and 6, and 9 and 10 were amplified as single amplikons. Primers, annealing temperatures and extension times are shown in Table 2. Reaction mixtures contained 0.25 mg DNA, 0.5 ml Taq-polymerase (USB, Amersham-Buchler, Braunschweig, Germany), 0.5 ml dNTP 20 mM each (Pharmacia LKB, Freiburg, Germany), 0.5 pmol of each primer, 5 mM MgCl2 (25 mM) were added and thermal cycling was begun. The samples were analyzed on 2% agarose gels, the two bands of patient B1 were cut out of the gel, soaked in 20 ml H2O for several hours and 1 ml was used for reamplification.
After purification over affinity columns (QiaQuick, Qiagen) the samples were manually sequenced with ThermoSequenase (USB, Amersham-Buchler) according to the manufacturer’s instructions using \(^{33}P\)ddNTPs (Amersham-Buchler). Exons 2, 3 and 4, and 9 and 10 were sequenced from both ends using 1 pmol PCR primers, exons 9 and 10 also with 1 pmol internal primer MEN1·10F (Table 2), exons 5 and 6, 7 and 8 using 1 pmol reverse PCR primer.

For restriction analysis in families M and W a 6 ml purified PCR sample was combined with 0.7 ml 1 unit enzyme buffer and 3 units Hpa II or Dde I (Gibco-BRL, Eggenstein, Germany) and incubated overnight at 37°C.

### Results

We identified four new heterozygous mutations in the menin gene in three families and a sporadic-disease patient. In patient M1 a T to G mutation of base 275 and insertion of a G at position 276 creates a conservative base change at codon 55 and a frameshift that truncates the protein after 55 normal and 64 nonsense amino acids. This particular mutation creates an Hpa II site and restriction analysis shows that only M1 is affected; her two children are not (Figs 1 and 3).

The disease phenotype of M1 with four neoplasms typical of MEN-1 is severe in comparison to family B where the large deletion of 32 bases from base 416 to 447 (Figs 2 and 3) creates a frameshift with truncation of the protein after 102 normal and 2 nonsense amino acids. The only manifestation in the index patient B1 until the age of 72 has been an insulinoma. Her son who inherited the deletion is also oligosymptomatic with only parathyroid adenoma and hypergastrinemia without a detectable tumor. His two asymptomatic children are currently under clinical, biochemical and genetic investigation. The C402T mutation in family W creates a stop codon and a new Dde I restriction site (Figs 1 and 3) and truncates the protein after 97 amino acids. The phenotype with parathyroid adenoma and gastrinoma in the mother and son of family W is a classical MEN-1 constellation, as is the combination of pituitary and parathyroid adenoma in patient J who carries a 5 bp insertion at position 317 creating a frameshift with truncation after 69 normal and 51 nonsense amino acids.

### Discussion

Our results confirm the findings of Chandrasekharappa et al. (6) and recently published results (13, 14) that...

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### Table 1

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Patient Age Sex</th>
<th>Pituitary</th>
<th>Parathyroid</th>
<th>Enteropancreatic</th>
<th>Other</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family M</td>
<td>45 F</td>
<td>Prolactinoma</td>
<td>Parathyroid adenoma</td>
<td>Gastrinoma</td>
<td>Thyroid adenoma</td>
<td>T275G, 276insG (P55)</td>
</tr>
<tr>
<td>M2</td>
<td>26 M</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>WT</td>
</tr>
<tr>
<td>M3</td>
<td>22 F</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>WT</td>
</tr>
<tr>
<td>Family B</td>
<td>72 F</td>
<td>—</td>
<td>—</td>
<td>Insulinoma</td>
<td>—</td>
<td>416del32 (D102)</td>
</tr>
<tr>
<td>B2</td>
<td>50 M</td>
<td>—</td>
<td>Parathyroid adenoma</td>
<td>Hypergastrinemia</td>
<td>—</td>
<td>416del32 (D102)</td>
</tr>
<tr>
<td>Family W</td>
<td>57 F</td>
<td>Parathyroid adenoma</td>
<td>—</td>
<td>Gastrinoma</td>
<td>—</td>
<td>C402T (R98X)</td>
</tr>
<tr>
<td>W2</td>
<td>34 M</td>
<td>Parathyroid adenoma</td>
<td>Gastrinoma</td>
<td>—</td>
<td>C402T (R98X)</td>
<td></td>
</tr>
<tr>
<td>Sporadic</td>
<td>60 M</td>
<td>Pituitary adenoma</td>
<td>—</td>
<td>Hyperparathyroidism</td>
<td>—</td>
<td>317ins5 (P69)</td>
</tr>
</tbody>
</table>

WT, wild-type.

### Table 2

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Annealing (°C)</th>
<th>Extension time (s)</th>
</tr>
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<tbody>
<tr>
<td>2</td>
<td>GAACTGCCCAGACCTCC</td>
<td>ACACCTGCCCAGACCTCAAG</td>
<td>61</td>
<td>40</td>
</tr>
<tr>
<td>3 and 4</td>
<td>TGCCCTCTTCCCACATGTTA</td>
<td>CAGGGTCCCCAGACGAAGAATCAAG</td>
<td>61</td>
<td>40</td>
</tr>
<tr>
<td>5 and 6</td>
<td>CTTCTCCCTCCTGTCCTCC</td>
<td>GTTCTCCCTTCTGACCCCTCC</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>7</td>
<td>CTCCTGCTCCACCCCTCCACTCA</td>
<td>GGACAGGCGTAGCCCTACTAAT</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>8</td>
<td>GGGTGACGGCAGACAGCCTC</td>
<td>CCTGACATCCCTCAGCAAGTA</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>9 and 10</td>
<td>TGGAGCCAGGGGTCTTCTTGCCT</td>
<td>CCCCAACAGCGGATCGAGAT</td>
<td>63</td>
<td>60</td>
</tr>
<tr>
<td>MEN1 x 10F</td>
<td>CACCTTGCTCTCCCACCTGCC</td>
<td>—</td>
<td>63</td>
<td>—</td>
</tr>
</tbody>
</table>
patients with MEN-1 syndrome carry heterozygous germline mutations in the menin gene. Three of four mutations we describe are novel; the R98X mutation in family W has recently been found in another European kindred (14) but not in 50 North American kindreds (13), possibly indicating a European founder effect. Interestingly the deletion in family B starts at C416, the same nucleotide where five single base 416delC deletions were found in five families (13), but a deletion of 32 bases has not yet been described in the menin gene. This could be important for diagnostic strategies, because single-exon PCR-based assays can miss large deletions spanning intron–exon borders. The remaining two mutations starting at codons 55 and 69 are entirely novel. All mutations we found are located in exon 2 as are 40% of all mutations described until now (6, 13, 14). Even if this indicates that this region might be particularly prone to mutations, there seems to be no hot spot region that might predict distinct functional domains or allow diagnostic strategies to focus on parts of the gene.

Since mapping of the MEN-1 gene to 11q13 (1) it is believed that inherited loss of a tumor suppressor protein or its function and somatic loss of the remaining allele are the key events in this disease. This fits with the Knudson two-hit hypothesis (15) and therefore the menin gene is believed to be a tumor suppressor. This hypothesis has been supported by loss of heterozygosity analysis in MEN-1 tumors (16). In addition, the variety of different mutations with missense as well as nonsense and frameshift mutations, found in our study and others (6, 13, 14), suggests that loss of function is the molecular mechanism that leads to the disease. Definite confirmation of this hypothesis, however, must await studies on structure and function of the menin protein. Although there is no apparent genotype-phenotype correlation (13), functional studies may identify function-phenotype correlations or other proteins acting as disease modifiers.

References

3 Wood TF, Srivatsan ES, Chakraborti R, Ma GC, Kuan N, Samara GJ et al. A 1.5-megabase physical map encompassing the multiple


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