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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, angiofibromas, 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 μg DNA, 0.5 μl Taq-polymerase (USB, Amersham-Buchler, Braunschweig, Germany), 0.5 μl dNTP 20 mM each (Pharmacia LKB, Freiburg, Germany), 25 pmol of each primer, 5 μl 10 × buffer and H_2O to 47 μl. For all exons except exon 2 the magnesium-free buffer supplied with Taq-polymerase was used, for exon 2 the 10 × buffer contained 160 mM $(NH_4)_2SO_4$, 670 mM Tris–HCl pH 8.8 and 0.1% Tween-20. For exons 9 and 10, 2.5 μl dimethylsulfoxide were added. The samples were pre-heated to 72 °C, 3 μl $MgCl_2$ (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 μl H_2O for several hours and 1 μl was used for reamplification.

Table 1 Patients, phenotype and *menin* mutations (base designation from GenBank U93236, the first codon with a base change given in brackets).

	Patient	Age	Sex	Phenotype				Genotype
				Pituitary	Parathyroid	Enteropancreatic	Other	
Family M	M1	45	F	Prolactinoma	Parathyroid adenoma	Gastrinoma	Thyroid adenoma	T275G, 276insG (P55)
	M2	26	M	—	—	—	—	WT
	M3	22	F	—	—	—	—	WT
Family B	B1	72	F	—	—	Insulinoma	—	416del32 (D102)
	B2	50	M	—	Parathyroid adenoma	Hypergastrinemia	—	416del32 (D102)
Family W	W1	57	F	—	Parathyroid adenoma	Gastrinoma	—	C402T (R98X)
	W2	34	M	—	Parathyroid adenoma	Gastrinoma	—	C402T (R98X)
Sporadic	J	60	M	Pituitary adenoma	Hyperparathyroidism	—	—	317ins5 (P69)

WT, wild-type.

After purification over affinity columns (QiaQuick, Qiagen) the samples were manually sequenced with ThermoSequenase (USB, Amersham-Buchler) according to the manufacturer's instructions using [α-^{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 μl purified PCR sample was combined with 0.7 μl 10× 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

Table 2 Primers for PCR and sequencing.

	Forward primer	Reverse primer	Annealing (°C)	Extension time (s)
Exon 2	GAACCTGCCCGACCCTCC	ACACCTGCCGAACCTCACAAG	61	40
Exon 3 and 4	TGGCCCCTTTCCCCATGTTA	CAGGGTCCCACAGCAAGTCAAG	61	40
Exon 5 and 6	CGTTCTCCTCCCTGTTCCGT	GTCTCCCTTCTGCACCCTCC	60	20
Exon 7	CCTCTGCCTCACCTCCATCCA	GGACAGGCTGCAGGCCCTAGT	60	20
Exon 8	GGGCTGACCCAGACAGCATC	CCTGCCATCCCTAATCCCGTA	60	20
Exon 9 and 10	TGGAGCCAGGGGTCTTTGCCT	CCCCACAAGCGGTCCGAAGT	63	60
MEN1 × 10F	CACCTTGCTCTCCCCACTGGC	—	63	—

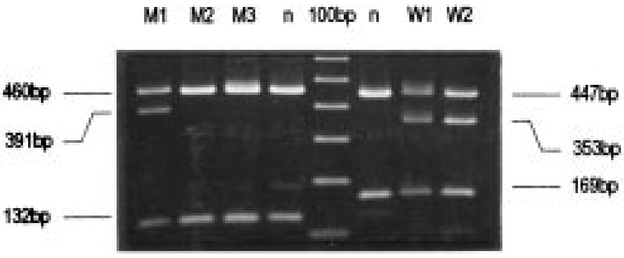

Figure 1 Restriction analysis of exon 2 in families M and W: patients M1, M2, M3 and a normal control (n) digested with Hpa II; 100 bp ladder (Pharmacia); a normal control (n) and samples from patients W1 and W2 digested with Dde I. Patients M1, W1 and W2 show a smaller fragment that results from the creation of a new restriction site by the mutation.

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.

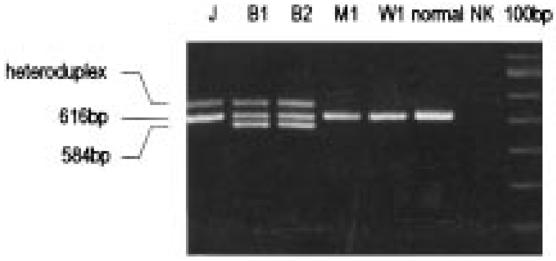

Figure 2 PCR samples of exon 2 separated on 2% agarose: patients J, B1, B2, M1, W1, normal control, PCR negative control (NK) and a 100 bp ladder (Pharmacia). Patients B1 and B2 show an additional smaller band that represents the allele with the 32 bp deletion, Patients J, B1 and B2 show a larger band that results from heteroduplex formation; the small insertions in patient J and M1 are not resolved.

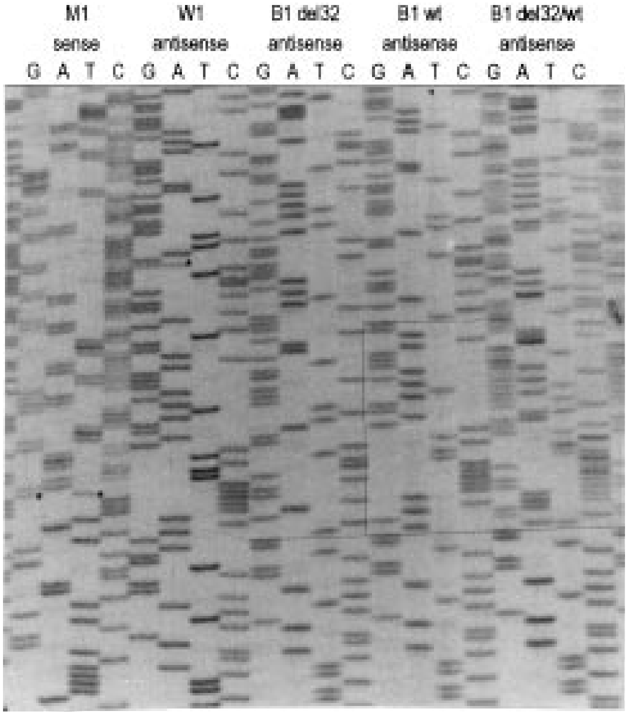

Figure 3 Direct sequencing of exon 2: Patient M1 (sense), W1(antisense), the mutated and wild-type (wt) alleles of patient B1 and his heterozygous genomic sample (antisense). Patient M1 shows a T to G and G insertion double mutation (asterisks), patient W1 a C to T mutation (asterisk) and patient B1 the deletion of 32 bases. The deletion point and the deleted bases are indicated by the faint, parallel, horizontal lines.

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