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Multiple endocrine neoplasia type 1

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Abstract

Combined clinical and laboratory investigations of multiple endocrine neoplasia type 1 (MEN1) have resulted in an increased understanding of this disorder which may be inherited as an autosomal dominant condition. Defining the features of each disease manifestation in MEN1 has improved patient management and treatment, and has also facilitated a screening protocol to be instituted. The application of the techniques of molecular biology has enabled the identification of the gene causing MEN1 and the detection of mutations in patients. The function of the protein encoded by the MEN1 gene has been shown to be in the regulation of JunD-mediated transcription but much still remains to be elucidated. However, these recent advances provide for the identification of mutant MEN1 gene carriers who are at a high risk of developing this disorder and thus require regular and biochemical screening to detect the development of endocrine tumours.

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Introduction

Multiple endocrine neoplasia (Thakker & Ponder 1988, Thakker 1995, Marx 1998) is characterised by the occurrence of tumours involving two or more endocrine glands within a single patient. The disorder has previously been referred to as multiple endocrine adenopathy (MEA) or the pluriglandular syndrome. However, glandular hyperplasia and malignancy may also occur in some patients and the term multiple endocrine neoplasia (MEN) is now preferred. There are two major forms of multiple endocrine neoplasia referred to as type 1 and type 2 and each form is characterised by the development of tumours within specific endocrine glands (Table 1). Thus, the combined occurrence of tumours of the parathyroid glands, the pancreatic islet cells and the anterior pituitary is characteristic of multiple endocrine neoplasia type 1 (MEN1), which is also referred to as Wermer's syndrome (Wermer 1954). In addition to these tumours, adrenal cortical, carcinoid, facial angiofibromas, collagenomas and lipomatous tumours have also been described in patients with MEN1 (Trump *et al.* 1996, Marx 1998). However, in multiple endocrine neoplasia type 2 (MEN2), which is also called Sipple's syndrome (Sipple 1961), medullary thyroid carcinoma (MTC) occurs in association with phaeochromocytoma, and three clinical variants, referred to as MEN2a, MEN2b and MTC-only, are recognised (Thakker & Ponder 1988, Thakker 1998). In MEN2a, which is the most common variant, the development of MTC is associated with phaeochromocytoma and parathyroid tumours. However, in MEN2b parathyroid involvement is absent and the occurrence of MTC and phaeochromocytoma is found in association with a marfanoid habitus, mucosal neuromas, medullated corneal fibres and intestinal autonomic ganglion dysfunction leading to a megacolon. In the variant of MTC-only, medullary thyroid carcinoma appears to be the sole manifestation of the syndrome. Although MEN1 and MEN2 usually occur as distinct and separate syndromes as outlined above, some patients occasionally may develop tumours which are associated with both MEN1 and MEN2. For example, patients suffering from islet cell tumours of the pancreas and phaeochromocytomas or from acromegaly and phaeochromocytoma have been described and these patients may represent 'overlap' syndromes. All these forms of MEN may either be inherited as autosomal dominant syndromes, or they may occur sporadically i.e. without a family history. However, this distinction between sporadic and familial cases may sometimes be difficult as in some sporadic the family history may be absent because the parent with the disease may have died before developing symptoms. In this review, the main clinical features, molecular genetics, and recent progress in the study of MEN1 will be discussed

Clinical features of MEN1

The incidence of MEN1 has been estimated from randomly chosen *post mortem* studies to be 0.25%, and to

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Table 1 The multiple endocrine neoplasia (MEN) syndromes, their characteristic tumours and associated genetic abnormalities

Type (chromosomal location)	**Tumours**	**Gene: most frequently** (%) **mutated codons**
MEN1	Parathyroids	MEN1:
(11q13)	Pancreatic islets	83/84, 4 bp del (≈6%)
	Gastrinoma	119, 3 bp del (≈2%)
	Insulinoma	209-211, 4 bp del (≈4%)
	Glucagonoma	514-516, del or ins (=7%)
	VIPoma	
	PPoma	
	Pituitary (anterior)	
	Prolactinoma	
	Somatotrophinoma	
	Corticotrophinoma	
	Non-functioning	
	Associated tumours	
	Adrenal cortical	
	Carcinoid	
	Lipoma	
	Angiofibromas	
	Collagenomas	
MEN2		
(10 cen-10q.11.2)		
MEN2a	MTC	ret: 634, missense e.g. Cys→Arg (≈85%)
	Phaeochromocytoma	
	Parathyroid	
MTC-only	MTC	ret: 618, missense (>50%)
MEN2b	MTC	ret: 918, Met →Thr (>95%)
	Phaeochromocytoma	
	Associated abnormalities	
	Mucosal neuromas	
	Marfanoid habitus	
	Medullated corneal nerve fibres	
	Megacolon	

Autosomal dominant inheritance of the MEN syndromes has been established.
Del, deletion, ins, insertion.
(Adapted from Thakker (1998), with permission)

be % amongst patients with primary hyperpara-thyroidism (Marx *et al.* 1982, Thakker 1995). The disorder affects all age groups, with a reported age range of 8-81 years, and >95% of patients have developed clinical manifestations of the disorder by the fifth decade (Thakker 1995, Trump *et al.* 1996, Bassett *et al.* 1998). The clinical

manifestations of MEN1 are related to the sites of tumours and to their products of secretion. In addition to the triad of parathyroid, pancreatic and pituitary tumours, which constitute the major components of MEN1, adrenal cortical, carcinoid, facial angiofibromas, collagenomas and lipomatous tumours have also been described (Trump *et al.* 1996, Marx 1998).

Parathyroid tumours

Primary hyperparathyroidism is the most common feature of MEN1 and occurs in more than 95% of all MEN1 patients (Benson *et al.* 1987, Marx *et al.* 1986,Thakker 1995, Trump *et al.* 1996). Patients may present with asymptomatic hypercalcaemia, or nephrolithiasis, or osteitis fibrosa cystica or vague symptoms associated with hypercalcaemia, for example polyuria, polydipsia, constipation, malaise or occasionally with peptic ulcers. Biochemical investigations reveal hypercalcaemia usually in association with raised circulating parathyroid hormone (PTH) concentrations. No effective medical treatment for primary hyperparathyroidism is generally available and surgical removal of the abnormally overactive parathyroids is the definitive treatment. However, all four parathyroid glands are usually affected with multiple adenomas or hyperplasia, although this histological distinction may be difficult, and total parathyroidectomy has been proposed as the definitive treatment for primary hyperparathyroidism in MEN1, with the resultant lifelong hypocalcaemia being treated with oral calcitriol (1,25-dihydroxy vitamin D_3) (Rizzoli *et al.* 1985). It is recommended that such total parathyroidectomy should be reserved for the symptomatic hypercalcaemic patient with MEN1, and that the asymptomatic hypercalcaemic MEN1 patient should not have parathyroid surgery but have regular assessments for the onset of symptoms and complications, when total parathyroidectomy should be undertaken.

Pancreatic tumours

The incidence of pancreatic islet cell tumours in MEN1 patients varies from 30 to 80% in different series (Thakker & Ponder 1988, Thakker 1995, Trump *et al.* 1996). The majority of these tumours produce excessive amounts of hormone, for example gastrin, insulin, glucagon or vasoactive intestinal polypeptide (VIP), and are associated with distinct clinical syndromes.

Gastrinomas

These gastrin-secreting tumours represent over 50% of all pancreatic islet cell tumours in MEN1, and are the major cause of morbidity and mortality in MEN1 patients. This is due to the recurrent severe multiple peptic ulcers which may perforate. This association of recurrent peptic ulceration, marked gastric acid production and non β-islet cell tumours of the pancreas is referred to as the Zollinger-Ellison syndrome (Zollinger & Ellison 1955). Additional prominent clinical features of this syndrome include diarrhoea and steatorrhoea. The diagnosis is established by demonstration of a raised fasting serum gastrin concentration in association with an increased basal gastric acid secretion (Wolfe & Jensen 1987). Medical treatment of MEN1 patients with the Zollinger-Ellison syndrome is directed at reducing basal acid output to less than 10 mmol/l, and this may be achieved by the parietal cell H^+-K^+-ATPase inhibitor, omeprazole. The ideal treatment for a non-metastatic gastrinoma is surgical excision of the gastrinoma. However, in patients with MEN1 the gastrinomas are frequently multiple or extra-pancreatic and surgery has not been successful (Delcore *et al.* 1989, Sheppard *et al.* 1989). The treatment of disseminated gastrinomas is difficult and hormonal therapy with octreotide, which is a human somatostatin analogue, chemotherapy with streptozotocin and 5-fluoroaracil, hepatic artery embolisation, and removal of all resectable tumour have all occasionally been successful (Thakker 1995).

Insulinoma

These β-islet cell tumours secreting insulin represent one-third of all pancreatic tumours in MEN1 patients (Thakker 1995, Trump *et al.* 1996). Insulinomas also occur in association with gastrinomas in 10% of MEN1 patients, and the two tumours may arise at different times. Patients with an insulinoma present with hypoglycaemic symptoms which develop after a fast or exertion and improve after glucose intake. Biochemical investigations reveal raised plasma insulin concentrations in association with hypoglycaemia. Circulating concentrations of C-peptide and proinsulin, which are also raised, may be useful in establishing the diagnosis, as may an insulin suppression test. Medical treatment, which consists of frequent carbohydrate feeds and diazoxide, is not always successful and surgery is often required. Most insulinomas are multiple and small and preoperative localisation with computed tomography scanning, coeliac axis angiography and pre-perioperative percutaneous transhepatic portal venous sampling is difficult and success rates have varied. Surgical treatment, which ranges from enucleation of a single tumour to a distal pancreatectomy or partial pancreatectomy, has been curative in some patients. Chemotherapy, which consists of streptozotocin or octreotide, is used for metastatic disease.

Glucagonoma

These α-islet cell, glucagon-secreting pancreatic tumours, have been reported in a few MEN1 patients (Thakker 1995, Trump *et al.* 1996, Bassett *et al.* 1997, Marx 1998).

The characteristic clinical manifestations of a skin rash (necrolytic migratory erythyema), weight loss, anaemia and stomatitis may be absent and the presence of the tumour is indicated only by glucose intolerance and hyperglucagonaemia. The tail of the pancreas is the most frequent site for glucagonomas and surgical removal of these is the treatment of choice. However, treatment may be difficult as 50% of patients have metastases at the time of diagnosis. Medical treatment of these with octreotide, or with streptozotocin has been successful in some patients.

VIPoma

Patients with VIPomas, which are vasoactive intestinal peptide (VIP)-secreting pancreatic tumours, develop watery diarrhoea, hypokalaemia and achlorhydria, referred to as the WDHA syndrome (Marx *et al.* 1967). This clinical syndrome has also been referred to as the Verner-Morrison syndrome (Verner & Morrison 1958) or the VIPoma syndrome (Bloom *et al.* 1973). VIPomas have been reported in only a few MEN1 patients and the diagnosis is established by documenting a markedly raised plasma VIP concentration (Thakker 1995). Surgical management of VIPomas, which are mostly located in the tail of the pancreas, has been curative. However, in patients with unresectable tumour, treatment with streptozotocin, octreotide, corticosteroids, indomethacin, metoclopramide and lithium carbonate has proved beneficial.

PPoma

These tumours, which secrete pancreatic polypeptide (PP) are found in a large number of patients with MEN1 (Friesen *et al.* 1980, Skogseid *et al.* 1987, Thakker 1995). No pathological sequelae of excessive PP secretion are apparent and the clinical significance of PP is unknown, although the use of serum PP measurements has been suggested for the detection of pancreatic tumours in MEN1 patients.

Pituitary tumours

The incidence of pituitary tumours in MEN1 patients varies from 15 to 90% in different series (Thakker 1995, Trump *et al.* 1996). Approximately 60% of MEN1-associated pituitary tumours secrete prolactin, 25% secrete growth hormone (GH), 3% secrete adenocorticotrophin (ACTH) and the remainder appear to be non-functioning. The clinical manifestations depend upon the size of the pituitary tumour and its product of secretion. Enlarging pituitary tumours may compress adjacent structures such as the optic chiasm or normal pituitary tissue and cause bitemporal hemianopia or hypopituitarism respectively. The tumour size and extension are radiologically assessed by computed tomography scanning and nuclear magnetic resonance imaging. Treatment of pituitary tumours in MEN1 patients is similar to that in non-MEN1 patients and consists of medical therapy or selective hypophysectomy by the transphenoidal approach if feasible, with radiotherapy being reserved for residual unresectable tumour.

Associated tumours

Patients with MEN1 may have tumours involving glands other than the parathyroids, pancreas and pituitary. Thus carcinoid, adrenal cortical, facial angiofibromas, collagenomas, thyroid and lipomatous tumours have been described in association with MEN1 (Thakker 1995, Trump *et al.* 1996, Marx 1998).

Carcinoid tumours

Carcinoid tumours which occur more frequently in patients with MEN1 may be inherited as an autosomal dominant trait in association with MEN1 (Duh *et al.* 1987). The carcinoid tumour may be located in the bronchi, the gastrointestinal tract, the pancreas, or the thymus. Most patients are asymptomatic and do not suffer from the flushing attacks and dyspnoea associated with the carcinoid syndrome, which usually develops after the tumour has metastasised to the liver.

Adrenal corticol tumours

The incidence of asymptomatic adrenal cortical tumours in MEN1 patients has been reported to be as high as 40% (Skogseid *et al.* 1992). The majority of these tumours are non-functioning. However, functioning adrenal cortical tumours in MEN1 patients have been documented to cause hypercortisolaemia and Cushing's syndrome, and primary hyperaldosteronism, as in Conn's syndrome (Thakker 1995, Trump *et al.* 1996).

Lipomas

Lipomas may occur in 20%-30% of patients (Darling *et al.* 1997, Marx 1998), and frequently they are multiple. In addition, pleural or retroperitoneal lipomas may also occur in patients with MEN1.

Thyroid tumours

Thyroid tumours, consisting of adenomas, colloid goitres and carcinomas have been reported to occur in over 25% of MEN1 patients (Thakker 1995, Marx 1998). However, the prevalence of thyroid disorders in the general population is high and it has been suggested that the association of thyroid abnormalities in MEN1 patients may be incidental and not significant.

Facial angiofibromas and collagenomas

Multiple facial angiofibromas, which are identical to those observed in patients with tuberous sclerosis, have been

observed in 88% of MEN1 patients (Darling *et al.* 1997, Marx 1998), and collagenomas have been reported in >70% of MEN1 patients (Darling *et al.* 1997, Marx 1998).

Molecular genetics of MEN1

Models of tumour development

The development of tumours may be associated with mutations or inappropriate expression of specific normal cellular genes, which are referred to as oncogenes (reviewed in Thakker & Ponder 1988, Thakker 1993, 1994 1995, Brown & Solomon 1997). Two types of oncogenes, referred to as dominant and recessive oncogenes, have been described. An activation of dominant oncogenes leads to transformation of the cells containing them, and examples of this are the chromosomal translocations associated with the occurrence of chronic myeloid leukaemia and Burkitt's lymphoma. In these conditions, the mutations which lead to activation of the oncogene are dominant at the cellular level, and therefore only one copy of the mutated gene is required for the phenotypic effect. Such dominantly acting oncogenes may be assayed in cell culture by first transferring them into recipient cells and then scoring the numbers of transformed colonies, and this is referred to as the transfection assay. However, in some inherited neoplasms which may also arise sporadically, such as retinoblastoma, tumour development is associated with two recessive mutations which inactivate oncogenes, and these are referred to as recessive oncogenes. In the inherited tumours, the first of the two recessive mutations is inherited via the germ cell line and is present in all the cells. This recessive mutation is not expressed until a second mutation, within a somatic cell, causes loss of the normal dominant allele. The mutations causing the inherited and sporadic tumours are similar but the cell types in which they occur are different. In the inherited tumours the first mutation occurs in the germ cell, whereas in the sporadic tumours both mutations occur in the somatic cell. Thus, the risk of tumour development in an individual who has not inherited the first germline mutation is much smaller, as both mutational events must coincide in the same somatic cell. In addition, the apparent paradox that the inherited cancer syndromes are due to recessive mutations but dominantly inherited at the level of the family is explained because, in individuals who have inherited the first recessive mutation, a loss of a single remaining wild type allele is almost certain to occur in at least one of the large number of cells in the target tissue. This cell will be detected because it forms a tumour, and almost all individuals who have inherited the germline mutation will express the disease, even though they inherited a single copy of the recessive gene. This model involving two (or more) mutations in the development of tumours is known as the 'two hit' or Knudson's hypothesis (Knudson 1971, 1993). The normal function of these recessive oncogenes appears to be in regulating cell

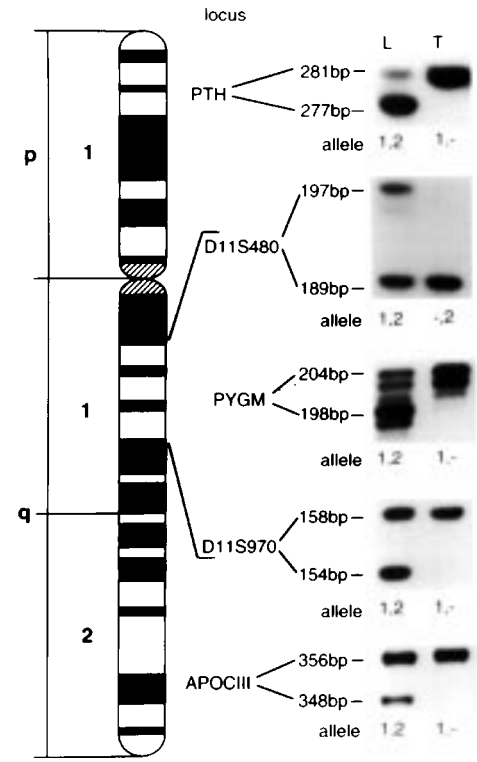

Figure 1 Loss of heterozygosity (LOH) involving polymorphic loci from chromosome 11 in a parathyroid tumour from a patient with familial MEN1. The microsatellite polymorphisms obtained from the patient's leucocyte (L) and parathyroid tumour (T) DNA at the PTH, D11S480, phosphorylase glycogen muscle (PYGM), D11S970 and apolipoprotein C-III (APOCIII) loci are shown. These microsatellite polymorphisms have been identified using specific primers for each of the loci which have been localised to chromosome 11, and are shown juxtaposed to their region of origin on the short (p) and long (q) arms of chromosome 11. The microsatellite polymorphisms are assigned alleles (see Fig. 2). For example D11S480 yielded a 197 bp product (allele 1) and a 189 bp product (allele 2) following PCR amplification of leucocyte DNA, but the tumour cells have lost the 197 bp product (allele 1) and are hemizygous (alleles -,2). Similar losses of alleles are detected using the other DNA markers, and an extensive loss of alleles involving the whole of chromosome 11 is observed in the parathyroid tumour of this patient with MEN1. In addition, the complete absence of bands suggests that this abnormality has occurred within all the tumour cells studied, and indicates a monoclonal origin for this MEN1 parathyroid tumour. (From Pang & Thakker (1994), with permission.)

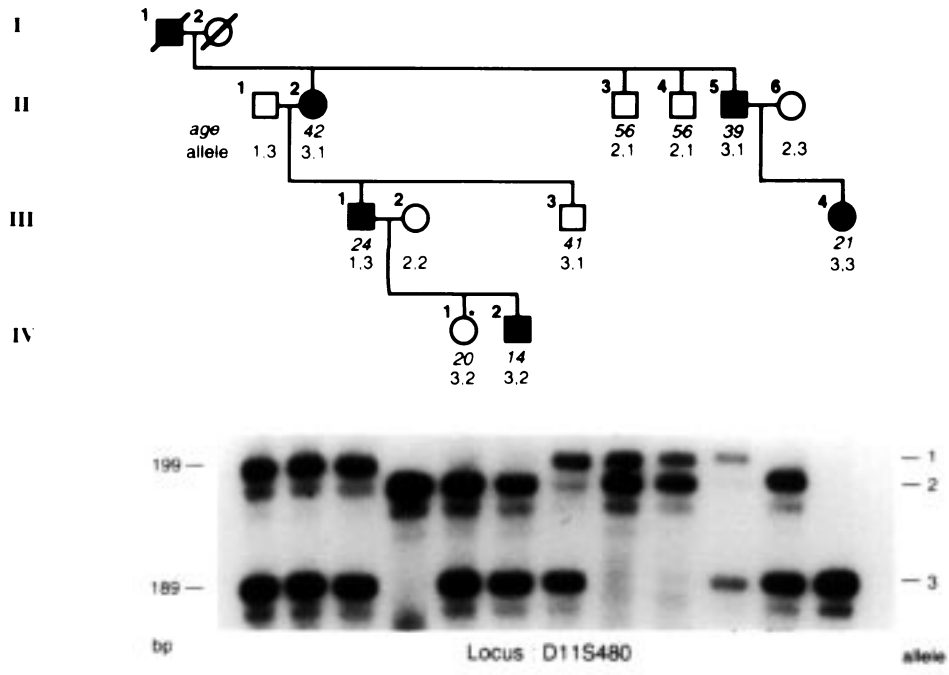

Figure 2 Segregation of D11S480, a polymorphic locus from chromosome 11q13, and MEN1 in a family. Genomic DNA from the family members (upper panel) was used with [γ-^{32}P]adenosine triphosphate (ATP) for PCR amplification of the polymorphic repetitive element $(CA)_n$ at this locus. The PCR amplification products were separated on a polyacrylamide gel and detected by autoradiography (lower panel); these ranged in size from 189 to 199 bp. Alleles were designated for each PCR product and are indicated on the right. For example, individuals II.1, II.2 and III.1 reveal 2 pairs of bands on autoradiography. The upper pair of bands is designated allele 1 and the lower pair of bands is designated allele 3; and these 3 individuals are therefore heterozygous (alleles 1, 3). A pair of bands for each allele is frequently observed in the PCR detection of microsatellite repeats. The upper band in the pair is the 'true' allele and the lower band in the pair is its associated 'shadow' which results from slipped-strand mispairing during the PCR. The segregation of these bands and their respective alleles together with the disease can be studied in the family members whose alleles and ages are shown. In some individuals, the inheritance of paternal and maternal alleles can be ascertained; the paternal allele is shown on the left. Individuals are represented as unaffected male (open square), affected male (filled square), unaffected female (open circle), and affected female (filled circle). Individual II.2 is affected and heterozygous (alleles 3, 1) and an examination of her affected child (III.1), grandchild (IV.2), sibling (II.5) and niece (III.4) reveals inheritance of allele 3 with the disease. The unaffected individuals II.3, II.4, and III.3 have not inherited this allele 3. However, the daughter (IV.1) of individual III.1 has inherited allele 3, but remains unaffected at the age of 20 years; this may either be a representation of age-related penetrance (see Fig. 5), or a recombination between the disease and D11S480 loci. Thus, in this family, the disease and D11S480 loci are co-segregating in 8 out of the 9 children, but in one individual (IV.1), assuming a 100% penetrance (see below) in early childhood, recombination is observed. Thus, MEN1 and D11S480 are co-segregating in 8/9 of the meioses and not segregating in 1/9 meioses, and the likelihood that the two loci are linked at θ=0.11, i.e. 11% recombination, is $(8/9)^8 \times (1/9)^1$. If the disease and the D11S480 loci were not linked, then the disease would be associated with allele 1 in one half (1/2) of the children and with allele 3 in the remaining half (1/2) of the children, and the likelihood that the two loci are not linked is $(1/2)^9$. Thus, the odds ratio in favour of linkage between the MEN1 and D11S480 loci at θ=0.11, i.e. 11% in this family, is therefore $(8/9)^8 \times (1/9)^1 \div (1/2)^9$=22.17:1, and the LOD score (i.e. $\log_{10}$ of the odds ratio favouring linkage)=1.34 (i.e. $\log_{10}$ 22.17). A LOD score of +3 which indicates a probability in favour of linkage of 1000:1 establishes linkage. LOD scores from individual families can also be summated, and such studies revealed that the peak LOD score between MEN1 and the DS11S480 locus was >+3 (Pang *et al.* 1996), thereby establishing linkage between MEN1 and D11S480 loci.

growth and differentiation, and these genes have also been referred to as anti-oncogenes or tumour suppressor genes. An important feature which has facilitated the investigation of these genetic abnormalities associated with tumour development is that the loss of the remaining allele (i.e. the 'second hit'), which occurs in the somatic cell and gives rise to the tumour, often involves a large scale loss of chromosomal material. This 'second hit' may be

detected by a comparison of the DNA sequence polymorphisms in the leucocytes and tumour obtained from a patient, and observing a loss of heterozygosity (LOH) in the tumour (Fig. 1).

Identification of the MEN1 gene

The gene causing MEN1 was localised to chromosome 11q13 by genetic mapping studies which investigated MEN1-associated tumours for LOH (Fig. 1) and by segregation studies in MEN1 families (Fig. 2) (Larsson *et al.* 1988, Friedman *et al.* 1989, Thakker *et al.* 1989, 1993, Byström *et al.* 1990). The results of these studies, which were consistent with Knudson's model for tumour development, indicated that the MEN1 gene represented a putative tumour suppressor gene. Further genetic mapping studies defined a <300 kb region as the minimal critical segment that contained the MEN1 gene and characterisation of genes from this region led to the identification, in 1997, of the MEN1 gene (The European Consortium on MEN1 1996, 1997, Chandrasekharappa *et al.* 1997, Debelenko *et al.* 1997*a*), which consists of 10 exons with a 1830 bp coding region (Fig. 3) that encodes a novel 610 amino acid protein, referred to as MENIN (Chandrasekharappa *et al.* 1997). Mutations of the MEN1 gene (Figs 3 and 4) have been identified and the total number of germline mutations of the MEN1 gene that have been identified by 21 studies (Agarwal *et al.* 1997, Debelenko *et al.* 1997*b*, Mayr *et al.* 1997, 1998, Shimizu *et al.* 1997, Toliat *et al.* 1997, Zhuang *et al.* 1997*a*, Bartsch *et al.* 1998, Bassett *et al.* 1998, Chico *et al.* 1998, Fujimori *et al.* 1998, Giraud *et al.* 1998, Kishi *et al.* 1998, Sakurai *et al.* 1998, Sato *et al.* 1998, Tanaka *et al.* 1998, Teh *et al.* 1998*a*,*b*,*c*, Gortz *et al.* 1999, Poncin *et al.* 1999) during the past 2 years in MEN1 patients is 262 (Table 2).

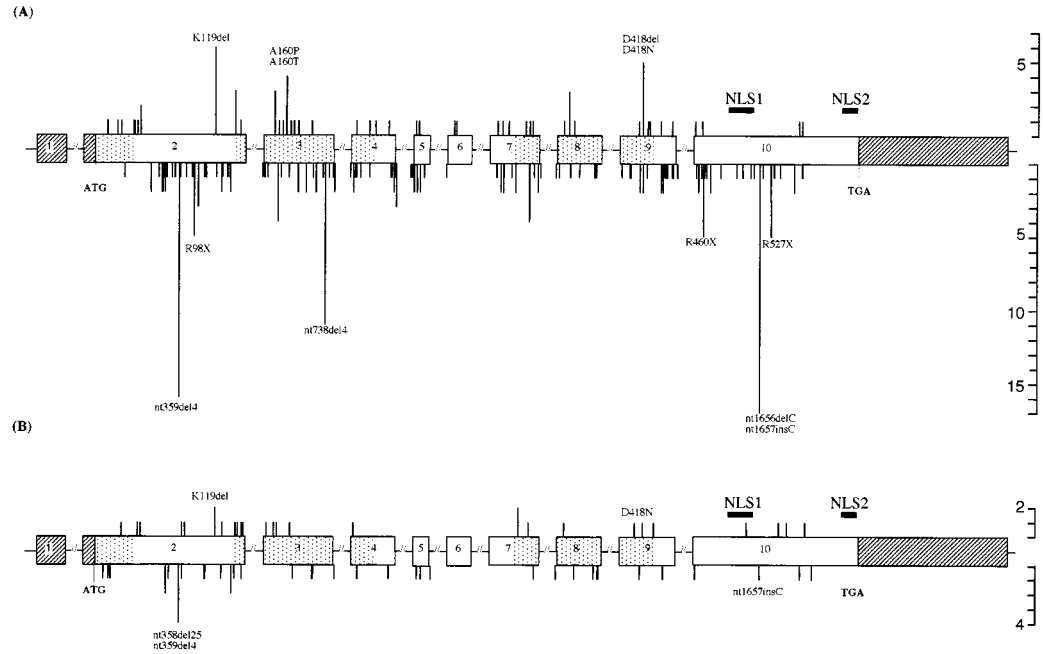

Figure 3 Schematic representation of the genomic organisation of the MEN1 gene illustrating germline (panel A) and somatic (panel B) mutations. The human MEN1 gene consists of 10 exons that span >9 kb of genomic DNA and encodes a 610 amino acid protein (Chandrasekharappa *et al.* 1997). The 1.83 kb coding region is organised into 9 exons (exons 2 to 10) and 8 introns (indicated by a line but not to scale). The sizes of the exons (boxes) range from 88 to 1312 bp and those of the introns range from 41 to 1564 bp. The start (ATG) and stop (TGA) sites in exons 2 and 10, respectively, are indicated. Exon 1, the 5' part of exon 2 and 3' part of exon 10 are untranslated (indicated by the hatched boxes). The locations of the two nuclear localisation sites (NLS), which are at codons 479 to 497, and 588 to 608 at the C-terminus, are represented by the thick horizontal lines, and the locations of the 3 domains, which are formed by codons 1 to 40 (exon 2), 139 to 242 (exons 2, 3 and 4) and 323 to 428 (exons 7, 8 and 9), that interact with JunD are indicated by the stippled boxes. The sites of the 262 germline mutations (panel A) and 67 somatic mutations (panel B) are indicated by the vertical lines; the missense and in-frame mutations are represented above the gene and the nonsense, frameshift and splice site mutations are represented below the gene. The detailed descriptions of these 329 mutations are given in Table 2. Mutations which have occurred more than 4 times (scale shown on the right) are indicated.

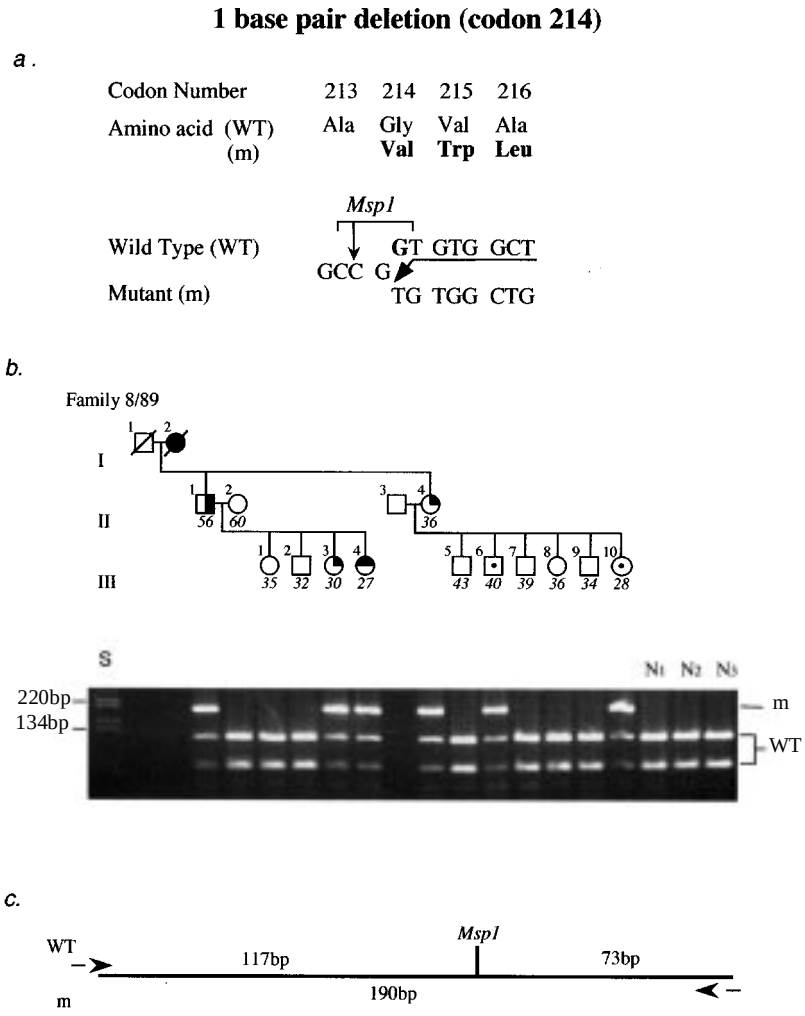

Figure 4 Detection of mutation in exon 3 in family 8/89 by restriction enzyme analysis. DNA sequence analysis of individual II.1 revealed a 1 bp deletion at the second position (GGT) of codon 214 (panel *a*). The deletion has caused a frameshift which continues to codon 223 before a stop codon (TGA) is encountered in the new frame. The 1 bp deletion results in the loss of an MspI restriction enzyme site (C/CGG) from the normal (wild type, WT) sequence (panel *a*) and this has facilitated the detection of this mutation in the other affected members (II.4, III.3, and III.4) of this family (panel *b*). The mutant (m) PCR product is 190 bp whereas the wild type (WT) products are 117 and 73 bp (panel *c*). The affected individuals were heterozygous, and the unaffected members were homozygous for the wild type sequence. Individuals III.6 and III.10, who are 40 and 28 years old respectively, are mutant gene carriers who are clinically and biochemically normal and this is due to the age-related penetrance of this disorder (see Fig. 5). Individuals are represented as: male (square); female (circle); unaffected (open); affected with parathyroid tumours (filled upper right quadrant), with gastrinoma (filled lower right quadrant), with prolactinoma (filled upper left quadrant); and unaffected mutant gene carriers (dot in the middle of the open symbol). Individual I.2 who is deceased but was known to be affected (tumour details not known) is shown as a filled symbol. The age is indicated below for each individual at diagnosis or at the time of the last biochemical screening. The standard size marker (S) in the form of the 1 kb ladder is indicated. Co-segregation of this mutation with MEN1 in family 8/89 and its absence in 110 alleles from 55 unrelated normal individuals (N_{1-3} shown) indicates that it is not a common DNA sequence polymorphism. (Adapted from Bassett *et al.* (1998), with permission.)

Approximately 22% are nonsense mutations, ≈48% are frameshift deletions or insertions, 8% are in-frame deletions or insertions, 5% are donor-splice site mutations and ≈17% are missense mutations. More than 10% of the MEN1 mutations arise *de novo* and may be transmitted to subsequent generations (Agarwal *et al.* 1997, Bassett *et al.* 1998, Teh *et al.* 1998*a*). It is also important to note that between 5% to 10% of MEN1 patients may not harbour mutations in the coding region of the MEN1 gene (Agarwal *et al.* 1997, Chandrasekharappa *et al.* 1997, The European Consortium on MEN1 1997, Bassett *et al.* 1998, Giraud *et al.* 1998, Teh *et al.* 1998*a*), and that these individuals may have mutations in the promoter or untranslated regions (UTRs), which remain to be investigated.

The majority (75%) of the MEN1 mutations are inactivating, and are consistent with those expected in a tumour suppressor gene. The mutations are not only diverse in their types but are also scattered (Fig. 3) throughout the 1830 bp coding region of the MEN1 gene with no evidence for clustering as observed in MEN2 (Table 1) (Gagel & Cotes 1996). However, some of the mutations have been observed to occur several times in unrelated families (Table 2 and Fig. 3), and the 4 deletional and insertional mutations involving codons 83 and 84, codon 119, codons 209 to 211, and codons 514 to 516, account for approximately 19% of all the germline MEN1 mutations, and thus these may represent potential 'hot' spots. Such deletional and insertional hot spots may be associated with DNA sequence repeats that may consist of long tracts of either single nucleotides or shorter elements, ranging from dinucleotides to octanucleotides. Indeed, the DNA sequence in the vicinity of codons 83 and 84 in exon 2, and codons 209 to 211 in exon 3, contains CT and CA dinucleotide repeats, respectively, flanking the 4 bp deletions (Table 2); these would be consistent with a replication-slippage model in which there is misalignment of the dinucleotide repeat during replica-tion, followed by excision of the 4 bp single-stranded loop (Bassett *et al.* 1998). A similar replication-slippage model may also be involved at codons 119 to 120 which both consist of AAG nucleotides encoding a lysine (K) residue (Table 2). The deletions and insertions of codon 516 involve a poly(C)7 tract, and a slipped-strand mispairing model is also the most likely mechanism to be associated with this mutational hot spot (Bassett *et al.* 1998). Thus, the MEN1 gene appears to contain DNA sequences that may render it susceptible to deletional and insertional mutations.

Correlations between the MEN1 mutations and the clinical manifestations of the disorder appear to be absent. For example, a detailed study of 5 unrelated families with the same 4 bp deletion in codons 210 and 211 (Table 3) revealed a wide range of MEN1-associated tumours (Bassett *et al.* 1998); all the affected family members had parathyroid tumours, but members of families 1, 3, 4 and 5 had gastrinomas whereas members of family 2 had insulinomas. In addition, prolactinomas occurred in members of families 2, 3, 4 and 5 but not members of family 1, which were affected with carcinoid tumours. The apparent lack of genotype-phenotype correlations, which contrasts with the situation in MEN2 (Table 1) (Gagel & Cotes 1996), together with the wide diversity of mutations in the 1830 bp coding region of the MEN1 gene, will make mutational analysis for diagnostic purposes in MEN1 time-consuming and expensive (Thakker 1998).

MEN1 mutations in sporadic non-MEN1 endocrine tumours

Parathyroid, pancreatic islet cell, and anterior pituitary tumours may occur either as part of MEN1 or, more commonly, as sporadic, non-familial, tumours. Tumours from MEN1 patients have been observed to harbour the germline mutation together with a somatic LOH involving chromosome 11q13 (Larsson *et al.* 1988, Friedman *et al.* 1989, Thakker *et al.* 1989, 1993, Byström *et al.* 1990), as expected from Knudson's model and the proposed role of the MEN1 gene as a tumour suppressor. However, LOH involving chromosome 11q13, which is the location of the MEN1, has also been observed in 5% to 50% of sporadic endocrine tumours, implicating the MEN1 gene in the aetiology of these tumours (Byström *et al.* 1990, Thakker *et al.* 1993). Somatic MEN1 mutations (Fig. 3 and Table 2) have been detected in 13% of sporadic parathyroid tumours (total number, n=150) (Heppner *et al.* 1997, Carling *et al.* 1998, Farnebo *et al.* 1998, Shan *et al.* 1998), 39% of gastrinomas (n=54) (Zhuang *et al.* 1997*a*, Wang *et al.* 1998, Mailman *et al.* 1999), 17% of insulinomas (n=18) (Zhuang *et al.* 1997*a*, Shan *et al.* 1998), 66% of VIPomas (n=3) (Shan *et al.* 1998, Wang *et al.* 1998), 13% of non-functioning pancreatic tumours (n=15) (Hessman *et al.* 1998), 100% of glucagonomas (n=2) (Hessman *et al.* 1998), 1.5% of adrenal cortical tumours (n=68) (Gortz *et al.* 1999), 36% of bronchial carcinoid tumours (n=11) (Debelenko *et al.* 1997*b*), 40% of anterior pituitary adenomas (n=117) (Zhuang *et al.* 1997*b*, Prezant *et al.* 1998, Tanaka *et al.* 1998), 10.5% of angiofibromas (n=19) (Boni *et al.* 1998) and 17% of lipomas (n=6) (Vortmeyer *et al.* 1998). These 67 somatic mutations are scattered through the 1830 bp coding region (Fig. 3), and 9% are nonsense mutations, 45% are frameshift deletions or insertions, 6% are in-frame deletions or insertions, 4% are donor-splice site mutations, and 36% are missense mutations (Table 2). A comparison of the locations of the somatic and germline mutations revealed a higher frequency (43% (somatic) versus 27% (germline), P<0.001) of somatic mutations in exon 2, and the significance of this observation (Pannett & Thakker

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
Exon 2					
1	8-12	nt134del13bp	fs	S (CAR)	Debelenko *et al.* 1997*b*
2	12	C<u>C</u>G→C<u>T</u>G	ms, P12L	G	Agarwal *et al.* 1997
3	13-33	nt147del61bp	fs	S (GAS)	Wang *et al.* 1998
4	15-20	nt154del16bp	fs	S (HPT)	Carling *et al.* 1998
5	22	C<u>T</u>G→C<u>G</u>G	ms, L22R	G	Agarwal *et al.* 1997
6	26	<u>G</u>AG→<u>A</u>AG	ms, E26K	G	Bartsch *et al.* 1998
7	26	<u>G</u>AG→<u>A</u>AG	ms, E26K	S (HPT)	Heppner *et al.* 1997
8	29	<u>C</u>GA→<u>T</u>GA	ns, R29X	G	Sato *et al.* 1998
9	38	nt226dup15bp (TGGTGCTCCTTTCCT)	if	G	Bassett *et al.* 1998
10	39	T<u>T</u>G→T<u>G</u>G	ms, L39W	G	Poncin *et al.* 1999
11	42	G<u>G</u>C→G<u>A</u>C	ms, G42D	G	Bassett *et al.* 1998
12	42	G<u>G</u>C→G<u>A</u>C	ms, G42D	S (GEP)	Toliat *et al.* 1997
13	45	G<u>A</u>G→G<u>G</u>G	ms, E45G	G	Sato *et al.* 1998
14	45	G<u>A</u>G→G<u>G</u>G	ms, E45G	G	Sato *et al.* 1998
15	45	GA<u>G</u>→GA<u>T</u>	ms, E45D	S (HPT)	Farnebo *et al.* 1998
16	55	nt275delTins2bp (CCT→CCGG)	fs	G	Bartsch *et al.* 1998
17	55	nt275delTins2bp (CCT→CCGG)	fs	G	Mayr *et al.* 1997, 1998
18	63	nt299ins5bp	fs	G	Tanaka *et al.* 1998
19	66	nt307delGins2bp (AGC→AAAC)	fs	G	Bartsch *et al.* 1998
20[d]	66	nt309dup10bp (CCAGCCCAGC)	fs	G	Bassett *et al.* 1998
21	67	nt310dup5bp	fs	G	Giraud *et al.* 1998
22	67	nt311insG	fs	G	Teh *et al.* 1998*a*
23	68	nt313delC	fs	G	Agarwal *et al.* 1997
24	69-73	nt315del11bp	fs	G	Bassett *et al.* 1998
25	69	nt317ins5bp	fs	G	Mayr *et al.* 1997, 1998
26	70-72	nt320del7bp	fs	S (GEP)	Mailman *et al.* 1999
27	70-75	nt320del16bp	fs	S (GAS)	Wang *et al.* 1998
28	71	nt322ins4bp	fs	G	Tanaka *et al.* 1998
29	73-76	nt328del9bp	fs	S (GAS)	Wang *et al.* 1998
30	76	nt337delC	fs	G	Giraud *et al.* 1998
31	79	nt345delC	fs	G	Tanaka *et al.* 1998
32	83-91	nt358del25bp	fs	S (GAS)	Zhuang *et al.* 1997*a*
33	83-84	nt359del4bp	fs	G	Poncin *et al.* 1999
34	83-84	nt359del4bp	fs	G	Poncin *et al.* 1999
35	83-84	nt359del4bp	fs	G	Agarwal *et al.* 1997
36	83-84	nt359del4bp	fs	G	Agarwal *et al.* 1997
37	83-84	nt359del4bp	fs	G	Sakurai *et al.* 1998

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank) (Continued)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
38	83-84	nt359del4bp	fs	G	Bassett *et al.* 1998
39	83-84	nt359del4bp	fs	G	Bassett *et al.* 1998
40	83-84	nt359del4bp	fs	G	Bassett *et al.* 1998
41	83-84	nt359del4bp	fs	G	Giraud *et al.* 1998
42	83-84	nt359del4bp	fs	G	Giraud *et al.* 1998
43	83-84	nt359del4bp	fs	G	Giraud *et al.* 1998
44	83-84	nt359del4bp	fs	G	Teh *et al.* 1998*a*
45	83-84	nt359del4bp	fs	G	Teh *et al.* 1998*a*
46	83-84	nt359del4bp	fs	G	Teh *et al.* 1998*a*
47	83-84	nt359del4bp	fs	G	Teh *et al.* 1998*a*
48	83-84	nt359del4bp	fs	G	Teh *et al.* 1998*a*
49	83-84	nt359del4bp	fs	S (GAS)	Zhuang *et al.* 1997*a*
50	83-84	nt359del4bp	fs	S (GAS)	Wang *et al.* 1998
51	83-84	nt359del4bp	fs	S (LIP)	Vortmeyer *et al.* 1998
52	84	nt360dupGT	fs	G	Giraud *et al.* 1998
53	86	ATC→TTC	ms, I86F	S (GAS)	Zhuang *et al.* 1997*a*
54	86-87	nt368del4bp	fs	S (HPT)	Carling *et al.* 1998
55	89	CTC→CGC	ms, L89R	S (GLU)	Hessman *et al.* 1998
56	90	nt379delAT	fs	G	Bassett *et al.* 1998
57	96	CAG→TAG	ns, Q96X	G	Giraud *et al.* 1998
58	98	CAG→TAG	ns, R98X	G	Mayr *et al.* 1997, 1998
59	98	CAG→TAG	ns, R98X	G	Bassett *et al.* 1998
60	98	CAG→TAG	ns, R98X	G	Giraud *et al.* 1998
61	98	CAG→TAG	ns, R98X	G	Giraud *et al.* 1998
62	98	CAG→TAG	ns, R98X	G	Teh *et al.* 1998*a*
63	102-113	nt416del32bp	fs	G	Mayr *et al.* 1997, 1998
64[e]	102	nt416delC	fs	G	Agarwal *et al.* 1997
65	102	nt416delC	fs	G	Teh *et al.* 1998*a*
66	108	CGA→TGA	ns, R108X	G	Giraud *et al.* 1998
67	108	CGA→TGA	ns, R108X	S (HPT)	Heppner *et al.* 1997
68	109	GAA→TAA	ns, E109X	S (ADR)	Gortz *et al.* 1999
69	110	nt437insGG	fs	G	Giraud *et al.* 1998
70	119	K119del	if	G	Bassett *et al.* 1998
71	119	K119del	if	G	Shimizu *et al.* 1997
72	119	K119del	if	G	Sakurai *et al.* 1998
73	119	K119del	if	G	Mayr *et al.* 1998
74	119	K119del	if	G	Agarwal *et al.* 1997
75	119	K119del	if	G	Agarwal *et al.* 1997
76	119	K119del	if	S (HPT)	Farnebo *et al.* 1998

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank) (Continued)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
77	119	K119del	if	S (GEP)	Toliat *et al.* 1997
78	120	AAG→TAG	ns, K120X	G	Agarwal *et al.* 1997
79	125	nt483delAT	fs	G	Tanaka *et al.* 1998
80	125	nt483delAT	fs	G	Shimizu *et al.* 1997
81	125	nt483delAT	fs	S (GAS)	Zhuang *et al.* 1997*a*
82	126	TGG→GGG	ms, W126G	S (GAS)	Zhuang *et al.* 1997*a*
83[d]	126	TGG→TAG	ns, W126X	G	Bassett *et al.* 1998
84	131	nt501delC	fs	G	Bassett *et al.* 1998
85	133	AAG→TAG	ns, Y133X	G	Giraud *et al.* 1998
86[f]	134	nt512delC	fs	G	Agarwal *et al.* 1997
87	134	nt512delC	fs	G	Bassett *et al.* 1998
88	135	AAG→TAG	ns, K135X	S (ANG)	Boni *et al.* 1998
89	135	nt515delG	fs	S (HPT)	Carling *et al.* 1998
90	138	nt522delG	fs	S (VIP)	Wang *et al.* 1998
91	139	CAC→GAC	ms, H139D	G	Agarwal *et al.* 1997
92	139	CAC→GAC	ms, H139D	G	Zhuang *et al.* 1997*b*
93	139	CAC→GAC	ms, H139D	S (HPT)	Carling *et al.* 1998
94	139	CAC→TAC	ms, H139Y	G	Agarwal *et al.* 1997
95	141	CAG→CGG	ms, Q141R	S (GAS)	Wang *et al.* 1998
96	144	TTC→GTC	ms, F144V	G	Agarwal *et al.* 1997
97	145	nt545insT	fs	S (INS)	Zhuang *et al.* 1997*a*
98	145	AGC→AGG	ms, S145R	S (NF)	Wang *et al.* 1998
99	147	I147del	if	S (GAS)	Wang *et al.* 1998
Intron 2					
100		nt556-3 (c→g)	sp	G	Teh *et al.* 1998*b*
Exon 3					
101	150	nt560insA	fs	G	Teh *et al.* 1998*a*
102	152	TTG→TGG	ms, L152W	S (HPT)	Carling *et al.* 1998
103	153	nt569delC	fs	G	Sato *et al.* 1998
104	159	TTT→TGT	ms, F159C	S (GAS)	Zhuang *et al.* 1997*a*
105	160	GCT→CCT	ms, A160P	G	Bassett *et al.* 1998
107	160	GCT→ACT	ms, A160T	G	Teh *et al.* 1998*a*
108	162	GTT→TGT	ms, V162C	S (GAS)	Wang *et al.* 1998
109[d]	163	nt597delG	fs	G	Teh *et al.* 1998*a*
110	163	nt598dup5bp (GTTGG)	fs	G	Bassett *et al.* 1998
111	163	nt599insA	fs	G	Teh *et al.* 1998*a*
112	163	nt599insA	fs	G	Teh *et al.* 1998*a*

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank) (Continued)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
113	164	GCC→GAC	ms, A164D	G	Bassett *et al.* 1998
114	166	CAG→TAG	ns, Q166X	G	Tanaka *et al.* 1998
115	168	CTG→CCG	ms, L168P	G	Bartsch *et al.* 1998
116	171-173	nt621del9bp	if	G	Sakurai *et al.* 1998
117	171-173	nt621del9bp	if	G	Sakurai *et al.* 1998
118	172	GAT→TAT	ms, D172Y	G	Poncin *et al.* 1999
119	172	GAT→TAT	ms, D172Y	G	Poncin *et al.* 1999
120	172	GAT→TAT	ms, D172Y	G	Poncin *et al.* 1999
121	172	GAT→TAT	ms, D172Y	G	Giraud *et al.* 1998
122[d]	174	nt630delC	fs	G	Bassett *et al.* 1998
123	175	CTC→CGC	ms, L175R	S (HPT)	Farnebo *et al.* 1998
124	176	GCC→CCC	ms, A176P	G	Agarwal *et al.* 1997
125	178	nt643insC	fs	S (GAS)	Mailman *et al.* 1999
126	179	GAG→GAT	ms, E179D	G	Poncin *et al.* 1999
127	183	TGG→TCG	ms, W183S	G	Bassett *et al.* 1998
128	183	na	ns, W183X	G	Agarwal *et al.* 1997
129	183	TGG→TGA	ns, W183X	G	Bassett *et al.* 1998
130[g]	184	GTA→GAA	ms, V184E	G	Fujimori *et al.* 1998
131	191	GAG→TAG	ns, E191X	G	Bassett *et al.* 1998
132	191	GAG→TAG	ns, E191X	G	Teh *et al.* 1998*a*
133	197	nt699insA	fs	S (HPT)	Farnebo *et al.* 1998
134	197	T197del	if	G	Giraud *et al.* 1998
135	198	na	ns, W198X	G	Agarwal *et al.* 1997
136	198	TGG→TGA	ns, W198X	S (VIP)	Shan *et al.* 1998
137	201	nt711delA	fs	G	Sato *et al.* 1998
138	201	nt713delG	fs	G	Agarwal *et al.* 1997
139	208	nt734delC	fs	G	Teh *et al.* 1998*a*
140[i]	209-210	nt735del4bp	fs	G	Agarwal *et al.* 1997
141[i]	209-210	nt735del4bp	fs	G	Agarwal *et al.* 1997
142	210-211	nt738del4bp	fs	G	Sakurai *et al.* 1998
143	210-211	nt738del4bp	fs	G	Bassett *et al.* 1998
144	210-211	nt738del4bp	fs	G	Bassett *et al.* 1998
145	210-211	nt738del4bp	fs	G	Bassett *et al.* 1998
146	210-211	nt738del4bp	fs	G	Bassett *et al.* 1998
147	210-211	nt738del4bp	fs	G	Bassett *et al.* 1998
148	210-211	nt738del4bp	fs	G	Giraud *et al.* 1998
149	210-211	nt738del4bp	fs	G	Teh *et al.* 1998*a*
150	210-211	nt738del4bp	fs	G	Teh *et al.* 1998*a*
151	214	nt751delG	fs	G	Bassett *et al.* 1998

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank) (Continued)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
152	218	nt764G→T	sp	G	Giraud *et al.* 1998
Intron 3					
153		nt764+1 (g→t)	sp	G	Teh *et al.* 1998*b*
154		nt764+1 (g→t)	sp	G	Teh *et al.* 1998*a*
155		nt764+3 (a→g)	sp	S (CAR)	Debelenko *et al.* 1997*b*
156		nt765-1 (g→t)	sp	G	Mayr *et al.* 1998
Exon 4					
157	220	na	ns, W220X	G	Teh *et al.* 1998*b*
158	220	na	ns, W220X	G	Teh *et al.* 1998*a*
159	220-223	nt770del9bp	if	S (GAS)	Wang *et al.* 1998
160	222	nt776delC	fs	G	Giraud *et al.* 1998
161	223	nt778delT	fs	G	Giraud *et al.* 1998
162	223	CTG→CCG	ms, L223P	G	Giraud *et al.* 1998
163	224-225	nt781del5bp	fs	S (HPT)	Farnebo *et al.* 1998
164	227	na	ns, Y227X	G	Teh *et al.* 1998*a*
165	236-239	nt817del9bp	if	G	Bassett *et al.* 1998
166	236	nt818insT	fs	G	Bartsch *et al.* 1998
167	242	GCC→GTC	ms, A242V	G	Agarwal *et al.* 1997
168	255	nt875insA	fs	S (GAS)	Zhuang *et al.* 1997*a*
169[g]	255	GAG→AAG	ms, E255K	G	Teh *et al.* 1998*c*
170	257	nt879ins7bp (GGAGCTT)	fs	G	Poncin *et al.* 1999
171	258	CAG→TAG	ns, Q258X	G	Poncin *et al.* 1999
172	260	CAG→TAG	ns, Q260X	G	Shimizu *et al.* 1997
173	260	CAG→TAG	ns, Q260X	G	Agarwal *et al.* 1997
174	261	CAG→TAG	ns, Q261X	G	Giraud *et al.* 1998
Intron 4					
175		nt893+1(g→c)	sp	G	Poncin *et al.* 1999
176		nt893+1(g→t)	sp	G	Giraud *et al.* 1998
177		nt893+1(g→t)	sp	G	Teh *et al.* 1998*a*
178		nt894-9 (g→a)	sp	G	Gortz *et al.* 1999
179		nt894-1 (g→c)	sp	G	Giraud *et al.* 1998
Exon 5					
180	262	nt894delAinsGG	fs	G	Bassett *et al.* 1998
181	262	AAG→TAG	ns, K262X	G	Bassett *et al.* 1998
182	264	CTC→CCC	ms, L264P	G	Poncin *et al.* 1999

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank) (Continued)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
183	265	nt904delG	fs	S (GEP)	Toliat *et al.* 1997
184[d]	265	na	ns, W265X	G	Agarwal *et al.* 1997
185	265	na	ns, W265X	G	Giraud *et al.* 1998
186[g]	267	CTC→CCC	ms, L267P	G	Poncin *et al.* 1999
187	268	nt912insT	fs	G	Chico *et al.* 1998
188	268	TAT→TAG	ns, T268X	G	Bartsch *et al.* 1998
189	269	nt916del22bp	fs	S (GH/PRL)	Tanaka *et al.* 1998
190	272	nt924insC	fs	G	Giraud *et al.* 1998
Intron 5					
191		nt934+1(g→a)	sp	S (HPT)	Heppner *et al.* 1997
192		nt935-2 (a→g)	sp	G	Toliat *et al.* 1997
Exon 6					
193[d]	284	GCA→GAA	ms, A284E	G	Bassett *et al.* 1998
194	286	CTA→CCA	ms, L286P	G	Agarwal *et al.* 1997
Exon 7					
195	308	na	ns, S308X	G	Agarwal *et al.* 1997
196	309	GCC→CCC	ms, A309P	G	Agarwal *et al.* 1997
197	312	na	ns, Y312X	G	Sato *et al.* 1998
198	312	na	ns, Y312X	G	Agarwal *et al.* 1997
199	314	CGG→CCG	ms, R314P	G	Giraud *et al.* 1998
200	315	nt1054insA	fs	G	Giraud *et al.* 1998
201	315	nt1055insA	fs	G	Poncin *et al.* 1999
202	320	CCC→CTC	ms, P320L	G	Tanaka *et al.* 1998
203	323	na	ns, Y323X	G	Agarwal *et al.* 1997
204	327	nt1089delT	fs	G	Bassett *et al.* 1998
205	327	nt1089delT	fs	G	Teh *et al.* 1998*a*
206	330	CGC→CCC	ms, R330P	S (GAS)	Wang *et al.* 1998
207	330	CGC→CCC	ms, R330P	S (GAS)	Wang *et al.* 1998
208	337	GCC→GAC	ms, A337D	G	Giraud *et al.* 1998
209	340	GCC→ACC	ms, A340T	S (HPT)	Shan *et al.* 1998
210	341	nt1132delG	fs	G	Agarwal *et al.* 1997
211	341	TGG→CGG	ms, W341R	G	Giraud *et al.* 1998
212	341	TGG→TAG	ns, W341X	G	Bassett *et al.* 1998
213	341	na	ns, W341X	G	Teh *et al.* 1998*a*
214	341	na	ns, W341X	G	Teh *et al.* 1998*a*
215	344	ACG→AGG	ms, T344R	G	Agarwal *et al.* 1997

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank) (Continued)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
216	345	nt1143delG	fs	G	Sakurai *et al.* 1998
217	345	nt1144delC	fs	S (HPT)	Farnebo *et al.* 1998
218	349	CAG→TAG	ns, Q349X	G	Bassett *et al.* 1998
219	349	CAG→TAG	ns, Q349X	G	Bassett *et al.* 1998
Intron 7					
220[d]		nt1159+1 (g→a)	sp	G	Bassett *et al.* 1998
221		nt1160-5del7bp	sp	S (GEP)	Toliat *et al.* 1997
222		nt1160-2 (a→g)	sp	G	Tanaka *et al.* 1998
Exon 8					
223[g]	353	TAC→TAA	ns, Y353X	G	Shimizu *et al.* 1997
224	358	E358del	if	G	Bassett *et al.* 1998
225	358-359	nt1184transvGG→AA	ms,E359K	S (ANG)	Boni *et al.* 1998
226	363	E363del	if	G	Agarwal *et al.* 1997
227	363	E363del	if	G	Agarwal *et al.* 1997
228	363	E363del	if	G	Zhuang *et al.* 1997*a*
229	364-365	nt1202del2bp		G	Agarwal *et al.* 1997
230	368	GCC→GAC	ms, A368D	G	Giraud *et al.* 1998
231	368-370	nt1212del7bp	fs	S (GAS)	Zhuang *et al.* 1997*a*
232	373	nt1229delC	fs	G	Bassett *et al.* 1998
233	387	nt1269delG	fs	S (GAS)	Wang *et al.* 1998
234	388	GAG→TAG	ns, E388X	G	Bassett *et al.* 1998
235	390	nt1280delG	fs	G	Agarwal *et al.* 1997
236	391	nt1279ins11bp (GAGGAGCGGCC)	fs	S (HPT)	Heppner *et al.* 1997
237	392	GAG→TAG	ns, E392X	S (GLU)	Hessman *et al.* 1998
Exon 9					
238	397	nt1300delC	fs	G	Giraud *et al.* 1998
239	405	nt1325delG	fs	G	Giraud *et al.* 1998
240	406-408	nt1328del5bp	fs	G	Bassett *et al.* 1998
241	410	na	ms, F410L	S (ACTH)	Zhuang *et al.* 1997*b*
242[g]	414	L414del	if	G	Sato *et al.* 1998
243	415	CGA→TGA	ns, R415X	G	Giraud *et al.* 1998
244	415	CGA→TGA	ns, R415X	G	Teh *et al.* 1998b
245	415	CGA→TGA	ns, R415X	S (NFpanc)	Hessman *et al.* 1998
246	418-421	nt1362del12bp	if	G	Giraud *et al.* 1998
247	418	D418del	if	G	Agarwal *et al.* 1997

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank) (Continued)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
248	418	GAC→AAC	ms, D418N	G	Bassett *et al.* 1998
249	418	GAC→AAC	ms, D418N	G	Giraud *et al.* 1998
250	418	GAC→AAC	ms, D418N	G	Teh *et al.* 1998*a*
251	418	GAC→AAC	ms, D418N	G	Heppner *et al.* 1997
252	418	nt1363delAC	fs	G	Poncin *et al.* 1999
253	418	nt1363delAC	fs	G	Giraud *et al.* 1998
254	422	nt1374delA	fs	G	Giraud *et al.* 1998
255	423	na	ms, W423S	G	Mayr *et al.* 1998
256	425	E425del	if	G	Giraud *et al.* 1998
257	429	ACG→AAG	ms, T429K	S (INS)	Shan *et al.* 1998
258	436	TGG→CGG	ms, W436R	G	Agarwal *et al.* 1997
259	436	TGG→TGA	ns, W436X	G	Mayr *et al.* 1998
260	436	na	ns, W436X	G	Agarwal *et al.* 1997
261	437	nt1419delG	fs	G	Bartsch *et al.* 1998
262	438	nt1422insA	fs	G	Sakurai *et al.* 1998
263	438-444	nt1423del19bp	fs	S (HPT)	Carling *et al.* 1998
264	438-439	nt1424del4bp	fs	G	Giraud *et al.* 1998
265	440	nt1424del2ins4bp (TT❑1GAAA)	fs	G	Bassett *et al.* 1998
266	442	CAG→TAG	ns, Q442X	G	Shimizu *et al.* 1997
267	447	TTT→CTT	ms, F447S	G	Agarwal *et al.* 1997
268	447-450	nt1449del11bp	fs	G	Giraud *et al.* 1998
269	448-450	nt1452del11bp	fs	G	Teh *et al.* 1998*a*
Intron 9					
270		nt1460+4del2bp	sp	G	Giraud *et al.* 1998
Exon 10					
271	451	nt1461delG	fs	S (CAR)	Debelenko *et al.* 1997*b*
272[h]	452-456	nt1466del12bp	if	G	Teh *et al.* 1998*a*
273	453	CAG→TAG	ns, Q453X	G	Bassett *et al.* 1998
274	455-456	nt1473del5bp	fs	G	Tanaka *et al.* 1998
275	455-456	nt1473del5bp	fs	G	Sakurai *et al.* 1998
276	458-461	nt1484del8bp	fs	G	Agarwal *et al.* 1997
277	460	CGA→TGA	ns, R460X	G	Agarwal *et al.* 1997
279	460	CGA→TGA	ns, R460X	G	Agarwal *et al.* 1997
280	460	CGA→TGA	ns, R460X	G	Bassett *et al.* 1998
281	460	CGA→TGA	ns, R460X	G	Giraud *et al.* 1998
282	461	nt1491insCC	fs	G	Giraud *et al.* 1998
284	463	nt1499dup8bp	fs	G	Giraud *et al.* 1998

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank) (Continued)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
285	467	nt1508ins	fs	G	Giraud *et al.* 1998
286	467	nt1509ins2bp	fs	G	Agarwal *et al.* 1997
287	477	nt1539insG	fs	G	Poncin *et al.* 1999
288	493	nt1587delCCinsG (CCG→GG)	fs	G	Bartsch *et al.* 1998
289	499	nt1607delA	fs	G	Giraud *et al.* 1998
290	502	A<u>A</u>G→A<u>T</u>G	ms, K502M	S (NFpit)	Zhuang *et al.* 1997*b*
291	507	nt1630insC	fs	G	Giraud *et al.* 1998
292	510	nt1639delCA	fs	G	Bartsch *et al.* 1998
293	516	nt1656delC	fs	G	Agarwal *et al.* 1997
294	516	nt1656delC	fs	G	Agarwal *et al.* 1997
295	516	nt1656delC	fs	G	Bassett *et al.* 1998
296	516	nt1656delC	fs	G	Giraud *et al.* 1998
297	516	nt1656delC	fs	G	Giraud *et al.* 1998
298	516	nt1657insC	fs	G	Agarwal *et al.* 1997
299	516	nt1657insC	fs	G	Sakurai *et al.* 1998
300	516	nt1657insC	fs	G	Sakurai *et al.* 1998
301	516	nt1657insC	fs	G	Sakurai *et al.* 1998
302	516	nt1657insC	fs	G	Giraud *et al.* 1998
303	516	nt1657insC	fs	G	Bassett *et al.* 1998
304	516	nt1657insC	fs	G	Bassett *et al.* 1998
305	516	nt1657insC	fs	G	Giraud *et al.* 1998
306	516	nt1657insC	fs	G	Giraud *et al.* 1998
307	516	nt1657insC	fs	G	Giraud *et al.* 1998
308	516	nt1657insC	fs	G	Teh *et al.* 1998*a*
309	516	nt1657insC	fs	G	Teh *et al.* 1998*a*
310	516	nt1657insC	fs	S (CAR)	Debelenko *et al.* 1997*b*
311	519	nt1666delC	fs	G	Poncin *et al.* 1999
312	527	<u>C</u>GA→<u>T</u>GA	ns, R527X	G	Agarwal *et al.* 1997
313	527	<u>C</u>GA→<u>T</u>GA	ns, R527X	G	Bassett *et al.* 1998
314	527	<u>C</u>GA→<u>T</u>GA	ns, R527X	G	Teh *et al.* 1998*a*
315	527	<u>C</u>GA→<u>T</u>GA	ns, R527X	G	Giraud *et al.* 1998
316	527	<u>C</u>GA→<u>T</u>GA	ns, R527X	G	Giraud *et al.* 1998
317	530-531	nt1699del3ins2bp (AAGG→AGC)	fs	G	Debelenko *et al.* 1997*b*
318	535	G<u>C</u>T→G<u>T</u>T	ms, A535V	S (INS)	Zhuang *et al.* 1997*a*
319	536	<u>C</u>AG→<u>T</u>AG	ns, Q536X	G	Giraud *et al.* 1998
320	543	T<u>C</u>A→T<u>T</u>A	ms, S543L	S (GAS)	Zhuang *et al.* 1997*a*
321	550	nt1768delT	fs	G	Giraud *et al.* 1998
322	550	nt1768delT	fs	G	Giraud *et al.* 1998
323	555	A<u>G</u>T→A<u>A</u>T	ms, S555N	G	Giraud *et al.* 1998

Table 2 Germline and somatic mutations in the MEN1 gene. Frameshift, in frame, and splice site mutations are numbered with reference to the cDNA sequence U93236 (GenBank) (Continued)

Location; number	Codon	Mutation[a]	Predicted effect[b]	Type: germline (G), or somatic (S)[c]	Reference
324	556	nt1777delA	fs	S (GH/PRL)	Tanaka *et al.* 1998
325	558	M558del	if	G	Giraud *et al.* 1998
326	558	nt1782delA	fs	G	Giraud *et al.* 1998
327	561	ATG→ACG	ms, M561T	S (HPT)	Heppner *et al.* 1997
328	568-569	nt1812del5bp	fs	S (HPT)	Heppner *et al.* 1997
329	whole gene			G	Kishi *et al.* 1998

[a]Mutation: na=mutation not available.
[b]Predicted effect: fs=frameshift mutation; ns=nonsense mutation; ms=missense mutation; if=in-frame deletion or insertion mutation; sp=splice site mutation.
[c]Somatic mutation - origin of tumour: HPT= parathyroid tumour; GAS= gastrinoma; INS= insulinoma; VIP=VIPoma; GLU=glucagon; GEP= neuroendocrine gastroenteropancreatic tumour; NFPpanc= non-functioning pancreatic tumour; PRL=prolactinoma; GH= somatotrophinoma; ACTH=corticotrophinoma; NFpit=non-functioning pituitary tumour; ADR=adrenal cortical tumour ; CAR=lung carcinoid; ANG=angiofibroma; LIP=lipoma; NF=non-functioning.
[d]Occurrence of *de novo* mutation demonstrated by familial analysis.
[e]Mutation reported in 5 related USA families.
[f]Mutation reported in 6 related USA families.
[g]Familial isolated primary hyperparathyroidism (FIHP) mutation.
[h]Mutation reported in 8 Finnish families; likely to be due to a founder effect.
[i]These mutations may also involve codons 210-211.
ins, insertion; del, deletion; dup, duplication; transv, transversion.

1999) in the context of the Knudson two-hit hypothesis remains to be elucidated. The tumours harbouring a somatic MEN1 mutation had chromosome 11q13 LOH as the other genetic abnormality, or 'hit', consistent with Knudson's hypothesis. These studies (Debelenko *et al.* 1997*b*, Heppner *et al.* 1997, Toliat *et al.* 1997, Zhuang *et al.* 1997*a*,*b*, Boni *et al.* 1998, Carling *et al.* 1998, Farnebo *et al.* 1998, Hessman *et al.* 1998, Prezant *et al.* 1998, Shan *et al.* 1998, Vortmeyer *et al.* 1998, Wang *et al.* 1998, Gortz *et al.* 1999, Mailman *et al.* 1999) indicate that although inactivation of the MEN1 gene may have a role in the aetiology of some sporadic endocrine tumours, the involvement of other genes with major roles in the aetiology of such sporadic endocrine tumours is highly likely.

Function of MEN1 protein (MENIN)

Initial analysis of the predicted amino acid sequence encoded by the MEN1 gene did not reveal homologies to any other proteins, sequence motifs, signal peptides, or consensus nuclear localisation signals (Chandrasekharappa *et al.* 1997), and thus the putative function of the protein (MENIN) could not be deduced. However, studies based on immunofluorescence, Western blotting of subcellular fractions, and epitope tagging with enhanced green fluorescent protein, revealed that MENIN was located primarily in the nucleus (Guru *et al.* 1998). Furthermore, enhanced green fluorescent protein-tagged MENIN deletional constructs identified at least two independent nuclear localisation signals that are located in the C-terminal quarter of the protein (Fig. 3) (Guru *et al.* 1998). Interestingly, none of the MEN1 germline missense or in-frame deletions (Agarwal *et al.* 1997, Debelenko *et al.* 1997*b*, Mayr *et al.* 1997, 1998, Shimizu *et al.* 1997, Toliat *et al.* 1997, Zhuang *et al.*1997*a*, Bartsch *et al.* 1998,

Table 3 MEN1-associated tumours in five unrelated families with a 4 bp deletion at codons 210 and 211

	Family				
Tumour	1	2	3	4	5
Parathyroid	+	+	+	+	+
Gastrinoma	+	–	+	+	+
Insulinoma	–	+	–	–	–
Glucagonoma	–	–	–	–	+
Prolactinoma	–	–	–	–	+
Carcinoid	+	–	–	–	–

+, presence; -, absence of tumours.
(Adapted from Thakker (1998), with permission).

Bassett *et al.* 1998, Chico *et al.* 1998, Fujimori *et al.* 1998, Giraud *et al.* 1998, Kishi *et al.* 1998, Sakurai *et al.* 1998, Sato *et al.* 1998, Tanaka *et al.* 1998, Teh *et al.* 1998*a*,*b*,*c*, Gortz *et al.* 1999, Poncin *et al.* 1999) alter either of these putative nuclear localisation signals (Fig. 3). However, all of the truncated MEN1 proteins that would result from the nonsense and frameshift mutations, if expressed, would lack at least one of these nuclear localisation signals (Fig. 3). The nuclear localisation of MENIN suggested that it may act either in the regulation of transcription, or DNA replication, or the cell cycle. In order to investigate this further and to identify proteins that may interact with MENIN, the yeast two hybrid system was utilised. This revealed that MENIN directly interacts with the N-terminus of the AP-1 transcriptional factor JunD, to repress JunD-activated transcription (Agarwal *et al.* 1999). Analysis of several MEN1 missense and deletional mutations indicated that the N-terminus and central domains of MENIN (Fig. 3) have a critical role in MENIN-JunD interaction. However, JunD inhibits cell growth (Hirai *et al.* 1989, Ryder *et al.* 1989, Pfarr *et al.* 1994), an action that differs from that of other AP-1 proteins, and thus the repressive effect of MENIN on JunD-mediated transcriptional activation would predict enhanced growth rather than the observed suppression in growth. This seeming paradox may be due to the involvement of other target genes and proteins that may be involved in cell proliferation and that may have interactions with the MENIN-JunD complex (Agarwal *et al.* 1999). These suggestions are further supported by the observation that disease-associated mutations which occur outside the domains interacting with JunD (Fig. 3), are associated with normal MENIN-JunD binding; this suggests that MENIN may interact with other proteins that may influence JunD-mediated transcription. Further investigations are needed to elucidate the role of MENIN-JunD interactions in the control of endocrine cell proliferation.

Screening in MEN1

MEN1 is inherited as an autosomal dominant disorder in the majority of patients. Occasionally, MEN1 may arise sporadically (i.e. without family history), although it may be difficult to make the distinction between sporadic and familial forms; in some cases the family history may be

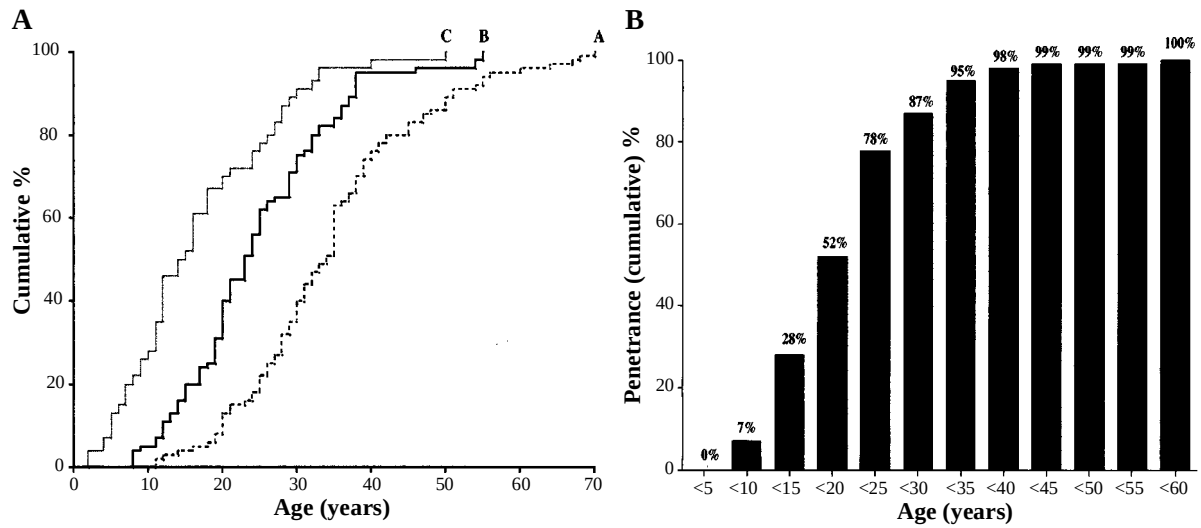

Figure 5 The age distributions (panel A) and age-related penetrances (panel B) of MEN1 determined from an analysis of 174 mutant gene carriers. The age distributions were determined for 3 groups of MEN1 mutant gene carriers from 40 families in whom mutations were detected (Bassett *et al.* 1998). The 91 members of group A presented with symptoms, whereas the 40 members of group B were asymptomatic and were detected by biochemical screening. The 43 members of group C represent those individuals who are MEN1 mutant gene carriers (Fig. 4) and who remain asymptomatic and biochemically normal. The ages included for members of groups A, B and C are those at the onset of symptoms, at the finding of the biochemical abnormality, and at the last clinical and biochemical evaluation respectively. Groups B and C contained members who were significantly younger than those in group A (P<0.001). The younger age of the group C mutant gene carriers is consistent with an age-related penetrance for MEN1, and this was calculated (panel B) for the first 5 decades. The age-related penetrances (i.e. the proportion of mutant gene carriers with manifestations of the disease by a given age) rose steadily from 7% in the <10 years group, to 52%, 87%, 98% and 100%, by the ages of 20, 30, 40, and 60 years respectively. (Adapted from Bassett *et al.* (1998), with permission.)

absent because the parent with MEN1 is not available, and has perhaps already died before developing any manifestations, and other cases may be due to *de novo* mutations, which will be transmitted in an autosomal dominant manner in future generations (Agarwal *et al.* 1997, Bassett *et al.* 1998, Teh *et al.* 1998*a*). MEN1 is an uncommon disorder, but because of its autosomal dominant inheritance, the finding of MEN1 in a patient has important implications for other family members; first-degree relatives have about a 50% risk of developing the disease. Screening for MEN1 in patients involves the detection of tumours and the ascertainment of the germline genetic state i.e. normal or mutant gene carrier. The detection of tumours entails clinical, biochemical and radiological investigations for MEN1-associated tumours in patients. The recent cloning of the MEN1 gene has facilitated the identification of individuals who have mutations and hence are at a high risk of developing the disease (Figs 3 and 4).

Genetic analysis

Mutational analysis for MEN1 could now be introduced to identify those individuals who are mutant carriers and thus at a high risk of developing tumours. The advantages of DNA analysis are that it requires a single blood sample and does not need, in theory, to be repeated. This is because the analysis is independent of the age of the individual and provides an objective result. Such mutational analysis could be undertaken in children around the first decade, as some children have developed tumours by the age of 8 years (Thakker 1995, Trump *et al.* 1996), and appropriate intervention in the form of biochemical testing and/or treatment has been considered. However, the great diversity together with the widely scattered locations of the MEN1 mutations (Fig. 3), and a lack of genotype-phenotype correlation (Table 3) will make such mutational screening time-consuming, arduous and expensive, and as yet this is not widely available. Nevertheless, an integrated programme of both mutational analysis, to identify mutant gene carriers, and biochemical screening, to detect the development of tumours, would be of advantage. Thus, a DNA test identifying an individual as a mutant gene carrier is likely to lead not to immediate medical or surgical treatment but to earlier and more frequent biochemical and radiological screening, whereas a DNA result that indicates that an individual is not at risk will lead to a decision for no further clinical investigations. Thus, the identification of MEN1 mutations may be of help in the clinical management of patients and their families with this disorder.

Detection of MEN1 tumours

Biochemical screening for the development of MEN1 tumours in asymptomatic members of families with MEN1 is of great importance, as earlier diagnosis and treatment of these tumours may help to reduce morbidity and mortality. The age-related penetrance (i.e. the proportion of gene carriers manifesting symptoms or signs of the disease by a given age) has been ascertained (Fig. 5) and the mutation appears to be non-penetrant below the age of 8 years. Thereafter, the mutant MEN1 gene has a high penetrance, being >50% penetrant by 20 years of age and >95% penetrant by 40 years (Bassett *et al.* 1998). Screening for MEN1 tumours is difficult because the clinical and biochemical manifestations in members of any one family are not uniformly similar (Trump *et al.* 1996). The attempts to screen for the development of MEN1 tumours in the asymptomatic relatives of an affected individual have depended largely on measuring the serum concentrations of calcium, gastrointestinal hormones and prolactin, and also on ultrasound and radiological imaging of the abdomen and pituitary. Parathyroid overactivity causing hypercalcaemia is almost invariably the first manifestation of the disorder and this has become a useful and easy biochemical screening investigation (Benson *et al*. 1987, Marx *et al*. 1986).

At present, it is suggested that individuals at high risk of expressing MEN1 (i.e. mutant gene carriers) should undergo biochemical screening at least once per annum, and also have baseline pituitary and abdominal imaging, which should then be repeated infrequently (at 5 to 10 yearly intervals). Screening should commence in early childhood, as the disease has developed in some individuals by the age of 8 years, and should continue for life, as some individuals have not developed the disease until the eighth decade. Screening history and physical examination should be directed towards eliciting the symptoms and signs of hypercalcaemia, nephrolithiasis, peptic ulcer disease, neuroglycopaenia, hypopituitarism, galactorrhoea and amenorrhoea in women, acromegaly, Cushing's disease, visual field loss and the presence of subcutaneous lipomata, angiofibromas, and collagenomas. Biochemical screening should include serum calcium and prolactin estimations in all individuals, and measurement of gastrointestinal hormones e.g. gastrin, and more specific endocrine function tests should be reserved for individuals who have symptoms or signs suggestive of a clinical syndrome.

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