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Clinical studies of multiple endocrine neoplasia type 1 (MEN1)

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Summary

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterized by the combined occurrence of parathyroid, pancreatic islet and anterior pituitary tumours. To facilitate a screening programme for MEN1, we investigated 709 people (364 males and 345 females, age range 1–84 years) from 62 MEN1 families, and 36 non-familial MEN1 patients. Of those investigated, 220 (95 males and 125 females, age range 8–79 years) suffered from MEN1. Parathyroid, pancreatic and pituitary tumours occurred in 95%, 41% and 30% of the patients, respectively. Parathyroid tumours were the first manifestation of MEN1 in 87% of patients, and amongst the pituitary and pancreatic tumours, somatotrophinomas and gastrinomas were more common in patients above the age of 40 years, whilst insulinomas occurred more frequently in patients below the age of 40 years. Biochemical screening indicated that the penetrance of MEN1 by the ages of 20, 35 and 50 years was 43%, 85% and 94%, respectively, and that the development of MEN1 was confined to first-degree relatives in 91% of patients and to second-degree relatives in 9% of patients. These findings have helped to define a proposed screening programme for MEN1.

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Introduction

Multiple endocrine neoplasia type 1 (MEN1)[1] is an autosomal dominant disorder characterized by the combined occurrence of tumours of the parathyroid glands, the pancreatic islet cells and the anterior pituitary.[1–5] Parathyroid tumours are the most common manifestation of the disorder[6–10] but patients may also suffer from pancreatic tumours which secrete gastrin, insulin, pancreatic polypeptide (PP), or glucagon, and anterior pituitary tumours which usually secrete prolactin, growth hormone (GH) or adrenocorticotrophin (ACTH).[4–10] Adrenal cortical tumours,[11] carcinoid tumours,[12] lipomatous tumours[7] and the McCune-Albright syndrome[13] have also been observed in association with MEN1. The disease may arise in families, and an autosomal dominant inheritance has been established.[2,3] Children of an affected individual are thus at a 50% risk of inheriting the mutant gene and of potentially developing these endocrine tumours. Earlier detection of these tumours by screening may help to reduce the morbidity and mortality in this high-risk population. However, screening for MEN1 is difficult as the combination of affected glands may differ in members of the same family.[1] In addition, the age-related penetrance (the proportion of gene carriers who have manifested symptoms or signs of the disease by a given age) has not been established. We used clinical and biochemical methods to investigate the members of 98 families with MEN1, aiming to characterize further its manifestations, to determine its age-related penetrance, and to define a suitable screening strategy.

Methods

Patients

Ninety-eight unrelated MEN1 patients were studied and their detailed family medical histories obtained. Patients and their family members were assessed for present and past manifestations of MEN1: polyuria, polydipsia, constipation, malaise, bone pains or nephrolithiasis suggesting hypercalcaemia, indigestion, recurrent peptic ulceration, hypoglycaemia, neuroglycopenia or diarrhoea suggesting the presence of a pancreatic islet-cell tumour, amenorrhoea, galactorrhoea, impotence or weight changes suggesting the presence of a pituitary tumour and a detailed questionnaire (Appendix I) completed. The medical records of deceased family members and of the known affected individuals were also examined.

Biochemical measurements

Venous blood samples were obtained from patients and family members, and serum calcium, albumin, creatinine and alkaline phosphatase were determined using a multi-channel autoanalyser.[14] Serum calcium concentrations were corrected to an albumin of 41 g/l[14] (normal range 2.25–2.55 mmol/l). Serum prolactin was also determined in all individuals using the Serono immunoradiometric assay[15] (normal range <500 mIU/l). Estimations of other anterior pituitary hormones, gastrointestinal hormones, serum insulin and glucose were made if indicated from the clinical history and examination.

Phenotype allocation

Individuals were taken to be affected if they had evidence of two or more MEN1-associated tumours, or if they were the relative of an affected individual and had evidence of one MEN1 tumour as determined by the following characteristics: (i) persistent hypercalcaemia (corrected calcium >2.55 mmol/l); (ii) persistent hyperprolactinaemia (defined by a serum prolactin of >800 mIU/l, so as to exclude stress-related hyperprolactinaemia) occurring in the absence of a known cause, such as pregnancy, hypothyroidism, or drugs, e.g. phenothiazines, together with other radiological or surgical evidence of a pituitary tumour; (iii) biochemical and/or radiological abnormalities demonstrating acromegaly or Cushing's disease (iv) biochemical and/or radiological abnormalities demonstrating a gastrinoma, insulinoma, PPoma, VIPoma or glucagonoma.

Kinship coefficient

Individuals were designated a kinship coefficient (KC) as an index of their proximity to affected members.[7] KCs were assigned prior to biochemical screening, and known affected individuals were designated a KC of 1.0; first-degree unaffected relatives, *i.e.* siblings and children of an affected individual, were designated a KC of 0.5; and second-degree unaffected relatives, i.e. grandchildren, nephews and nieces, were assigned a KC of 0.25. Spouses, who were also screened for MEN1 to ensure that they were unaffected and therefore not transmitting MEN1 to their children, were assigned a KC of 0.

Data analysis

The clinical and biochemical information for each individual was entered into a database using the DATAEASE computer program on an IBM PC, and statistical analysis was performed with the MINITAB package. The incidence of insulinomas by age and its correlation with the number of mutations required for its development was assessed by plotting the proportion of patients not yet diagnosed (S) at a

given age against the age (t), as previously described for the development of retinoblastoma and Knudson's two-hit hypothesis.[16] Thus, the incidence by age of tumours that develop after a single mutation, *e.g.* familial forms, would conform to a first-order equation $\log_{10} S = a - kt$, whereas that of tumours developing as a result of two mutations, e.g. the sporadic forms, would conform to a second-order equation $\log_{10} S = a - kt^2$. The ages at which insulinomas developed in familial MEN1 patients and sporadic non-MEN1 patients were determined from our study and a previously reported study,[17] respectively, and used for these calculations.

Results

Details of patients and families

Clinical and biochemical results were obtained from 745 individuals (380 males and 365 females) aged 1–84 years, of whom 220 (95 males and 125 females) were affected with MEN1, and 525 (283 males and 242 females) were unaffected (Table 1). In the unaffected group, 364 (204 males and 160 females) were siblings and 161 (79 males and 82 females) were spouses. Spouses were included in the analysis to ensure that none was affected with MEN1. Of the 220 affected individuals, 184 were family members from 62 families (Figure 1 and Appendix II) and 36 individuals were sporadic cases (Table 1) in whom no family history of MEN1 could be established; 118 of the 184 familial MEN1 patients presented with symptoms and the remaining 66 individuals, who were asymptomatic, were detected by biochemical screening. The mean ages of the affected ($\mu \pm \sigma = 33.3 \pm 15.5$ years), the total unaffected (33.9 ± 17.9 years) and the unaffected sibling (28.9 ± 16.5 years) groups did not differ significantly (Table 1). However, the mean age of the screened asymptomatic group (24.8 ± 11.6 years) was significantly ($p < 0.001$) lower than that of the symptomatic (36.4 ± 15.7 years) and sporadic (37.7 ± 15.6) groups, thereby demonstrating the value of biochemical screening in the earlier detection of MEN1. The male to female ratio of the affected group (M : F = 95 : 125) differed significantly ($p < 0.05$) from the whole, which may reflect a higher penetrance of MEN1 in females, and it is of interest that the two individuals in whom non-penetrance was observed above the age of 55 years (see below) were both males.

Analysis of tumour types

The 220 affected individuals had a total of 384 tumours (Appendix II); 120 of these patients had two or more tumours and the remaining 100 patients had one tumour. The distribution of the tumours is shown in Figure 2. Hypercalcaemia was found in 208 (94.5%) patients; the diagnosis of primary hyperparathyroidism had been confirmed in 87% of these patients by either parathyroidectomy (71%) or persistently raised serum PTH concentrations (16%). In the remaining 5.5% ($n = 12$) of MEN1 patients (2.2/91 I.1, 4/89 V.6, 8.7/87 II.1, 11/89 II.7, 11.2/90 III.4, 13.3/90 III.24, 13.3/92 III.1, 16/90 IV.20, 19/89 III.4, 19.2/92 II.1, S15, S19, Appendix II) who had no evidence of parathyroid tumours, five patients (4 males, 1 female) had a pancreatic tumour (2 insulinomas, 1 combined gastrinoma-glucagonoma, 2 non-secreting), six patients (2 males, 4 females) had a pituitary tumour (5 prolactinomas, 1 somatotrophinoma) and one patient (13.3/92 III.1, Appendix II) had an insulinoma and a non-functioning pituitary tumour. Of these 12 patients, eight were under 30 years of age and thus may develop parathyroid tumours which represent the most common manifestation of MEN1 (Figures 1 and 3) and which occurred as the sole endocrinopathy in 42.3% of MEN1 patients. Pancreatic tumours occurred in 40% of patients, with gastrinomas being the most common (63%) and with insulinomas being the next most common (27%) tumours; three of these tumours secreted both gastrin and insulin, and two of the

Table 1 Ages and sex ratios of MEN1 patients and families ($n = 745$)

Age ($\mu \pm \sigma$) (years)	Affected ($n = 220$) 33.3 ± 15.5			Unaffected ($n = 525$) 33.9 ± 17.9	
M : F	95 : 125			283 : 242	
	Familial		Sporadic	Siblings	Spouses
	Asymptomatic	Symptomatic			
n	66	118	36	364	161
Age ($\mu \pm \sigma$) (years)	24.8 ± 11.6	36.4 ± 15.7	37.7 ± 15.6	28.9 ± 16.5	46.6 ± 14.5
M : F	26 : 40	53 : 65	16 : 20	204 : 160	79 : 82

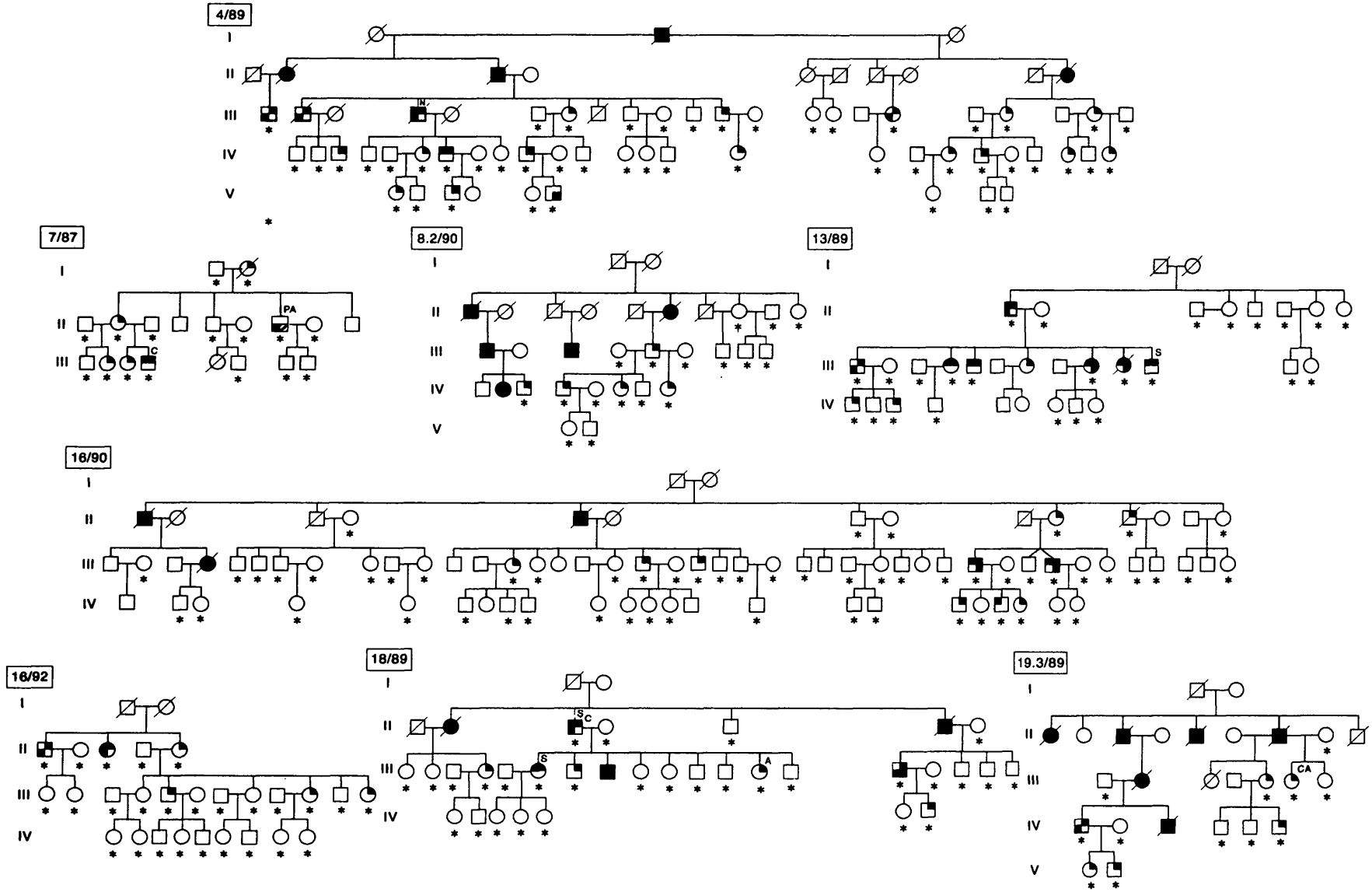

Figure 1. Eight MEN1 pedigrees in whom there were five or more affected members are shown. Males are indicated by a square, females by a circle and deceased family members are indicated by a diagonal line across their symbol. The identification code of each family is as indicated in Appendix II. The individuals from whom blood samples were obtained are denoted by (*). The clinical details of the six affected members of 7/87, the nine affected members of family 18/89, 5/13 affected members of 16/90, eight of the 24 affected members of family 4/89 have been previously reported.[17,25] The presence of tumours is indicated as follows: ◪ ◔ Parathyroid tumour, ◪ ◕ Gastrinoma, ◧ ◔ Insulinoma, ■ ◕ Pancreatic tumour: type unknown, ◧ ◔ Anterior pituitary tumour (for details of: prolactinoma; somatotrophinoma; corticotrophinoma; non-functioning tumour see Appendix 11), $\square^{CA}$ $\bigcirc^{CA}$ Carcinoid tumour, $\square^{A}$ $\bigcirc^{A}$ Adrenocortical tumour. Unaffected members are represented by □ ○, and those affected members who were unavailable for this study but were reported to suffer from MEN1 tumours by their relatives are represented as ■ ●.

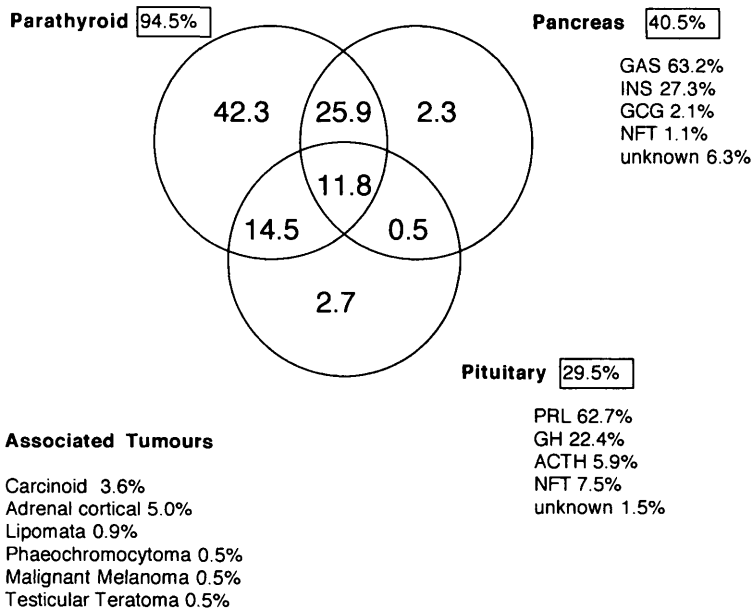

Figure 2. Schematic representation of the distribution of 384 MEN1 tumours in the 220 MEN1 patients. The proportions of patients in whom parathyroid, pancreatic or pituitary tumours occurred are shown in the respective boxes, for example, 94.5% of the patients had a parathyroid tumour. The Venn diagram indicates the proportions of patients with each combination of tumours, for example, 33.7% (25.9% + 11.8%) of the patients had both a parathyroid and pancreatic tumour, whereas 2.3% of the patients had a pancreatic tumour only. The hormones secreted by each of these tumours are indicated: GAS, gastrin; INS, insulin; GCG, glucagon; NFT, non-functioning tumour; PRL, prolactin; GH, growth hormone; ACTH, adrenocorticotrophic hormone. Thus, parathyroid tumours represent the most common form of MEN1 tumours.

gastrinomas also secreted glucagon. The gastrinomas were confirmed surgically or radiologically in 80% of patients and the insulinomas were confirmed surgically in 96% of patients. Pituitary tumours occurred in 30% of patients. Over three-fifths were prolactinomas and the majority of the remainder were somatotrophinomas. Ninety-six percent of the prolactinomas and 93% of the somatotrophinomas were confirmed by surgery or radiology. Additional endocrine tumours were found in 21 individuals: carcinoid tumours in eight patients (4%), adrenal cortical tumours in 12 patients (5%), lipomata in two patients (1%), a phaeochromocytoma in one patient, and a testicular teratoma and a malignant melanoma in one patient. Of these 21 patients, one patient had both a non-functioning adrenal tumour and a phaeochromocytoma, one patient had both an adrenal tumour and a carcinoid tumour, and the patient with a testicular teratoma and a malignant melanoma also had a carcinoid tumour.

The combinations of these MEN1 tumours differed in members of the same family, and this variable expression of MEN1 is illustrated by family 13/89 (Figure 1). In this family the father (generation II) suffered from parathyroid tumours, an insulinoma and a prolactinoma. All of his six affected children

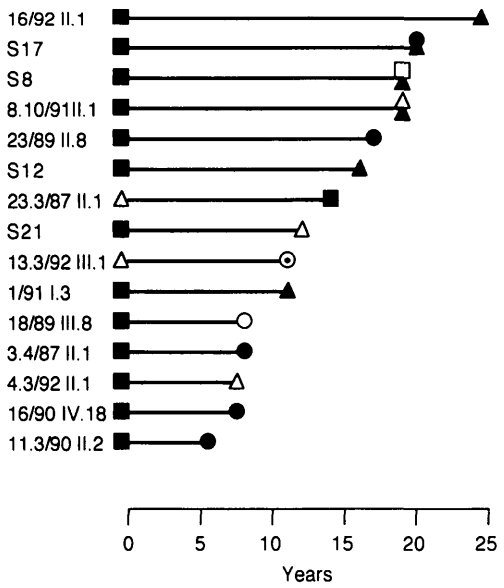

Figure 3. Order of tumour development in 15 MEN1 patients. The time interval between the occurrence of the first tumour (time = 0 years) and subsequent tumours in each patient, who is identified by the family and individual number (Appendix II), are shown. The tumours are: ■, PTH-secreting; ▲, gastrinoma; △, insulinoma; ●, prolactinoma; ○, corticotrophinoma; □, somatotrophinoma; ⊙, non-functioning pituitary tumour. Parathyroid tumours were the first manifestation of MEN1 in 13/15 patients, and in the remaining two patients, insulinomas represented the first manifestation of MEN1. The time interval for the occurrence of the subsequent tumours ranged from 6 to 24 years and there was no correlation between the time interval and the tumour types.

(generation III) suffered from parathyroid tumours but three daughters and one son had a prolactinoma whilst another son had a somatotrophinoma. In addition, one of his daughters suffered from an insulinoma whereas a son suffered from a gastrinoma. The interval between the appearance of each tumour in a patient also varied and ranged from 6 to 24 years in the 15 MEN1 patients in whom the order of tumour development could be unequivocally established (Figure 3).

Age-related penetrance and a mutational model

The ages at which the first manifestation of an MEN1 tumour occurred could be established in 219/220 MEN1 patients and ranged from 8 to 79 years. Sixty-six of these 219 patients were asymptomatic and had been detected by biochemical screening; the remaining 153 patients had presented with symptoms. The cumulative percentages of patients who had developed MEN1 in the symptomatic group at the ages of 20, 35 and 50 years were 18%, 52% and 78%, respectively, whereas in the biochemically-screened asymptomatic group, these respective cumulative percentages were increased to 43%, 85% and 94% (Figure 4**a**). Thus biochemical screening detected an earlier onset ($p<0.001$) of MEN1 in all age groups.

The age-related penetrance (the proportion of gene carriers who have manifested symptoms or signs of the disease by a given age) of MEN1 was determined

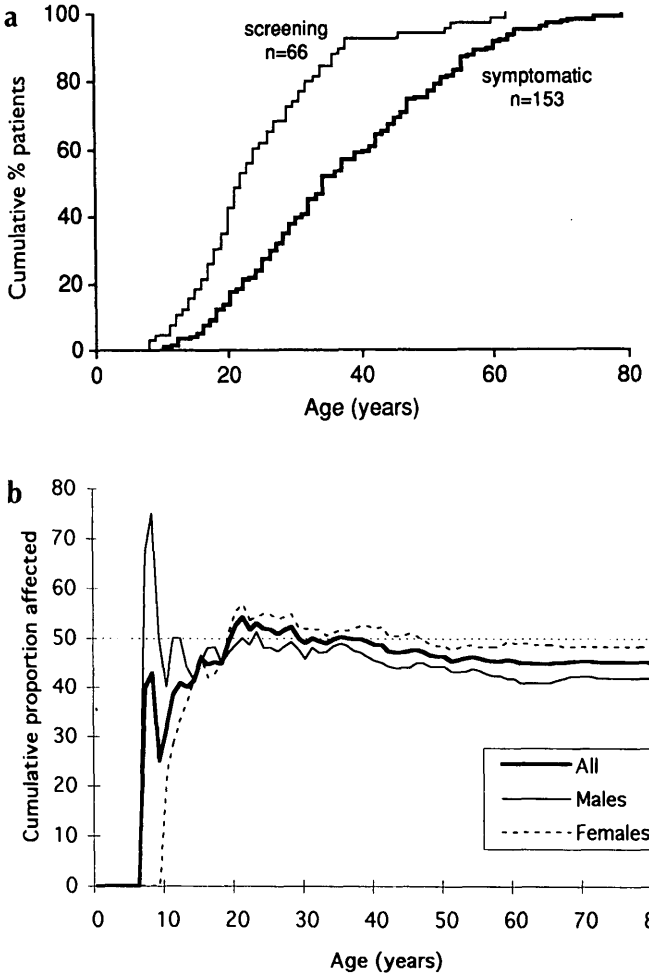

Figure 4. Age-related onset and penetrance of MEN1. The ages and cumulative percentages of patients (**a**) who had developed the first manifestation of MEN1 are shown for the symptomatic presenting group (bold line) and for the asymptomatic, biochemically detected group (faint line). The ratio of the affected individuals (■) to the total number (■ affected + □ unaffected) of individuals at different ages (**b**) represents an estimation of the penetrance of MEN1. The initial sharp rise in this ratio below the age of 10 years that was particularly observed in males is due to five individuals (4 males and 1 female) who are 4/89 V.3. 7/87 III.4, 13.3/92 III.1, 18/89 IV.7, and S7 (Appendix II). All four males had primary hyperparathyroidism, one also had an insulinoma and another also suffered from a corticotrophinoma; the one female, who suffered from an insulinoma and a non-functioning pituitary tumour, did not have hypercalcaemia. However, the expected 50% ratio for an autosomal dominant disorder was attained by 21 years and maintained until 40 years of age, after which it decreased to 42%. This decrease was attributed to the higher mortality associated with the gastrinomas and somatotrophinomas (Figure 5). These results indicate that MEN1 has a high penetrance by the age of 21 years.

and the results from 288 offspring (129 affected, 159 unaffected) of 101 affected parents are shown in Figure 4**b**. The 1 : 1 ratio for affected to unaffected individuals that would be expected for an autosomal dominant disorder was first achieved at 21 years, thereby indicating a near complete penetrance for MEN1 by this age. Interestingly, the ages of conversion from an unaffected to affected phenotype that were established for two individuals, 19/92 III.4 and 16/90 IV.20 (Appendix II), who had undergone annual biochemical screening from their early teens were found to be 20 and 21 years respectively; individual 19/92 III.4 developed primary hyperparathyroidism and underwent parathyroidectomy and individual 16/90 IV.20 developed a microprolactinoma and was treated with bromocriptine. However, the penetrance of MEN1 above the age of 21 years was not complete, as two male obligate carriers (the father of the affected male 7.2/89 III.1 and the father of the affected female 3/92 III.2, Appendix II) had no clinical or biochemical manifestations of MEN1 by the ages of 52 years and 53 years, respectively. Thus, 2/162 carriers remain unaffected, thereby indicating a 98.8% penetrance of the MEN1 gene by the age of 53 years.

The decline observed above the age of 40 years in the ratio of affected individuals to the total number of individuals could result either from an increased number of unaffected individuals or a decreased number of affected individuals. The former possibility is clearly implausible, and the latter possibility, which may be due to a greater death rate in MEN1 patients above the age of 40 years was indirectly assessed by investigating the age distribution (Figure 5) of the 384 tumours (Figure 1) in the affected individuals. The results revealed that above the age of 40 years, there was a higher occurrence of gastrinomas and somatotrophinomas, which would be associated with a higher mortality. Such differences were not observed for the occurrence of parathyroid tumours and prolactinomas in the two age groups (Figure 5) but insulinomas were found to occur more frequently in the younger age group, and this may partly contribute to the decline in the ratio of affected individuals to the total between the ages of 13 and 17 years (Figure 4**b**).

This significantly ($p<0.01$) higher occurrence of insulinomas in the younger age group (Figure 5) may also be due to their neuroglycopenic presentation, which would result in their earlier detection when compared with other MEN1 tumours. A further analysis of the ages at which the 26 MEN1 insulinomas occurred and their comparison to the previously reported ages at which 57 non-MEN1 insulinomas[17] occurred helped to estimate the number of mutations which were likely to be required for their development. The mean age ($\pm$SD) at which insulinomas were detected in the MEN1 patients (28.86 ± 19.09) was significantly ($p<0.04$) lower than that in the non-MEN1 patients (45.98 ± 16.96). An analysis of the age of incidence for MEN1 insulinomas and for non-MEN1 insulinomas gave similar results to those reported for bilateral and unilateral retinoblastomas,[16] respect-

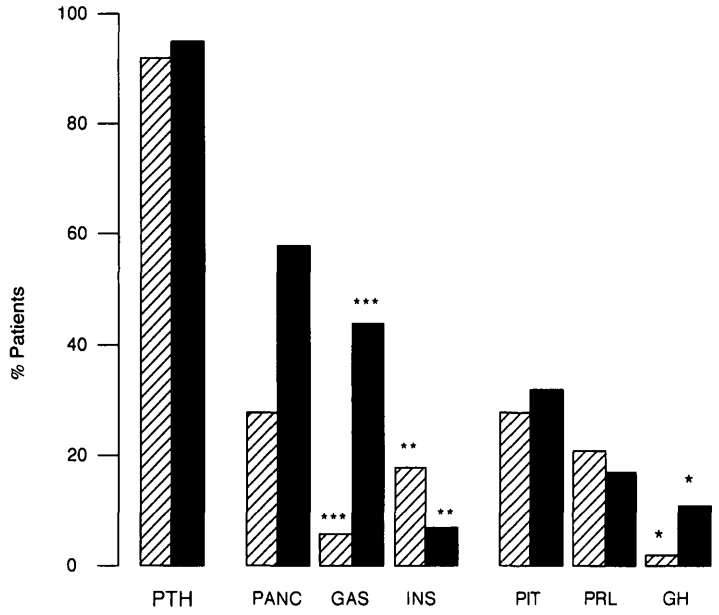

Figure 5. Development of 384 MEN1 tumours below (▨) and above (■) 40 years of age in 220 affected individuals (Figure 1). The proportion of patients developing parathyroid tumours (PTH), all pancreatic tumours (PANC), gastrinomas (GAS), insulinomas (INS), all pituitary tumours (PIT), prolactinomas (PRL) and somatotrophinomas (GH) is shown for each group. Significant differences (*$p<0.05$, **$p<0.01$ and ***$p<0.0001$) are indicated.

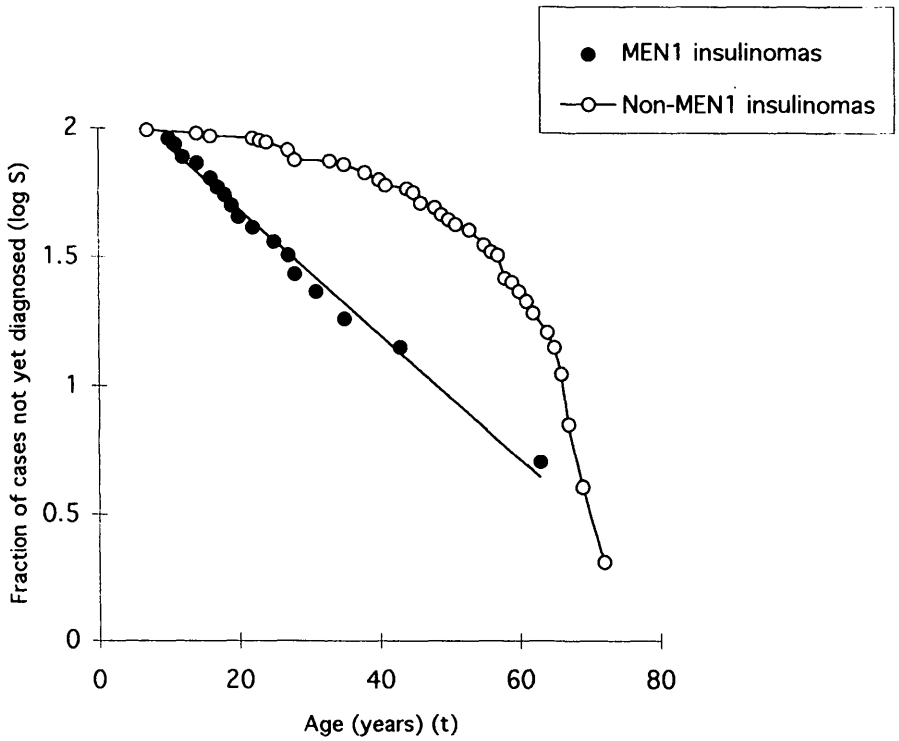

Figure 6. Age incidence of insulinoma in 26 familial MEN1 patients (●) and in 57 sporadic non-MEN1 patients[17] (○), based on the Knudson retinoblastoma model.[16] The age is plotted on the x axis and the corresponding $\log_{10}S$, which is the proportion of cases not yet diagnosed, is plotted on the y axis. The incidence by age of the familial MEN1 insulinomas ($n=26$) is best described by the first-order equation $y=0.024x+2.169$ ($r=0.994$), whereas that of the sporadic non-MEN1 insulinomas is best described by the second-order equation $y=-0.0004653x^2$ rather than a first-order equation ($p<0.001$). These results indicate that a single mutation is likely to be associated with the development of MEN1 insulinomas whereas two mutations are likely to be involved in the development of sporadic non-MEN1 insulinomas.

ively (Figure 6). Thus, the relationship between $\log_{10}$ S (the proportion of cases not yet diagnosed) and t (the age) was best described by a first-order equation for the MEN1 insulinomas and by a second-order equation for the non-MEN1 insulinomas. This situation is analogous to that for the retinoblastoma model in which two recessive mutations of a tumour suppressor gene are involved in oncogenesis; thus, MEN1 insulinomas require a single mutation, whereas non-MEN1 insulinomas require additional mutations.

Hypercalcaemia and coefficient of kinship

Parathyroid tumours are the most common and usually the first manifestation of MEN1 (Figures 2 and 3) and the detection of hypercalcaemia in members of MEN1 families represents a useful biochemical screening investigation. In order to assess which relatives of an MEN1 patient should be screened, we analysed the pre-treatment corrected serum calcium concentrations from 602 individuals according to their coefficient of kinship, KC (Figure 7). The corrected serum calcium concentrations for those individuals without previous parathyroid surgery (mean ± SD) in the KC 1.0 group

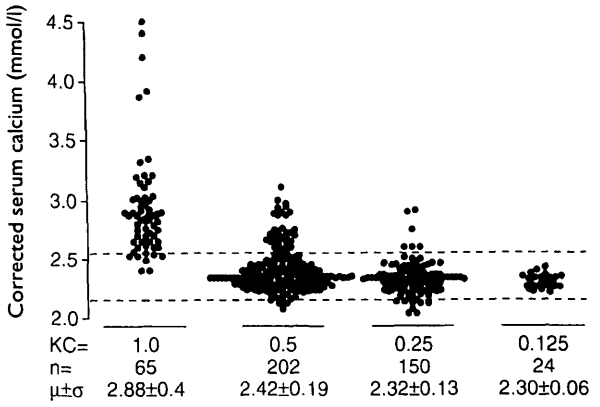

Figure 7. Corrected serum calcium concentrations by coefficient of kinship (KC). The normal range (2.20–2.55 mmol/l) for the corrected serum calcium is indicated by the broken lines. The mean corrected serum calcium concentrations and standard deviation (mean ± SD) for each of the KC groups 1.0, 0.5, 0.25, and 0.125 are shown. Data from KC group 0, i.e. the spouses ($n=161$, mean ± SD = 2.31 ± 0.09) are not shown. Hypercalcaemia was detected in only the first- (KC = 0.5) and second- (KC = 0.25) degree relatives of affected individuals (KC = 1.0).

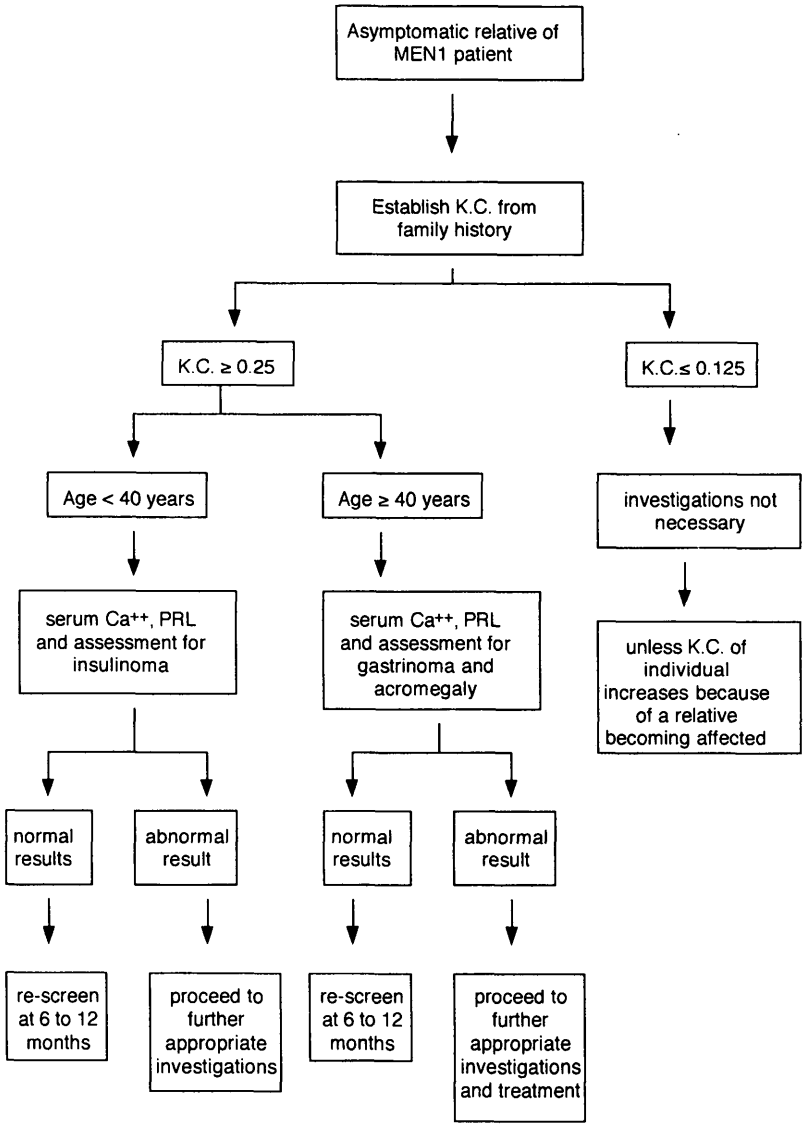

Figure 8. Screening protocol for an asymptomatic relative of a patient with MEN1. The relative should first have undergone a detailed clinical evaluation for MEN1-associated tumours to establish that the individual is asymptomatic. Relatives who are symptomatic should proceed to appropriate investigations and management. The KC refers to the coefficient of kinship (Figure 7), and helps to identify those members at a higher risk of developing MEN1. The MEN1 gene is located on chromosome 11q13[25,26] and the molecular genetic markers[24] which are not yet widely available, will help to identify those individuals with an affected haplotype[27] who have the greater likelihood of developing MEN1 tumours. Individuals identified either by KC or molecular genetic analysis as at higher risk should be entered into the screening programme as outlined.

(2.88 ± 0.40, $n=65$), was significantly ($p<0.001$) higher than in the KC 0.5 (2.42 ± 0.19, $n=202$), KC 0.25 (2.32 ± 0.13, $n=150$) and KC 0.125 (2.30 ± 0.06, $n=24$) groups. In addition, hypercalcaemia was observed in 91%, 19%, and 4% of the individuals in KC 1.0, 0.5 and 0.25 groups, respectively, but in 0% of the KC 0.125 group. These results indicate that hypercalcaemia is a useful investigation for the biochemical screening of MEN1 which has a high penetrance, and that such screening could be restricted to the first (KC = 0.5) and second (KC = 0.25) degree relatives of affected individuals.

Discussion

Our extensive study of 220 MEN1 patients has helped to define the manifestations and age-related penetrance of this autosomal dominant disorder. Parathyroid tumours were the first and most common of the lesions to develop, occurring in 87% and 94% of patients, respectively (Figures 2 and 3). In addition, pancreatic islet-cell tumours and anterior pituitary adenomas occurred in 40% and 30% of patients, respectively, with gastrinomas and prolactinomas representing the majority of

tumours in each of these respective categories. The occurrence of carcinoid tumours, adrenal cortical tumours and lipomata was 4%, 5%, and 1% in the patients, respectively. Our results, which represent the largest study of MEN1 patients and are comparable to those of previous reports,[1–4, 6–9, 18–20] also demonstrated the highly variable expression of MEN1. Thus, although the majority of patients had parathyroid tumours, the types of subsequent tumours originating from the pancreatic islets and the anterior pituitary showed considerable inter- and intra-familial variation (Figure 1). In addition, the time interval between the development of the first and subsequent tumours in a patient also varied considerably from 6 to 24 years (Figure 3). Our results are the first to demonstrate that gastrinomas and somatotrophinomas occur more frequently in MEN1 patients who are above the age of 40 years, that insulinomas occur more frequently in MEN1 patients who are below the age of 40 years (Figure 5), and that the genetic aetiology of MEN1 insulinomas (Figure 6) is consistent with Knudson's two-hit hypothesis.[16,21–23] In addition, our defining of the age-related onset for MEN1 tumours, which were confined to the first (KC = 0.5) and second (KC = 0.25) degree relatives (Figure 7), helped in the estimation of residual risks (i.e. 100% — [age-related onset %], Figure 4**a**), for these individuals. Thus, the residual risks for developing MEN1 tumours in such unaffected relatives who are biochemically normal are estimated to be 57%, 15% and 6% at the ages of 20, 35, and 50 years, respectively.

These results have facilitated the development of a proposed screening programme to help in the management of members from MEN1 families (Figure 8). Screening should be initiated before 8 years of age, as affected children (III.4 from family 7/87 and IV.7 from family 18/89, Appendix II) have been observed by this age. Biochemical screening should be undertaken in those with a KC $\geqslant$ 0.25. In addition, the use of molecular genetic markers that are close to the MEN1 gene[24] which is located on chromosome 11q13[25,26] will help further to identify those individuals who have inherited the affected haplotype[27] and are therefore at an increased risk of developing the disease. Serum calcium and prolactin concentrations should be determined in all such asymptomatic individuals, as parathyroid tumours or prolactinomas occur alone or in combination in 97.7% of MEN1 patients. In addition, further clinical and biochemical assessments should be particularly performed for insulinomas in individuals below the age of 40 years and for gastrinomas and somatotrophinomas in individuals above the age of 40 years. Family members who remain asymptomatic and biochemically normal should be rescreened at 6- to 12-monthly intervals until the age of 50 years, by which age the disease will have developed in 94% of individuals at risk. An application of this proposed screening protocol which has been derived from our comprehensive analysis of MEN1 patients will help in the earlier detection of MEN1 tumours and thereby appropriate management of patients with this inherited endocrine disorder.

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Appendix I: MEN1 patient questionnaire

Please supply details of patients with MEN1 (i.e. 2 or more tumours), or patients who have one tumour and a family history of MEN1, or any individual who has been screened in a family with MEN1.

NAME AND ADDRESS and/or identification code (e.g.hospital number)

DATE OF BIRTH: **SEX:**

SYMPTOMATIC/ASYMPTOMATIC

		List order (1,2,3...) Date/age of onset
renal stones	yes/no	
bone pain	yes/no	
indigestion	yes/no	
ulcers	yes/no	
diarrhoea	yes/no	
fits	yes/no	
amenorrhoea	yes/no	
galactorrhoea	yes/no	

TREATMENT:	**Drug** (date)	**Surgery** (date)	**pathology report** (precis and reference numbers)
parathyroid tumour			
pancreatic tumour			
pituitary tumour			
renal stones			
ulcers			

KNOWN FAMILY HISTORY yes/no

CONSULTANT/GP in charge:(name address and telephone number)

Blood obtained for:	cell line	(10-20 ml heparin)	yes/no
	DNA	(10-20 ml EDTA)	yes/no
	Growth factor EDTA and heparin)	(10-20 ml each of clotted	yes/no

SERUM BIOCHEMISTRY RESULTS: (indicate "ND" if not done)

	date:	**date:**	**date:**	**date:**
Calcium				
Albumin				
Corrected calcium				
Ionised calcium				
Creatinine				
Phosphate				
PTH				
Prolactin				
GH				
ACTH				
Gastrin				
Insulin/glucose				
Pancreatic polypeptide				
Others				

Please provide a **FAMILY TREE** below; include all affected and unaffected members, give dates of birth whether alive/dead (age at death) and maiden name or origin, if possible

Please return completed form to Professor RV Thakker, MRC Molecular Endocrinology Group, Collier Building, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 ONN, UK, tel: (44) (0)181 740 3014; fax (44) (0)181 749 8341

Appendix II: Clinical details of 220 MEN1 patients

Family	Patient no.	Sex	Age of detection	Tumour			
				Parathyroid	Pancreatic	Pituitary	Other
1/90	III.4	F	18	PTX			
1/91	I.3	M	28	PTX	GAS_r		
1/92	III.3	M	27	PTX	GAS_s		
2/92	II.3	F	21	PTX			
2.2/91	I.1	M	45		TUM_{pm}		ADR_{pm}
	II.1	M	34	PTX	GAS_r		
	III.1	M	11	PTX	INS_s		
2.4/90	II.8	F	62	PTX	GAS_s		
	II.10	F	60	PTX			
3/92	II.5	M	33	PTX		GH_s	
	III.2	F	22	PTX			
3.3/87	II.1	M	16	Ca	GAS_r		
3.4/87	II.1	M	23	PTX		PRL_r	
3.5/87	I.1	M	20	Ca	INS_s		
	II.1	M	34	PTX			
3.6/87	II.1	M	29	PTX	GAS_a		
4/89	III.1	M	67	PTH	GAS_r		
	III.2	M	46	PTX	GAS_s		
	III.4	F	45	PTX	GAS_{pm}	NFT_{pm}	
	III.7	F	42	PTX			
	III.12	M	39	Ca			
	III.17	F	54	Ca	GAS_r		
	III.19	F	20	PTX			
	III.21	F	55	Ca			
	IV.3	M	26	Ca			
	IV.6	F	31	Ca			
	IV.7	M	31	PTH		PRL_r	
	IV.10	M	24	PTX			
	IV.16	F	21	Ca			
	IV.19	F	36	Ca			
	IV.20	M	27	PTH			
	IV.23	F	38	Ca			
	IV.25	F	23	Ca			
	V.1	F	15	PTX			
	V.3	M	9	PTX			
	V.6	M	14		INS_s		
4.2/90	I.2	F	36	PTX			
	II.2	F	18	PTX			LIP_s
	II.3	M	20	PTX			
4.3/92	II.1	F	55	PTX	INS_s		
	II.4	M	35	PTX			
	III.2	F	16	PTX	INS_s		
5/87	II.1	M	37	PTX			
6/91	II.1	M	25	PTX	GAS_{gx}		
	III.1	F	17	Ca		PRL_r	
6.2/92	I.1	F	55	PTX	GAS_a	PRL_r	
	I.2	M	63	PTX	TUM_{pm}		
	II.1	M	32	PTX	GAS_u		ADR_sPHAE_s
	III.2	M	16	PTH		PRL_r	
	III.1	M	16	PTH			
6.4/91	II.1	F	26	PTX	GAS_r	PRL_r	
	II.2	F	62	PTX			
	III.1	F	26	PTX			
7/87	I.2	F	50	PTX			

Family	Patient no.	Sex	Age of detection	Tumour			
				Parathyroid	Pancreatic	Pituitary	Other
	II.2	F	38	PTX			
	II.7	M	28	PTX	TUM_{pm}	PRL_r	
	III.2	F	19	PTX			
	III.3	F	14	PTH			
	III.4	M	8	PTH		$ACTH_r$	
7.2/89	II.4	M	29	PTH	GAS_a		CRN_s
	II.6	F	29	Ca		PRL_s	
	III.1	M	22	Ca			
	III.3	F	25	PTX		PRL_s	
7.3/90	I.2	F	67	PTX			
	II.2	M	29	PTX			
	III.1	M	18	Ca			
	III.2	M	17	Ca			
7.4/91	II.1	M	42	PTX	GAS_u		
	II.2	F	52	PTX	GAS_u		
8/89	II.1	M	55	PTX	GAS_r		
	II.4	F	35	PTX			
	III.3	F	29	PTH			
	III.4	F	26	PTX		PRL_r	
8.2/90	III.5	M	39	PTH			
	IV.3	M	24	Ca			
	IV.4	M	35	Ca			
	IV.6	F	32	PTX			
	IV.8	F	17	PTH			
8.5/90	II.1	F	51	PTX			
8.7/87	II.1	M	75		TUM_r		
	II.2	F	20	Ca			
8.10/91	II.1	F	30	PTX	INS_sGAS_s		
10/90	I.1	M	45	PTX		PRL_s	
11/89	II.7	F	25			PRL_r	
	II.5	M	42	PTX	GAS_r		
	III.5	M	18	PTX		PRL_s	
11.2/90	II.3	M	55	PTH			
	II.14	F	21	PTX	GAS_r	PRL_s	ADR_r
	III.2	M	32	PTX	GAS_s		
	III.4	F	25			PRL_r	
11.3/90	II.2	F	22	Ca			
	II.4	F	20	PTX			
12.3/87	II.2	F	18	PTX			
	III.1	F	52	PTX	GAS_r		
13/89	II.1	M	33	PTX	GAS_u	PRL_r	
	III.1	M	27	PTX	GAS_s		
	III.4	F	37	PTX		PRL_g	
	III.5	M	32	PTH		PRL_r	
	III.7	F	33	PTH			
	III.8	F	25	Ca	INS_s	PRL_r	
	III.10	F	15	PTH	INS_s	PRL_r	
	III.11	M	20	PTH		GH_s	
	IV.1	M	17	PTH			
	IV.3	M	12	PTH			
13/92	II.2	F	60	PTX			
	III.4	F	53	Ca			
	III.2	F	53	PTX	GAS_r	GH_s	
13.2/90	II.1	M	47	PTX			
	II.3	F	54	PTX	GAS_s		
	III.1	F	15	PTX			
13.3/90	II.5	F	60	PTX			

Family	Patient no.	Sex	Age of detection	Tumour			
				Parathyroid	Pancreatic	Pituitary	Other
	II.8	M	24	PTX			
	III.24	F	11			PRL_r	
13.3/92	II.2	M	47	PTX	GAS_r		
	III.1	F	10		INS_s	NFT_s	
13.10/87	II.1	F	25	PTX	GAS_{gx}		
	III.1	F	20	PTH			
13.5/90	III.1	F	18	PTX	INS_s	PRL_r	
13.7/87	II.1	F	29	PTX	GAS_s		
13.8/92	I.2	F	20	PTX	GAS_u	PRL_r	
	II.1	F	21	PTX			
16/90	II.10	F	70	PTX			
	II.11	M	68	PTH			
	III.19	M	37	PTH			
	III.21	M	39	PTH			
	III.14	F	46	PTH			
	III.32	M	28	PTX	GAS_r	PRL_r	
	III.35	M	32	PTX	GAS_r	PRL_r	
	IV.18	M	14	PTX			
	IV.20	M	21			PRL_r	
	IV.21	F	13	PTH			
16/92	III.5	M	24	PTX			
	III.10	F	23	Ca			
	II.5	F	36	PTX			
	III.12	F	32	Ca			
	II.1	M	36	PTX	GAS_r		
	II.3	F	35	PTX	GAS_r	PRL_r	
16.3/87	II.1	M	22	PTX	TUM_s		
18/89	II.3	M	23	PTX	GAS_s	GH_rACTH_r	NF
	III.4	F	30	PTH			
	III.6	F	29	PTX		$ACTH_r$	
	III.7	M	24	PTH			
	III.15	M	32	PTX	GAS_sINS_s		
	III.13	F	19	PTX			ADR_r
	IV.7	M	8	PTH			
18.2/91	II.4	F	26	Ca		PRL_r	
	II.7	F	21	PTX			
19/89	II.2	M	41	PTX		TUM_s	
	III.4	F	16		INS_s		
19/92	II.2	M	47	PTX			CRN_{pm}‡, ADR_{pm}
	II.3	F	39	PTX	GAS_sINS_s		ADR_{pm}
	III.4	F	20	PTX			
	III.6	M	17	Ca			
19.1/92	II.6	F	22	PTH		PRL_r	
	I.2	F	61	PTX			
19.2/89	I.2	F	56	PTX	GAS_r	NFT_r	
	II.2	F	28	PTX		PRL_r	
19.2/92	II.1	M	71			PRL_h	
	III.2	F	35	PTX	GAS_s		
	IV.3	F	11	PTX			
19.3/89	III.5	F	41	PTX			
	III.6	F	40	PTX			CRN_s
	IV.1	M	30	PTX	GAS_s		
	IV.6	M	35	PTX			
	V.1	F	12	PTX			
	V.2	M	12	PTX			
19.4/91	II.3	F	31	PTX	INS_s		
19.6/87	II.6	F	62	PTX			

Family	Patient no.	Sex	Age of detection	Tumour			
				Parathyroid	Pancreatic	Pituitary	Other
	II.7	F	56	PTX			
	II.8	F	51	PTX		GH_s	
20/89	I.2	F	40	PTX			
	II.2	F	50	PTX	GAS_s		ADR_r
	III.2	M	26	PTH			
23/89	II.6	F	60	PTX	GAS_a		
	II.8	F	51	PTX		PRL_r	
	III.12	F	20	PTX	TUM_{pm}	NFT_{pm}	ADR_{pm}
	III.13	F	27	PTX	NFT_s		
23.2/87	I.1	F	44	PTX	GAS_r		
23.3/87	II.1	F	12	PTX	INS_s		
23.5/87	II.3	F	57	PTX	GAS_u		$CRN_s^{\dagger}$
23.6/90	II.2	M	35	PTX		GH_r	
25/90	III.6	F	19	Ca		PRL_r	
	V.4	M	20	PTH			
25.1/92	II.1	F	34	PTX	INS_s	PRL_r	
S1		F	47	PTH	GAS_s		
S2		F	59	PTX		GH_r	
S3		M	37	PTX	GAS_s		ADR_s
S4		F	27	PTX	INS_s		
S5		F	79	PTX	INS_s		
S6		F	52	PTX		GH_s	
S7		M	10	PTX	INS_s		
S8		F	48	PTX	GAS_r	GH_g	
S9		F	50	PTX		GH_s	
S10		M	17	Ca	INS_s		
S11		F	57	PTH		GH_r	
S12		M	18	PTX	GAS_r		
S13		M	47	PTX		GH_s	
S14		M	42	PTX	GAS_sGCG_s	PRL_sGH_s	$CRN_sT_sMM_s$
S15		M	42		GAS_sGCG_s		ADR_r
S16		M	44	PTX	GAS_{pm}	PRL_r	
S17		F	30	PTX	GAS_s	PRL_s	
S18		M	46	PTX	GAS_s		
S19		F	20			GH_s	CRN_s
S20		F	37	PTX	GAS_s		
S21		F	31	PTX	INS_s		
S22		M	47	PTX			CRN_s
S23		F	19	PTX	INS_s	PRL_r	
S24		F	63	PTH	INS_s		
S25		F	32	PTX	GAS_s		
S26		F	28	PTX	INS_s	PRL_r	
S27		F	43	PTX	GAS_r	PRL_r	
S28		M	12	PTX	INS_s		
S29		M	44	PTX		NFT_s	
S30		F	54	PTX		PRL_s	ADR_s*
S31		M	43	PTX			$CRN_s^{\dagger}$
S32		F	24	PTX		$ACTH_s$	
S33		M	32	PTX		GH_s	
S34		F	18	PTX	GAS_sINS_s	PRL_r	LIP_s
S35		M	36	PTX	GAS_s		
S36		M	22	PTX	GAS_s		

Ca, persistent hypercalcaemia; PTH, high serum PTH level and hypercalcaemia; PTX, parathyroidectomy.

GAS, pancreatic tumour secreting gastrin confirmed on the basis of: $_a$ acid output studies, $_g$ gastrectomy, $_u$ repeated gastric ulceration, $_r$ radiology, $_s$ surgery, $_{pm}$ post-mortem.
INS, pancreatic tumour secreting insulin, confirmed by: $_b$ biochemistry, $_s$ surgery.

GCG, pancreatic tumour secreting glucagon: $_{s}$ surgery.
TUM, tumour type unknown: $_{r}$ radiology, $_{pm}$ at post mortem.
NFT, non-functioning tumour: $_{s}$ surgery.

PRL, prolactinoma on basis of: $_{g}$ galactorrhoea, $_{h}$ persistent hyperprolactinaemia and/or confirmed by: $_{r}$ radiology, $_{s}$ surgery, $_{pm}$ post-mortem.
GH, somatotrophinoma on basis of biochemical data and: $_{s}$ surgery, $_{r}$ radiology.
ACTH, corticotrophinoma on basis of biochemical data and: $_{s}$ surgery, $_{r}$ radiology, $_{s}$ glucose tolerance test.
NFT, non-functioning tumour found at: $_{s}$ surgery, $_{r}$ radiology, $_{pm}$ post-mortem.
TUM, tumour of unknown type found at: $_{s}$ surgery.

ADR, adrenocortical tumour found at: $_{r}$ radiology, $_{s}$ surgery, $_{pm}$ post-mortem, *carcinoma.
PHAE, phaeochromocytoma.
CRN, carcinoid tumour demonstrated biochemically and: $_{s}$ surgery, $_{r}$ radiology, $_{pm}$ post-mortem, †gastric, ‡thymic.
LIP, lipomata: $_{s}$ surgery.
T, testicular teratoma: $_{s}$ surgery.
MM, malignant melanoma.