Outi Vierimaa

MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 (MEN1) AND PITUITARY ADENOMA PREDISPOSITION (PAP) IN NORTHERN FINLAND
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Academic dissertation to be presented, with the assent of the Faculty of Medicine of the University of Oulu, for public defence in Auditorium 5 of Oulu University Hospital, on June 27th, 2008, at 12 noon

OULUN YLIOPISTO, OULU 2008
Abstract

Multiple endocrine neoplasia type 1 (MEN1) is an inherited syndrome characterized by parathyroid, gastrointestinal and pituitary neuroendocrine tumours. In Northern Finland, two founder mutations of the MEN1 gene (1466del12, 1657insC) accounting for the majority of the MEN1 cases, have common ancestors born in the 18th and 19th centuries, respectively. Three small clusters of familial pituitary adenoma have also been detected, two of which could be linked by genealogy to a common ancestral couple born in the 18th century.

Clinical evaluation of 82 MEN1 mutation carriers showed that age was a risk factor for most of the MEN1-related manifestations. In the whole group, nonfunctional pancreatic tumour (NFPT) was more common in the frameshift/nonsense mutation carriers (odds ratio 3.26; 95% confidence interval 1.27–8.33, P = 0.014), whereas gastrinoma was more common in the in-frame/missense mutation carriers (OR 6.77, CI 1.31–35.0, P = 0.022). In the founder mutation carriers, the 1657insC mutation predicted the risk for NFPT (OR 3.56, CI 1.29–9.83, P = 0.015), while the 1466del12 mutation was associated with the risk for gastrinoma (OR 15.1, CI 1.73–131.9, P = 0.014).

The mean ages at death of the 32 obligatory MEN1 founder mutation carriers born between 1728 and 1929 were compared to those of the 29 spouses and sex-matched life expectancy estimates derived from Finnish national statistics. The ages at death of the mutation carrier males (61.1 ± 12.0 years) and females (67.2 ± 10.7 years) did not differ from the control groups.

PAP (pituitary adenoma predisposition) locus was mapped in the chromosome region 11q12–11q13 by whole-genome single-nucleotide polymorphism genotyping. Combining the linkage and the gene expression array data, AIP (aryl hydrocarbon receptor interacting protein) was chosen for sequencing. The nonsense mutation Q14X was identified in the affected (acromegaly, gigantism, prolactinoma) family members and in four other patients. Loss of heterozygosity was detected in pituitary adenomas of AIP mutation carriers.

Mutation analysis of MEN1, HRPT2 (hyperparathyroidism 2), CASR (calcium-sensing receptor), CDKN1B (cyclin-dependent kinase inhibitor 1B) and AIP genes was performed in primary hyperparathyroidism patients with features of inherited predisposition. One out of 29 patients was found to have the 1466del12 mutation, while no mutations were detected in other genes.

Keywords: genetic predisposition, multiple endocrine neoplasia type 1, pituitary adenoma, primary hyperparathyroidism
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Haukipudas, May 2008

Outi Vierimaa
Abbreviations

ACTH  adrenocorticotropic hormone
AHR  aryl hydrocarbon receptor
AIP  *aryl hydrocarbon receptor interacting protein gene*
CASR  *calcium-sensing receptor gene*
CDKN1B  *cyclin-dependent kinase inhibitor 1B gene*
CGA  chromogranin A (CgA)
CGB  chromogranin B (CgB)
CHES1  chekpoint suppressor 1
CI  confidence interval
CNC  Carney complex
CS  Cushing’s syndrome
CT  computed tomography
DNA  deoxyribonucleic acid
ECLoma  enterochromaffin-like cell tumour
EUS  endoscopic ultrasound
FHH  familial hypocalciuric hypercalcaemia
FIHP  familial isolated hyperparathyroidism
FIPA  familial isolated pituitary adenoma
FMTC  familial medullary thyroid cancer
FSH  follicle-stimulating hormone
GEP  gastroenteropancreatic
GH  growth hormone
GHRH  growth hormone-releasing hormone
GHRHoma  growth hormone-releasing hormone-secreting tumour
HPT-JT  hyperparathyroidism-jaw tumour syndrome
HRPT2  *hyperparathyroidism 2 gene*
IFS  isolated familial somatotropinoma
IGF-1  insulin-like growth factor 1
kD  kilodalton
LCCST  large-cell calcifying Sertoli cell tumor
LH  luteinizing hormone
LOH  loss of heterozygosity
LOD  logarithm of odds
MEN1  multiple endocrine neoplasia type 1
*MEN1  multiple endocrine neoplasia type 1 gene*
<table>
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<th>Acronym</th>
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<td>MEN2</td>
<td>multiple endocrine neoplasia type 2</td>
</tr>
<tr>
<td>MEN4</td>
<td>multiple endocrine neoplasia type 4</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
</tr>
<tr>
<td>NFPT</td>
<td>nonfunctional pancreatic (neuroendocrine) tumour</td>
</tr>
<tr>
<td>NLS</td>
<td>nuclear localization signals</td>
</tr>
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<td>NM23H1</td>
<td>nonmetastatic protein 23, homolog 1</td>
</tr>
<tr>
<td>OMIM</td>
<td>Online Mendelian Inheritance in Man</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
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<td>PAP</td>
<td>pituitary adenoma predisposition</td>
</tr>
<tr>
<td>PHPT</td>
<td>primary hyperparathyroidism</td>
</tr>
<tr>
<td>PP</td>
<td>pancreatic polypeptide</td>
</tr>
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<td>PPNAD</td>
<td>primary pigmented nodular adrenocortical disease</td>
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<tr>
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<td>PTH</td>
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<td>vasoactive intestinal peptide</td>
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<td>vs.</td>
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<td>ZES</td>
<td>Zollinger-Ellison syndrome</td>
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</tbody>
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List of original publications

This thesis is based on the following articles, which are referred to in the text by their Roman numerals:


## Contents

Abstract

Acknowledgements

Abbreviations

List of original publications

## Contents

5 Introduction 13

6 Review of the literature 17

6.1 Multiple endocrine neoplasia type 1 (MEN1)................................. 17

6.1.1 History and definition ................................................................. 17

6.1.2 Clinical features ....................................................................... 18

6.1.3 Clinical management of MEN1 mutation carriers .................. 33

6.1.4 Mortality .................................................................................. 34

6.1.5 Genetics of MEN1 ................................................................... 35

6.2 Other disorders with inherited predisposition to primary
hyperparathyroidism (PHPT) and/or anterior pituitary adenomas........ 38

6.3 Multiple endocrine neoplasia type 2A (MEN2A)............................ 42

6.3.1 Hyperparathyroidism-jaw tumour syndrome (HPT-JT) .......... 43

6.3.2 Familial hypocalciuric hypercalcaemia (FHH) .......................... 44

6.3.3 Familial isolated hyperparathyroidism (FIHP) ......................... 45

6.3.4 Carney complex (CNC) .............................................................. 45

6.3.5 Familial isolated pituitary adenoma (FIPA) ............................. 47

6.3.6 Isolated familial somatotropinoma (IFS) ................................. 48

6.3.7 Multiple endocrine neoplasia type 4 (MEN4)............................ 49

7 Purpose of the present study 51

8 Subjects and methods 53

8.1 Subjects and families.................................................................... 53

8.1.1 Clinical features and genotype-phenotype correlation of
MEN1 in Northern Finland (I) ......................................................... 53

8.1.2 Study on Premature Mortality in MEN1 founder mutation
carriers (II) ..................................................................................... 53

8.1.3 Pituitary adenoma predisposition (III) .................................... 54

8.1.4 Primary hyperparathyroidism patients with features of
 genetic predisposition (IV) ............................................................ 55
8.2 Biochemical and imaging studies of MEN1 mutation carriers and PHPT patients with features of genetic predisposition (I, IV) ........... 55
8.3 Obtaining clinical data and causes of death (I) .............................. 56
8.4 Comparing mean values of age at death (II) ................................. 57
8.5 Histopathology and immunohistochemistry (III) ............................ 57
8.6 SNP array and linkage analyses (III) ............................................. 57
8.7 Expression profiling (III) ............................................................... 59
8.8 Mutation screening and search for loss of heterozygosity (LOH) (III, IV) ............................................................................... 59
8.9 Ethical issues .................................................................................. 60

9 Results 63
9.1 MEN1 in Northern Finland; clinical features and genotype-phenotype correlation (I) ................................................................. 63
9.2 Effect of MEN1 founder mutations on premature mortality (II) ...... 67
9.3 Characterization of clinical and genetic aspects of pituitary adenoma predisposition (PAP) (III) ...................................................... 70
9.4 Mutation analysis of MEN1, HRPT2, CASR, CDKN1B and AIP genes in PHPT patients with features of genetic predisposition (IV) .... 72

10 Discussion 75
10.1 Clinical features of MEN1 and genotype-phenotype correlation (I) ...... 75
10.2 Mortality in MEN1 (I, II) ................................................................. 78
10.3 Pituitary adenoma predisposition and AIP (III) ............................... 82
10.4 PHPT patients with features of genetic predisposition (IV) ............. 85

11 Conclusion and future prospects 89

References

Original publications
1 Introduction

Multiple endocrine neoplasia type 1 (MEN1) (Online inheritance in man, OMIM 131100) characterized by tumours of the parathyroid, neuroendocrine cells of the pancreas, duodenum and anterior pituitary is one of numerous hereditary tumour predisposition syndromes (Marx 2001). In addition to MEN1, there are also other tumour predisposition conditions with target organs including endocrine glands. The variety of target organs and clinical manifestations are distinctive to a certain syndrome, although overlapping exists as well. The feature almost all of these syndromes have in common is the mode of inheritance, which is autosomal dominant. Therefore, the offspring of the mutation carrier parent are at 50-percent risk of inheriting the disease associating gene, and thus at risk of being affected by the disorder.

The first patient described with presumable MEN1 was reported more than 100 years ago (Erdheim 1903), and the gene behind this disorder, MEN1, was identified in 1997 (Chandrasekharappa et al. 1997, Lemmens et al. 1997). Despite the well-characterized clinical features of MEN1, it is noteworthy that the frequency of the manifestations tends to vary considerably between different studies (Table 1). Whether any of this variation of clinical features is dependent on the genotype of affected heterozygotes is under debate, although the majority of studies doubts the existence of genotype-phenotype correlation in MEN1 (Agarwal et al. 1997, Bassett et al. 1998, Giraud et al. 1998, Wautot et al. 2002).

The majority of the MEN1-associated manifestations are due to benign tumours of neuroendocrine cells, although those locating in foregut-derived tissues (especially duodenum, thymus, ventricle) and pancreas may be malignant (Falchetti et al. 2008). Several studies suggest that MEN1 patients are at increased risk of premature death compared to their non-affected relatives or normal population (Vasen et al. 1989, Wilkinson et al. 1993, Carty et al. 1998, Dean et al. 2000, Geerdink et al. 2003). The major causes of MEN1-related deaths have changed during the past decades. Previously, peptic ulcer disease and renal complications of hypercalcaemia due to primary hyperparathyroidism were among the most important causes of death in MEN1 patients (Ballard et al. 1964, Majewski & Wilson 1979, Vasen et al. 1989). The most recent studies suggest that malignant MEN1-related tumours are the major cause of death in MEN1 (Wilkinson et al. 1993, Carty et al. 1998, Doherty et al. 1998, Dean et al. 2000, Geerdink et al. 2003, Ferolla et al. 2005). It is estimated that approximately one third of the patients die from a MEN1-related cancer (Falchetti et al. 2008).
<table>
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<td>38</td>
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G, gastrinoma; NF, nonfunctional; I, insulinoma; PRL, prolactin-secreting tumour; GH, growth hormone-secreting tumour; NA, not available.

*One of the pituitary adenomas was a mixed growth hormone- and prolactin-producing tumour.

*Carcinoid (neuroendocrine) tumours were found in 8 (4%) patients.

*Carcinoid tumours were found in 10 (4%) patients.

*Thymic or bronchial carcinoids were present in 25 (8%) patients.

*The study population was divided into 5 birth cohorts and figures (penetrance in percentages) were given for each cohort. The figures shown here are the maximal penetrance percentages of each tumour category.
It is estimated that approximately 5% of the patients with primary hyperparathyroidism (PHPT) or pituitary adenoma have a hereditary form of the disease (Miedlich et al. 2003, Daly et al. 2006). MEN1 is the most common aetiologic factor for both of these tumours in familial setting. Other syndromes seen in association with dominantly inherited PHPT are multiple endocrine neoplasia type 2 (MEN2), hyperparathyroidism-jaw tumour syndrome (HPT-JT), familial hypocalseuric hypercalcaemia (FHH), and a newly discovered MEN1-like entity, multiple endocrine neoplasia type 4 (MEN4). The underlying genes in the corresponding order are RET, HRPT2, CASR and CDKN1B (Mulligan et al. 1993, Pollak et al. 1993, Carpten et al. 2002, Pellegata et al. 2006). Familial isolated hyperparathyroidism (FIHP) can result from the incomplete expression of a syndromic form of familial hyperparathyroidism, but it can also represent some other entity yet to be determined (Simonds et al. 2002). In addition to MEN1, pituitary tumours are seen in familial setting in Carney complex (CNC), familial isolated pituitary adenoma (FIPA), isolated familial somatotropinoma (IFS), and MEN4 (Daly et al. 2006). Germline mutations in PRKARIA are found in the majority of CNC patients (Kirschner et al. 2000). The locus for IFS has been mapped to chromosome 11q13 by several studies (Gadelha et al. 2000, Luccio-Camelo et al. 2004, Soares et al. 2005), but the genetic background for other types of FIPA families has remained unknown.

In the present work, one of the principal aims was to study the clinical features and possible genotype-phenotype correlation in Northern Finnish MEN1 patients. Another purpose of this study was to evaluate the mortality in MEN1 mutation carriers comparing the age at death to that of non-carrier spouses and to statistical life expectancy values. In addition, one of the main purposes of this work was to define the clinical features and genetic background of previously uncharacterized pituitary adenoma predisposition observed in the area. One further goal of this study was to investigate PHPT patients with features suggestive of genetic predisposition in order to evaluate the role of different genes in the aetiology of this endocrine disorder.
2 Review of the literature

2.1 Multiple endocrine neoplasia type 1 (MEN1)

2.1.1 History and definition

The main features of multiple endocrine neoplasia type 1 (MEN1) are parathyroid adenoma, enteropancreatic and foregut neuroendocrine tumours and anterior pituitary adenoma (Marx SJ 2001). Nonendocrine manifestations include lipoma, facial angiofibroma, skin collagenoma, leiomyomas of different sites, meningioma and ependymoma (Giraud et al. 1997, Pack et al. 1998, McKeeby et al. 2001, Asgharian et al. 2004). Neuroendocrine tumours may cause various symptoms depending on their hormonal activity. In addition, hormonally silent or active tumours may cause symptoms due to local compression or metastatic growth to distant organs.

The prevalence of MEN1 is estimated to be 10–17.5/100,000 (Betts et al. 1980, Eberle & Grun 1981, Watson et al. 1989, Öberg et al. 1989), and according to autopsy series as high as 2.5/1,000 (Lips et al. 1962, Lips et al. 1984).

The first description of a MEN1 patient was published in 1903. Erdheim reported the autopsy of a patient with acromegaly, pituitary adenoma, and four enlarged parathyroid glands (Erdheim 1903). Thirty-six years later, two sisters were reported with nephrolithiasis in association with tumours of the parathyroid glands and the pancreatic islets (Rossier & Dressler 1939). There was also a family history of peptic ulcer disease on the father’s side of the family; however, it was not until 1961 that it could be linked to the sisters’ endocrine tumours (Schmid et al. 1961). The hereditary nature of MEN1 was first suggested in the 1950s by Moldawer and Wermer (Moldawer 1953, Moldawer et al. 1954, Wermer 1954). Prolactinoma was first proposed as a component of MEN1 in 1974 by Cryer et al. The MEN1 syndrome has also been called Wermer’s syndrome and multiple endocrine adenomatosis type 1 (MEA1).

The genetic basis of MEN1 began to be resolved in 1988, when Larsson et al. mapped the MEN1 locus to chromosome 11q13 by family studies. They also found out that the tumours (insulinomas) showed loss of the normal allele of chromosome 11, providing evidence for MEN1 acting as a tumour suppressor gene (Larsson et al. 1988). In 1997, the MEN1 gene was characterized almost simultaneously by two groups (Chandrasekharappa et al. 1997, Lemmens et al. 1997).
2.1.2 Clinical features

Primary hyperparathyroidism (PHPT)

Primary hyperparathyroidism (PHPT) is in general a relatively common endocrinopathy. PHPT is diagnosed by persistent hypercalcaemia in the presence of inappropriately normal or elevated levels of parathyroid hormone (PTH) (Bilezikian et al. 2002). The prevalence of PHPT in postmenopausal women is estimated to be 21/1,000, or 3/1,000 in the general population with an annual incidence of approximately 20/100,000, depending on the screening methods used (Melton 1991, Adami et al. 2002). It has been estimated that approximately 2–3% of PHPT is associated with MEN1 (Marx 2001). PHPT is the most common and usually the earliest clinical manifestation of MEN1 showing almost total age-dependent penetrance among MEN1 mutation carriers (Marx 2001). A typical sporadic case of PHPT is a postmenopausal woman, whereas PHPT in MEN1 occurs equally in both sexes and is in most cases detectable by the age of 40 years (Burgess et al. 1998, Brandi et al. 2001, Schussheim et al. 2001). In the sporadic cases, PHPT is usually caused by a single adenoma. In MEN1, multiple parathyroid glands are usually affected, although the enlargement is highly asymmetric (Marx et al. 1991). According to Marx (2001), parathyroid lesions in MEN1 should be regarded as independent clonal adenomas, although an early hyperplastic phase has been suggested, but not proven in their development (Brandi et al. 2001). Nevertheless, parathyroid carcinoma is a rare cause of PHPT with a prevalence of <1% in sporadic cases (Koea & Shaw 1999). In addition, there are only few reported cases of parathyroid carcinoma in association with MEN1 (Shepherd 1985, Sato et al. 2000, Dionisi et al. 2002, Agha et al. 2007).

The classical phrase “painful bones, renal stones, abdominal groans, and psychic moans” was used to describe the signs and symptoms of PHPT decades ago, but nowadays the patients are often diagnosed without any symptoms through routine biochemical screening. Modern-day patients may present with subtle neurocognitive symptoms including fatigue, lethargy, muscle weakness, depression and cognitive impairment (Suliburk & Perrier 2007).

PHPT associated with urolithiasis is still a common feature in MEN1 patients. In a recent study on 26 MEN1 patients, 17 (65%) had been diagnosed with urolithiasis (Christopoulos et al. 2005). In a review of Japanese MEN1 patients from 1966 to 1995, 90% of the patients were found to have PHPT, and it was complicated by urolithiasis in 35% of cases (Yoshimoto 2000).
Osteoporosis is an important complication of PHPT, since reduced bone mineral density (BMD) is a known risk factor for skeletal fracture (Ross 1998). Burgess et al. (1999) examined 29 women with MEN1 belonging to a single family in order to find out the relationship of bone mass to PHPT, fracture risk, and parathyroidectomy in the setting of MEN1. Osteopoenia and osteoporosis were diagnosed in 41% and 44% of the patients, respectively, and reduced BMD was associated with an increased likelihood of skeletal fracture. In addition, parathyroidectomy improved femoral neck and lumbar spine BMDs by a mean ±SEM of 5.2±2.5% and 3.2±2.9, respectively (Burgess et al. 1999).

Peptic ulcer disease is classically associated with sporadic PHPT (Suliburk & Perrier 2007). According to Bilezikian et al. (2002), asymptomatic PHPT is not associated with peptic ulcer disease unless the patient is affected by MEN1 at the same time. It has been shown that PHPT can exacerbate hypergastrinaemia in patients with Zollinger-Ellison syndrome and MEN1, and the degree of hypergastrinaemia has been reduced by parathyroidectomy (Norton et al. 1987, Metz et al. 1994).

Patients with PHPT are also associated with an increased risk of cardiac morbidity, hypertension, and metabolic disturbances such as impaired glucose tolerance, type 2 diabetes mellitus and dyslipidaemia (Mihai et al. 2008). There is some evidence that PHPT in MEN1 patients may be associated with impaired glucose intolerance and type 2 diabetes (Furuto-Kato et al. 1994, McCallum et al. 2006).

According to some European studies, there is an increased risk of death among patients with PHPT persisting years after parathyroidectomy (Hedbäck et al. 1991, Nilsson et al. 2002, Øgard et al. 2004). However, overall mortality has not been different from normal population in patients undergoing parathyroidectomy more recently (after 1985) (Øgard et al. 2004) or in North American patients (Søreide et al. 1997).

For both sporadic cases and MEN1 patients, the overt symptoms related to PHPT are indications for parathyroid surgery. There are guidelines for parathyroid surgery in asymptomatic PHPT (Bilezikian et al. 2002, Mihai et al. 2008), but there is no consensus as to timing or which operation to perform in MEN1 patients with PHPT (Brandi et al. 2001, Hubbard et al 2006). Due to multiglandular involvement in MEN1, either subtotal parathyroidectomy (identifying four glands and removing at least three but leaving a parathyroid remnant the size of a normal parathyroid in the neck), or total parathyroidectomy (the resection of at least four glands combined with the transplantation of 10–20 1-mm³ pieces of parathyroid
tissue into individual pockets created in the brachioradialis muscle of the forearm) are the methods usually chosen. Common features of MEN1 related parathyroidectomy are postoperative persistence of PHPT or late recurrence, but on the other hand, hypoparathyroidism as well. (Hellman et al. 1992, Hubbard et al. 2006.)

Neuroendocrine gastroenteropancreatic (GEP) tumours

Neuroendocrine gastroenteropancreatic tumours are the second most common manifestation in MEN1 occurring in 27–74% of the patients (Table 1). They develop from the pancreatic islets and the endocrine cells of the duodenal and gastric mucosa (Tonelli et al. 2005). GEP tumours associated with MEN1 occur at younger ages and are multicentric compared to sporadic cases (Marx 2001). Typically, microadenomas are diffusely spread in the pancreas and duodenum (Åkerström et al. 2005). GEP tumours may be hormonally active and produce symptoms depending on the hormone in question, or they may be hormonally silent. Even though non-functional tumours may produce hormonal peptide(s), they do not secrete enough to cause a recognizable syndrome. GEP tumours have generally a benign course, although there is a subgroup showing aggressive behaviour, being a major cause of death among MEN1 patients (Tonelli et al. 2005).

Nonfunctional pancreatic tumour (NFPT). Today, it is estimated that nonfunctional pancreatic neuroendocrine tumour (NFPT) is the most common neuroendocrine GEP tumour in MEN1, as well as among sporadic cases (Tonelli et al. 2005, Kazanjian et al. 2006, Triponez et al. 2006a). The frequency of NFPT is steadily increasing, probably due to earlier diagnosis after genetic testing and more sensitive imaging and laboratory testing (Triponez et al. 2006a, b). However, in the studies shown in Table 1, gastrinoma was more frequent than NFPT in all (n=14), and insulinoma in eight, respectively. In a prospective endoscopic ultrasonography (EUS) evaluation of 51 MEN1 patients with a median age of 39 years, the frequency of NFPTs was 54.9%, and the lesions were multiple with a median of three lesions per patient. It was also shown that the size and number of the NFPTs can increase over time (Thomas-Marques et al. 2006). In addition, it has been shown that NFPTs are commonly detectable even at young age; for example, in the Tasmanian MEN1 study population 40% of the subjects in the age range of 10–20 years were found to have NFPTs (Burgess et al. 1998), and in the other study (Tho-
(Mas-Marques et al. 2006) the youngest patient detected with a NFPT was 16 years old.

There is evidence that the tumour size of a NFPT correlates with the risk of metastases and death. According to Triponez et al. (2006b), these risks are low for patients with tumours \( \leq 20 \) mm. However, the correlation between the size and metastasis potential has not been observed in some other studies (Lowney et al. 1998). The timing and procedure of surgical treatment of NFPTs has not reached consensus. Åkerström et al. (2005) consider surgery (enucleation of tumours in the pancreatic head, and concomitant distal 80\% subtotal pancreatic resection) when a tumour is detectable by EUS, e.g. with size exceeding arbitrarily 5–10 mm, to prevent further growth and malignant development. In contrast, Triponez et al. (2006b) concluded that surgery may not be beneficial for MEN1 patients with NFPTs \( \leq 20 \) mm.

**Gastrinoma.** Gastrinomas are the most frequent hormonally active type of GEP tumours, being present in approximately 40\% of MEN1 patients (Brandi et al. 2001), although prevalence as high as 60\% has been reported (Burgess et al. 1998, Table 1). Zollinger-Ellison syndrome (ZES) is a clinical condition caused by hypersecretion of gastrin, which in turn causes overproduction of acid in the parietal cells of the ventricle (Marx 2001). The first description of ZES included fulminating peptic ulcer disease, gastric acid hypersecretion, and non-beta islet cell tumours of the pancreas (Zollinger & Ellison 1955). Approximately 20–25\% of patients with ZES have MEN1 (Cadiot et al. 1999). In a study by Roy et al. (2000) including 261 patients with ZES (58, or 22\%, with MEN1 as well), the most common symptoms were abdominal pain and diarrhoea, present in 75\% and 73\% of the patients, respectively, whereas heartburn and weight loss were present in 44\% and 17\% of the patients, respectively. Gastrointestinal bleeding was the initial presentation in one in every four patients, and an important presenting sign suggesting ZES was prominent gastric body folds noted in endoscopy in 94\% of the patients. Mean age at onset of ZES was lower in MEN1 patients than in sporadic cases; 33.7±1.3 versus 43.2±0.8 years, \( p<0.0001 \) (Roy et al. 2000). In a study by Trump et al. (1996) including 220 MEN1 patients, it was found that there was a higher occurrence of gastrinomas above the age of 40 years.

The MEN1-associated gastrinoma is predominantly duodenal in origin and frequently multicentric, whereas sporadic gastrinomas are usually pancreatic and single (Pipeleers-Marichal et al. 1993, Tonelli et al. 2005). Although duodenal gastrinomas of MEN1 patients are usually small in size (<5 mm in diameter), they are frequently associated with regional lymph node metastases (Tonelli et al. 2005,
Åkerström et al. 2005). In a prospective study of the natural history of gastrinoma in patients with MEN1, 23% (13/57) of the patients were found to have liver metastases, and in 14% (8/57) gastrinomas showed aggressive growth (Gibril et al. 2001). The 5-year survival of patients with aggressive disease in which disease-related deaths were assessed was 88% (95% confidence interval, CI, 53–98%) whereas it was 100% (CI, 92–100%) for patients with non-aggressive disease with or without liver metastases (Gibril et al. 2001). In a study by Norton et al. (2001) the 15-year survival was 52% even if the patients had metastatic disease. Gibril et al. (2001) found several factors predictive of aggressive growth of gastrinomas including age at MEN1 diagnosis (<35 years), age at ZES onset (27 years) and diagnosis (33 years), duration of ZES before diagnosis (<2.1 years), fasting gastrin levels 10,000 pg/ml, pancreatic tumour size >30 mm, presence of liver and bone metastases, and gastric carcinoids.

Surgery for ZES in MEN1 is controversial. There is conflicting evidence as to whether cure of hypergastrinaemia can be achieved by any kind of surgery (Thompson 1998, Norton & Jensen 2004, Åkerström et al. 2005). On the other hand, modern antisecretory therapy with proton pump inhibitors, or occasionally H₂ receptor blockers, is efficient in controlling hypergastrinaemia (Brandi et al. 2001, Marx 2001). Some surgeons recommend routine surgical exploration to decrease the probability of malignant spread (Skogseid et al. 1996, Lowney et al. 1998, Thompson 1998). In this case, the operation includes distal pancreatectomy, intraoperative ultrasound and enucleation of tumours in the pancreatic head, duodenotomy, and removal of lymph nodes along the coeliac trunk and hepatic ligament (Norton & Jensen 2004). Others perform surgical exploration only when a tumour of 20 to 30 mm is imaged (Jensen 1998, Cadiot et al. 1999, Norton et al. 1999). Some authors suggest that a more aggressive surgical procedure, pancreatoduodenectomy, should be adopted more frequently (Tonelli et al. 2005). In addition, other treatment options in malignant gastrinoma may include biotherapy (somatostatin analogues, interferon), systemic chemotherapy, selective embolization of liver metastasis alone or in combination with intra-arterial chemotherapy (chemoembolization) and radiofrequency ablation (Plöckinger et al. 2004).

Insulinoma. Insulinoma is the second most prevalent hormonally active GEP tumour in MEN1, being present in approximately 10–20% of the patients (Marx 2001, Table 1). The median age at diagnosis of 56 insulinoma patients with MEN1 was 34 years (range, 5–69 years) (Lévy-Bohbot et al. 2004). Typically, patients affected with insulinoma suffer from hypoglycaemic attacks and dizziness. A combination of hypoglycaemia and hyperinsulinaemia during a fasting test of 48–72
hours is required for the diagnosis of insulinoma (Marx 2001, Alexakis & Neoptolemos 2008). In most cases with MEN1 insulinoma, there are multiple islet cell tumours, although most of them are nonfunctional and a single tumour of at least 5 mm in size is expected to cause the hyperinsulinism. Malignancy rate is higher with MEN1-associated than with sporadic insulinoma, although 85% of MEN1-related insulinomas are benign (Veldhuis et al. 1997, Åkerström et al. 2005). Surgery is the main treatment for all patients with hypoglycaemia due to an insulinoma (Alexakis & Neoptolemos 2008). During the exploration, any identified islet cell tumours are enucleated, and some surgeons perform concomitant distal (80%) pancreatic resection to minimize the risk of recurrent tumours. Regardless of the operative method chosen, most patients are cured (Veldhuis et al. 1997, Åkerström et al. 2005).

Rare functional GEP tumours. There are also some rare functional neuroendocrine GEP tumours such as glucagonoma, VIPoma, somatostatinoma and GHRHoma that are seen in association with MEN1. Glucagonomas are pancreatic islet tumours that oversecrete glucagon, which is a counter-regulatory hormone in the insulin control of glucose metabolism. A specific syndrome of diabetes mellitus, a skin rash (migratory neurolytic erythema), stomatitis, glossitis, cheilosis, hypoaminoacidaemia, cachexia, and a tendency for deep venous thrombosis is associated with glucagonoma (Doherty 2005). In a study by Lévy-Bohbot et al. (2004) 5/307 (1.6%) of the MEN1 patients with duodenopancreatic lesions or 5/580 (0.86%) of the whole MEN1 cohort had glucagonoma at a median age of 31 years (range, 19–51 years). It is estimated that 3% of glucagonoma patients have also MEN1 (Doherty 2005). Most glucagonomas are usually large with high risk of malignancy, and are suggested to be treated with radical surgery if possible (Åkerström et al. 2005).

VIPomas are mainly pancreatic tumours, with occasional duodenal location. They oversecrete vasoactive intestinal polypeptide (VIP), which is a neuropeptide that is distributed throughout the nervous system. Peripherally, VIP is located in peptidergic neurons adjacent to blood vessels throughout the splanchnic nervous system, including the small intestine, the colon and the exocrine ducts of the pancreas. The syndrome associated with the oversecretion of VIP is called Verner-Morrison syndrome, WDHA syndrome (Watery Diarrhoea, Hypokalaemia, Achlorhhydria) or pancreatic cholera. These patients have very severe, watery, secretory diarrhoea. Approximately 40% of the tumours are malignant, and 3% are associated with MEN1. (Doherty 2005.) In the study by Lévy-Bohbot et al. (2004) 3/307 (0.98%) of the MEN1 patients with duodenopancreatic lesions, or 3/580 (0.5%) of
the whole MEN1 cohort had VIPomas. It is recommended that VIPomas should also be treated surgically (Åkerström et al. 2005). It is also of note that 80–90% of VIPoma and glucagonoma patients have a symptomatic response to somatostatin analogues (Plöckinger et al. 2004).

Somatostatinoma may locate in the pancreas or duodenum. It oversecretes somatostatin, which is a cyclic polypeptide 14 amino acids in length. Somatostatin-secreting cells have been described in the hypothalamus, gastrointestinal tract and pancreas. Somatostatin inhibits the release and function of many other hormones. The somatostatinoma syndrome consists of diabetes mellitus, cholelithiasis, and steatorrhea. Most of the somatostatinomas are malignant, and approximately 1% is associated with MEN1, and of the duodenal somatostatinomas, up to 50% are associated with neurofibromatosis type 1 (NF1). (Doherty 2005.) Lévy-Bohbot et al. (2004) found out that 2/307 (0.65%) of the MEN1 patients with duodenopancreatic lesions, or 2/580 (0.34%) of the whole MEN1 cohort had somatostatinoma. It is also recommended that somatostatinomas be treated surgically (Åkerström et al. 2005).

GHRHoma is a tumour that oversecretes growth hormone-releasing hormone (GHRH). It stimulates the pituitary to release excessive growth hormone, resulting in acromegaly if untreated. GHRHomas occur in the lung, pancreas, adrenal and intestine. Approximately one third of GHRHomas are associated with MEN1, and the same proportion are malignant (Marx 2001, Doherty 2005). The treatment consists of surgery and medical therapy by somatostatin analogue (Doherty 2005).

Enterochromaffin-like (ECL) cell tumour (ECLoma). Gastric neuroendocrine tumours are found in up to 7% of MEN1 patients (Table 1). Gastric endocrine tumours are commonly referred to as gastric carcinoids, although a more appropriate term according to WHO’s classification is neuroendocrine tumours (Kloppel et al. 2004). They are categorized into well- or poorly-differentiated tumours. Well-differentiated tumours are more frequent, and among them, tumours composed of enterochromaffin-like (ECL) cells are the most common ones. They are also called ECL-cell carcinoids or ECLomas. ECL cells are the main endocrine cells in the oxyntic mucosa in the corpus and fundus of the stomach secreting histamine with a key role in the mechanism of acid secretion (Bordi et al. 2001, Plöckinger et al. 2004). Three subtypes of well-differentiated ECL cell tumours are recognized. Type 1 tumours are often multiple and benign, and secondary to hypergastrinaemia and associated with chronic atrophic gastritis. Type 2 tumours are also multiple and mainly benign lesions associated with hypergastrinaemia in the context of ZES and MEN1. Type 3 is a sporadic tumour, usually solitary and malignant. Less
than 5% of these tumours can cause the so-called ‘atypical carcinoid syndrome’ due to histamine production. (Marx 2001, Plöckinger et al. 2004, Tonelli et al. 2005.).

Patients with sporadic ZES rarely develop gastric ECLomas despite hypergastrinaemia for a long period of time. In contrast, ECLomas occur in 13–30% of patients with ZES related to MEN1 (Debelenko et al. 1997). It is thought that hypergastrinaemia is associated with ECL cell stimulation and hyperplasia, and loss of heterozygosity (LOH) at 11q13 detected in type 2 ECLomas may be a promoting factor towards tumour transformation (Debelenko et al. 1997).

In addition to fundus and corpus of the stomach, antral mucosa was shown to be another tissue type to harbour gastric endocrine tumours in MEN1 (Bordi et al. 1997). These tumours were not shown to express the phenotype of normal endocrine cells of the gastric antrum, and in at least two cases they could be identified as ectopic ECL cell carcinoids by VMAT-2 (vesicular monoamine transporter) immunostaining (Bordi et al. 1997). In addition, there are at least two descriptions of MEN1 patients with neuroendocrine tumours in the stomach in the absence of hypergastrinaemia (Bordi et al. 1997, Hosoya et al. 1999).

Optimal treatment of MEN1 ECLomas is not yet well established (Tonelli et al. 2005). It is recommended by the European neuroendocrine tumour society (ENETS) that small (<10 mm) polyps be surveyed and that >10 mm in size should be endoscopically resected after EUS. If there are > 6 polyps and >10 mm in size, extension to muscular wall or repeated recurrences, either surgical resection or antrectomy may be undertaken (Plöckinger et al. 2004). Partial or total gastrectomy with lymph node dissection may be necessary in malignant development, or in recurrences despite local surgical resection (Plöckinger et al. 2004).

Anterior pituitary tumours

Pituitary tumours account for approximately 15% of intracranial tumours, and the overwhelming majority of them are histologically benign, nonmetastatic adenomas arising from the hormone-secreting cells of the anterior lobe (Thapar et al. 1995, Heaney & Melmed 2004). Normal anterior pituitary gland comprises five different cell types capable of producing prolactin (PRL), growth hormone (GH), adrenocorticotropic hormone (ACTH), thyroid-stimulating hormone (TSH) and gonadotrophins (luteinizing hormone (LH) and follicle-stimulating hormone (FSH)). In a series of 3,000 surgically removed pituitary adenomas, the most common hormonally active tumours were prolactin cell adenoma (prolactinoma)
and growth hormone cell adenoma (somatotropinoma), the proportion being 27% and 14%, respectively (Thapar et al. 1995). In addition, different types of pituitary adenomas producing both prolactin and growth hormone (mixed GH-PRL cell adenoma, mammosomatotroph adenoma, acidophil stem cell adenoma) constituted 8% of the cases. Approximately one third of the adenomas were hormonally silent or nonfunctional, even though a subset of them might show positive immunohistochemical staining for one or more of the anterior pituitary hormones, e.g. silent corticotroph cell adenoma, accounting for 6% of all adenomas. (Thapar et al. 1995.)

**Prolactinoma.** Among patients with a pituitary tumour, the prevalence of MEN1 has been 2.7–5%, but among prolactinoma patients as high as 14% (Scheithauer et al. 1987, Schaaf et al. 1990, Corbetta et al. 1997). Anterior pituitary adenomas are the third major group of tumours in MEN1 being present in up to 65% of the patients (Table 1). In a study by Vergès et al. (2002) the age at diagnosis of pituitary adenoma did not differ between 136 MEN1 patients and 110 control, non-MEN1 patients (38.0±15.3 vs. 36.2±14.6 years). Furthermore, the distribution of different types of pituitary tumours did not differ between the groups, but clinical signs related to tumour size (headache, visual defects) were more common in MEN1 patients (29% vs. 14%, P<0.01) (Vergès et al. 2002).

Prolactinoma is the most common pituitary adenoma among MEN1 patients, with an average prevalence of 22±4% in different studies reviewed by Hao et al. (2004), where the overall prevalence of pituitary adenomas averaged 35±6%. In the studies reviewed in Table 1, the prevalence of prolactinoma varies between 0 and 53%. The symptoms relating to prolactinoma in women of reproductive age are galactorrhoea and hypogonadism with menstrual dysfunction and infertility. In men, the symptoms are decreased libido, impotence and infertility. At least partly due to the less recognized symptoms, tumours in men are diagnosed as larger in size after tumour growth causes symptoms of mass effect (Thapar et al. 1995). In general, women are four times more likely to present with prolactinoma than men, particularly at reproductive age (Thapar et al. 1995). This difference between sexes seen clinically is not evident in unselected autopsy series where prolactinomas are identified with equal frequency (McComb et al. 1983).

In a large multicentre study of 324 MEN1 patients including 136 pituitary adenomas (85 prolactinomas; 62.5%), an increased female-to-male ration (50% vs. 31%, P>0.001) was seen (Vergès et al. 2002). In the same study, a high frequency of (84%) macroadenomas was found in MEN1-related prolactinomas compared with those observed in non-MEN1 patients (24%). MEN1 prolactinomas showed
worse response to therapy, since normalization of plasma PRL level was significantly less frequent in MEN1 patients than in non-MEN1 subjects (44% vs. 90%, \(P<0.001\)) (Vergès et al. 2002). In addition, Burgess et al. (1996c) concluded that prolactinomas occurring in MEN1 may behave more aggressively than sporadic prolactinomas; the majority of their untreated prolactinoma patients developed micro- or macroadenomas during follow-up compared to the literature data by Schlechte et al. (1989) with a rate of 29% of untreated patients with sporadic prolactinoma developing radiologically detectable pituitary tumours. In addition, a high rate of referral to surgery (for failed treatment with bromocriptine) for macroadenomas was noticed (Burgess et al. 1996c). More evidence on behalf of an aggressive natural history of pituitary adenoma in MEN1 patients was provided by the fact that in their retrospective group of Tasmanian MEN1 patients, 4/34 had died of pituitary disease (Burgess et al. 1996c).

The management of MEN1-related prolactinoma does not differ from that of their sporadic counterparts. In most cases dopamine agonists are the first-line treatment, and in some cases surgery is necessary. Radiotherapy is needed only rarely to control the tumour expansion (Beckers et al. 2003).

Nonfunctional pituitary adenoma. Nonsecreting or nonfunctional pituitary adenomas may present with mass effect (hypopituitarism, pituitary apoplexy, visual compromise) (Marx 2001). Earlier, occasional cases of nonfunctional pituitary adenomas had been noted in MEN1, but Burgess et al. (1996c) found that 5 out of 41 (12%) patients studied had nonfunctional pituitary adenoma. In the other studies (Table 1) the prevalence of nonfunctional pituitary adenoma has varied between 0–11%. If treatment is needed, surgery by a transsphenoidal approach is the first choice in most cases (Beckers et al. 2003).

GH-secreting pituitary adenoma. Hypersecretion of GH results in gigantism and excessive linear growth prior to closure of the epiphyseal growth plates, and after that, in acromegaly (Eugster & Pescovitz 1999). Hypersecretion of GH is usually caused by pituitary adenoma, except for rare cases where the primary cause is oversecretion of GHRH from a hypothalamic or ectopic neuroendocrine (primarily pancreas or bronchus) tumour (Ayuk & Sheppard 2006). Gigantism is extremely rare. Acromegaly has an estimated annual incidence of three to four cases per million and a current estimated prevalence of 40–60 cases per million (Colao et al. 2004, Kauppinen-Mäkelin et al. 2005, Ayuk & Sheppard 2006).

Clinical features of acromegaly include direct effects of the tumour (headache, visual impairment, hyperprolactinaemia, hypopituitarism) and systemic effects of GH/IGF-1 (insulin-like growth factor type 1) excess. The latter include soft tissue
and skin changes (acral enlargement, increased skin thickness and soft tissue hyperplasia, increased sweating, skin tags, acanthosis nigricans), cardiovascular disorders (cardiomyopathy, hypertension, arrhythmias), metabolic features (impaired glucose and lipid metabolism, increased nitrogen retention), respiratory distress (upper airway obstruction, sleep apnoea, macroglossia), bone and joint fractures (increased articular cartilage thickness, arthropathy, carpal tunnel syndrome, osteopenia), and other endocrine consequences (multinodular thyroid goitre, thyrotoxicosis, hypercalciuria, hyperparathyroidism) (Colao et al. 2004). Acromegaly has been associated with a 2- to 3-fold increased mortality, but there is evidence that the normalization of post-treatment basal GH concentration (less than 2.5–1 µg/l) is associated with a normal life-span (see Kauppinen-Mäkelin et al. 2005).

In the early reports on MEN1 the majority of the pituitary lesions were associated with acromegaly (Erdheim 1903, Underdahl et al. 1953, Ballard et al. 1964). In the more recent study, GH-secreting tumours were present in 12/324 (3.7%) of the MEN1 patients in the France-Belgium multicentre study, or 12/136 (8.8%) of those with pituitary adenomas (Vergès et al. 2002). The mean age at diagnosis was 43.6±16.1 years. In the other series, the prevalence of GH-producing adenoma has ranged from 0 to 7% (Table 1). It is of note that the records of 124 MEN1 patients from the large Tasman 1 kindred were reviewed, and the authors did not find any cases of acromegaly or gigantism (Burgess et al. 1996b). In addition, a subset of 44 patients was screened for elevated levels of serum IGF-1, with no abnormal levels found in any of them (Burgess et al. 1996b,c).

The first-line treatment of GH-producing adenoma is transsphenoidal surgery, which is sometimes combined with somatostatin analogues. In cases of mixed GH-PRL-secreting adenomas or in the 10–20% of cases resistant to somatostatin analogues, dopamine agonists may be used. (Beckers et al. 2003.) The GH receptor antagonist pegvisomant is a new treatment option in acromegaly (Stewart 2003).

**ACTH-secreting pituitary adenoma.** ACTH-secreting pituitary adenomas (functioning corticotroph adenomas) constituted 8% of the tumours in a series of 3,000 surgically treated pituitary adenoma patients (Thapar et al. 1995). In MEN1 patients the prevalence in different studies has ranged from 1.7 to 7% (Trump et al. 1996, Marx et al. 1998, Vergès et al. 2002). Excessive secretion of ACTH leads to hypercortisolism and Cushing’s syndrome. Approximately 80% of the Cushing’s syndrome (CS) cases are ACTH-dependent (secondary to a tumour hypersecreting ACTH), whereas 20% are ACTH-independent (in most cases adrenal tumours). An ACTH-secreting pituitary tumour is present in approximately 80% of ACTH-
dependent CS referred to as Cushing’s disease in this case. ACTH may also be secreted from a non-pituitary tumour (small cell lung carcinoma, bronchial neuroendocrine tumours), constituting CS referred to as ectopic CS or occult ectopic CS. Hypercortisolism has multiple, diverse effects on tissues and metabolic functions. The most prevalent signs are obesity, plethora, moon face, hypertension, bruising, red-purple striae, muscle weakness and ankle oedema. The most common symptoms include weight gain, menstrual disturbances, hirsutism, psychiatric disturbances, backache, muscle wasting, fractures and scalp hair loss. (Makras et al. 2006.) In most cases, the treatment of pituitary adenomas secreting ACTH consists of surgery, and in addition, in some cases radiotherapy (Beckers et al. 2003).

Rare functional pituitary adenomas. Pituitary tumours secreting LH/FSH or TSH are among the least common hormonally active pituitary tumour types in general, and in association with MEN1 as well (Thapar et al. 1995, Marx 2001). Gonadotroph adenoma may cause ovarian hyperstimulation, but in most cases the secretion of adenoma is insufficient to cause a recognizable clinical syndrome (Välimäki et al. 1999). Thus, most gonadotroph adenomas appear clinically as nonfunctional, and are only found through immunohistochemical staining (Daneshdoost et al. 1993). In the series of Thapar et al. 6.4% of the tumours were gonadotrophinomas. Virtually all gonadotropinomas are macroadenomas at presentation (Thapar et al. 1995). Only very recently it has been shown that clinically apparent gonadotroph adenoma may exist in association with MEN1 (Benito et al. 2005). TSH secreting adenoma is the least common pituitary tumour type accounting for only 1% of all pituitary adenomas. It is a rare cause of hyperthyroidism in clinical practice. As their diagnosis is often delayed, these tumours are mostly diagnosed as macroadenomas (Foppiani et al. 2007). Primary therapy for gonadotroph adenomas is neurosurgery, but there may be a limited role for medical therapy in patients with residual disease (Shomali & Katznelson 1999). TSHomas are best treated by transsphenoidal surgery. Radiotherapy is indicated for inoperable or incompletely resected tumours. Octreotide administration is a useful adjunct for reducing tumor size preoperatively and for the medical management of surgical treatment failures (McDermott & Ridgway 1998).

Adrenal lesions

Adrenal involvement in MEN1 has recently been getting more attention due to the wide range of prevalence (3–55%) in different series of patients (Table 1 and 2). The first reports referred to autopsy studies or to incidental findings at laparotomy,
and the impact of adrenal involvement was underestimated (Waldmann et al. 2007). In general, clinically silent adrenal masses discovered by imaging studies performed for unrelated reasons are called adrenal incidentalomas. They have become a rather common finding in clinical practice, with a prevalence of 0.5–2% at abdominal CT scan, and in most cases, these masses are non-hypersecreting and benign (reviewed in Barzon et al. 2003). An even higher prevalence rate was found in a prospective evaluation on subjects undergoing high-resolution CT scan of the chest in a lung cancer screening programme; 23 out of 520 (4.4%) subjects with adrenal masses were identified; 21 adrenal adenomas, 1 myelolipoma, and 1 metastasis of lung cancer (Bovio et al. 2006). The prevalence of clinically unapparent adrenal masses detected at autopsy is less than 1% for ages <30 years, and increases to 7% in those 70 years of age or older (Anonymous 2002). The risk of malignancy over time for masses defined as benign at diagnosis is estimated to be 1/1,000, even though 5–25% of the masses increase in size during follow-up. Hormonal hyperfunction develops in about 1.7% (0–11%) of cases, the risk being higher in patients with lesions larger than 30 mm and/or with unilateral radiotracer uptake at scintigraphy (Barzon et al. 2003).

In most cases, MEN1-associated adrenal lesions consist of diffuse or nodular hyperplasia, or adenomas. In studies specially analysing adrenal involvement (Table 2) in MEN1, Cushing syndrome was present in 0 to 5% of the entire patient population, or in 0 to 17% of those with adrenal involvement. Pheochromocytoma was found less frequently with a prevalence of 0 to 3% in the whole study population, or 0 to 6% in those with adrenal involvement. Adrenal cortical carcinoma (ACC) was most common in the series by Skogseid et al. (1995) with a prevalence of 7% in the whole study group. Among the patients with adrenal lesions, ACC was most common in the study by Langer et al. (2002) with a prevalence of 22%. In addition, primary hyperaldosteronism due to adrenal adenoma has been reported in association with MEN1 (Beckers et al. 1992).

There is no consensus on MEN1-related adrenal lesions, but based on their own experience Waldmann et al. (2007) recommend surgery in MEN1 patients with adrenal tumours exceeding 30 mm, and a closer follow-up by EUS in patients with newly diagnosed adrenal lesions.
Thymic neuroendocrine carcinoma

Thymic neuroendocrine carcinoma (carcinoid) is a rare type of tumour with about 170 cases reported after its first description in 1972 (Rosai & Higa 1972, Teh et al. 1998c). MEN1-related thymic neuroendocrine carcinomas constitute approximately 25% of all cases (Teh et al. 1998c), and they occur in 1–8% of MEN1 patients (Marx et al. 1998, Gibril et al. 2003, Table 1). It is the most aggressive tumour in MEN1, diagnosed on average in the fifth decade, and has a clear male predominance in both sporadic and MEN1-related cases (Gibril et al. 2003). Sporadic thymic carcinoids are associated with Cushing’s syndrome in one third of the cases, but ACTH or other hormone-secreting thymic carcinoids are exceptions in association with MEN1 (Yano et al. 2006). The majority of patients are asymptomatic until late stage of the disease, when they may present with chest pain or discomfort and dyspnoea (Teh et al. 1998c). MEN1-associated thymic neuroendocrine carcinoids are often seen in familial clusters and the patients are often heavy smokers or have been exposed to other carcinogens (Teh et al. 1998a, 1998c, Ferolla et al. 2005).

It is suggested that cervical thymectomy at the time of parathyroid surgery might prevent thymic neuroendocrine carcinoma development (Brandi et al. 2001). However, it has been recognized that this procedure does not totally eliminate, but rather reduces the risk of subsequent formation of thymic carcinoid (Burgess et al. 2001, Lim et al. 2006). In case of thymic carcinoid, cure is uncommon even with early surgery. Therefore, Gibril et al. (2003) suggest that perioperative radiation should be considered.
Bronchial neuroendocrine tumour

Bronchial neuroendocrine tumours (bronchopulmonary carcinoids) are present in approximately 5% of MEN1 patients (Table 1), but prevalence as high as 31% has been suggested (Sachithanandan et al. 2004). In contrast to thymic carcinoids, bronchial tumours usually show a much more indolent natural history and have a female predominance. They also are multicentric and develop both synchronously and metasynchronously in the same patient with a mean age of diagnosis of 37 years (range 20–55) in a study by Sachithanandan et al. (2004). They are usually nonfunctional, but as thymic carcinoids may occasionally cause atypical carcinoid syndrome with facial flushing, lacrimation, headache, and bronchoconstriction, the biochemical cause remains unknown (Marx 2001). It is suggested by Sachithanandan et al. (2004) that radiological surveillance may be sufficient for a proportion of patients who have pulmonary nodules identified during MEN1 screening; clinical symptoms and size of the tumour (for example 30 mm or rapid growth) could serve as possible criteria for surgery.

Nonendocrine manifestations

Multiple facial angiofibromas were exclusively associated with tuberous sclerosis until Darling et al. (1997) observed these lesions in 88% (28/32) of their consecutive MEN1 patients, whereas collagenomas were found in 72% (23/32), and lipomas in 34% (11/32). The authors also suggested that confetti-like hypopigmented macules (2/28; 6%), and multiple gingival papules (2/28; 6%) are cutaneous manifestations of MEN1 (Darling et al. 1997). Facial angiofibromas were found in 43% (12/28) of the Japanese patients with MEN1 (Sakurai et al. 2000). LOH studies have shown allelic deletion of the MEN1 gene in angiofibromas, collagenomas, and lipomas of MEN1 patients, thus providing further evidence for these tumours being manifestations of MEN1 (Pack et al. 1998, Vortmeyer et al. 1998). Also, leiomyomata of different sites has been occasionally documented in MEN1 patients, and 11q13 LOH has been detected in oesophageal and uterine smooth muscle tumours in association with MEN1 (Vortmeyer et al. 1999, McKeeby et al. 2001). Spinal ependymoma is estimated to be an infrequent (<1%) manifestation of MEN1 (Marx 2001). One of the first descriptions of a patient with spinal ependymoma in association with MEN1 was reported in 1996 by Kato et al. Giraud et al. (1997) reported another case along with the results of genetic studies showing allelic loss of the wild-type MEN1 in
the ependymoma. In addition, 6/74 (8%) of the MEN1 patients in a prospective study by Asgharian et al. 2004 were found to have meningiomas. They also observed LOH at 11q13 in a resected meningioma, thus concluding that MEN1 plays a role in its pathogenesis (Asgharian et al. 2004).

2.1.3 Clinical management of MEN1 mutation carriers

*MEN1* Mutation testing can be offered to index case with MEN1 or with atypical MEN1 and to their relatives (Brandi et al. 2001). In approximately 10–20% of index cases for familial MEN1 cases, no mutation in the *MEN1* gene can be found (Brandi et al. 2001). In such cases, haplotype analysis around the MEN1 locus at chromosome 11q13 can allow screening for MEN1 carrier status (Brandi et al. 2001). *MEN1* genetic testing of an unaffected child is rarely justified, since it will not have a major effect on their immediate care (Marx 2001, American Society of Human Genetics Board of Directors and the American College of Medical Genetics Board of Directors 1995). Karges et al. (2002) suggest that genetic testing of family members (with the possible result of starting clinical follow-up of mutation-positive individuals) is generally not advisable before the age of 10–12 years.

Table 3 shows the screening programme suggested by the international consensus statement reported by Brandi et al. (2001). Biochemical screening (Table 3) allows the detection of MEN1-associated tumours one to two decades prior to clinically overt disease. Therefore, medical or surgical treatment will start earlier, which may result in reduced rate of morbidity and mortality, although this has not been proven (Clerici et al. 2001, Brandi et al. 2001). The screening programme for MEN1 mutation carriers includes annual clinical examination together with patient history and biochemical testing, and imaging testing every 3–5 years (Brandi et al. 2001, Karges et al. 2002, Kopp et al. 2001). Based on the patient’s detailed history, physical examination, or basic laboratory and imaging findings, additional diagnostic procedures may be required (Karges et al. 2002). Some authors would consider starting the screening protocol at a later age than suggested by Brandi et al. (2001), e.g. Johnston et al. (2000) recommended that the age to start could be 10–15 years, and Öberg and Skogseid (1998) suggest starting screening from the onset of adolescence. Some groups have also measured pancreatic polypeptide (PP) as a non-specific marker of islet cell tumours, and performed a standardized meal stimulation test for gastrin and PP for detecting GEP tumours (Öberg & Skogseid 1998, Kopp et al. 2001). In the OUH the genetic counselling for *MEN1* mutation
testing is usually offered when the person at risk of MEN1 reaches adulthood. In only rare cases, the mutation testing and/or screening have been started earlier. All the MEN1 mutation-positive individuals are offered follow-ups in order to screen MEN1 manifestations by endocrinologist. The frequency of biochemical testing and imaging studies depends on the clinical findings of the MEN1 heterozygote. (Unpublished data, original publication I).

Table 3. Screening programme for follow-up of MEN1 (multiple endocrine neoplasia type 1) mutation carriers according to the consensus statement by an international group consisting mostly of clinical endocrinologists (modified after Brandi et al. 2001).

<table>
<thead>
<tr>
<th>Tumours screened for</th>
<th>Age to begin (year) testing</th>
<th>Biochemical tests annually</th>
<th>Imaging tests every 3 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid adenoma</td>
<td>8</td>
<td>Calcium, PTH</td>
<td>None</td>
</tr>
<tr>
<td>Gastrinoma</td>
<td>20</td>
<td>Gastrin, gastric acid output*; secretin stimulated gastrin</td>
<td>None</td>
</tr>
<tr>
<td>Insulinoma</td>
<td>5</td>
<td>Fasting glucose; insulin</td>
<td></td>
</tr>
<tr>
<td>Other enteropancreatic</td>
<td>20</td>
<td>Chromogranin-A; glucagon; proinsulin</td>
<td>111In-DTPA octreotide scan; CT or MRI</td>
</tr>
<tr>
<td>Anterior pituitary</td>
<td>5</td>
<td>PRL; IGF-1</td>
<td>MRI</td>
</tr>
<tr>
<td>Foregut carcinoids</td>
<td>20</td>
<td>None</td>
<td>CT</td>
</tr>
</tbody>
</table>

*Gastric acid output measured if gastrin is high; secretin-stimulated gastrin measured if gastrin is high or if gastric acid output is high. Stomach best evaluated for carcinoids (ECLomas) incidental to gastric endoscopy. Thymus removed partially at parathyreoidectomy in MEN1. PTH, parathyroid hormone; PRL, prolactin; IGF-1, insulin-like growth factor type 1; 111In-DTPA octreotide, Indium-111-diethylenetriaminepenta-acetic acid-octreotide; CT, computerized tomography, MRI, magnetic resonance imaging.

2.1.4 Mortality

Several studies suggest that MEN1 patients are at increased risk of premature death compared to their non-affected relatives or to normal population (Vasen et al. 1989, Wilkinson et al. 1993, Carty et al. 1998, Dean et al. 2000, Geerdink et al. 2003, Table 4). In a study by Doherty et al. (1998) the MEN1-specific deaths (n=27) occurred at a younger age (median 47 years) than either MEN1 patients’ nonendocrine deaths (n=32) (median 60 years, p<0.02) or all non-MEN1-related deaths in kindred members (n=44) (median 55 years, p<0.05). However, there was no difference in survival between MEN1 carriers and unaffected kindred members. The authors concluded, however, that there is a substantial subset of MEN1 carriers who die early, often from malignant endocrine neoplasms. Therefore they suggest aggressive screening programmes to identify and initiate treatment of malignancies in the setting of MEN1 (Doherty et al. 1998). In 1993 Wilkinson et al. published a retrospective analysis of the natural history of untreated MEN1 in
one of the Tasmanian MEN1 kindreds with data available over a period of 130 years. Most cases were unrecognized as MEN1 at the time of the patient’s death. They concluded that MEN1 leads to premature death and that neoplasia rather than peptic ulcer disease is the main cause of death. A retrospective review of all MEN1 patients treated at the Mayo Clinic during the period 1951–1997 showed that the overall 20-year survival of MEN1 patients was 64% (95% confidence interval was 56–72%), and that of an age and gender matched upper Midwest population 81% (P<0.001).

In the earlier reports the major cause of death among MEN1 patients was peptic ulcer disease (Ballard et al. 1964, Majewski & Wilson 1979, Vasen et al. 1989). Other repeatedly seen causes of MEN1-related death were renal complications of hypercalcaemia and pituitary tumour. Today, malignant neuroendocrine tumours, mainly islet cell tumours and thymic carcinoids, have become the most important cause of death among MEN1 patients (Wilkinson et al. 1993, Carty et al. 1998, Doherty et al. 1998, Dean et al. 2000, Geerdink et al. 2003, Ferolla et al. 2005, Table 4).

Table 4. Published studies indicating causes of death related to multiple endocrine neoplasia type 1 (MEN1), modified from Geerdink et al. (2003).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Cases n</th>
<th>Peptic Ulcer Disease</th>
<th>Malignant Tumours GEP</th>
<th>Carcinoids</th>
<th>Renal Complications*</th>
<th>Pituitary Tumour</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ballard et al. 1964</td>
<td>8</td>
<td>88%</td>
<td>13%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Majewski &amp; Wilson 1979</td>
<td>91</td>
<td>9%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Vasen et al. 1989</td>
<td>15</td>
<td>73%</td>
<td>13%</td>
<td>0%</td>
<td>13%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Wilkinson et al. 1993</td>
<td>20</td>
<td>10%</td>
<td>15%</td>
<td>20%</td>
<td>30%</td>
<td>15%</td>
<td>10%</td>
</tr>
<tr>
<td>Doherty et al. 1998</td>
<td>27</td>
<td>22%</td>
<td>44%</td>
<td>22%</td>
<td>11%</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Carty et al. 1998</td>
<td>6</td>
<td>83%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>17%</td>
</tr>
<tr>
<td>Dean et al. 2000</td>
<td>17</td>
<td>6%</td>
<td>59%</td>
<td>18%</td>
<td>0%</td>
<td>0%</td>
<td>12%</td>
</tr>
<tr>
<td>Geerdink et al. 2003</td>
<td>17</td>
<td>24%</td>
<td>35%</td>
<td>35%</td>
<td>6%</td>
<td>0%</td>
<td>0%</td>
</tr>
</tbody>
</table>

GEP (gastroenteropancreatic). *Due to hypercalcaemia caused by primary hyperparathyroidism.

2.1.5 Genetics of MEN1

MEN1 gene and its product, menin

The MEN1 gene was identified in 1997; it is located at chromosome 11q13, and consists of 10 exons that span approximately > 9 kb of genomic DNA, and
encodes a 610-amino acid protein referred to as menin (Chandrasekharappa et al. 1997, Lemmens et al. 1997). The main transcript of the MEN1 gene is a 2.8-kb mRNA, although at least seven alternative transcripts have been reported (Lemos & Thakker 2008). Menin is ubiquitously expressed and is located predominantly in the nucleus in nondividing cells (Guru et al. 1998), but in dividing cells it is found mainly in the cytoplasm (Huang et al. 1999). It has no homology to any known proteins or sequence motifs. Menin has at least three nuclear localization signals (NLSs) and five putative guanosine triphosphatase (GTPase) sites (La et al. 2006, Lemos & Thakker 2008). Menin has been shown to interact with several proteins that are involved in transcriptional regulation, genome stability, cell division and proliferation (see Lemos & Thakker 2008). Menin has also been shown to bind to a broad range of gene promoters suggesting that it functions as a general transcriptional regulator (Scacheri et al. 2006).

MEN1 gene acts as a tumour suppressor gene. The presumed mechanism for tumour formation in MEN1 involves loss of menin functions in a tumour precursor cell. The first hit according to Knudson’s “two-hit” hypothesis is the inherited MEN1 gene defect and therefore it is present in every cell of the body. When the first hit is combined with a somatic loss of the normal allele of the MEN1 gene (the second-hit; loss of heterozygosity, LOH) in one cell, neoplastic clonal expansion from that cell is initiated (Brandi et al. 2001, Pannett & Thakker 2001).

MEN1 mutations

The mutations are scattered throughout the entire coding region and splice-sites of the MEN1 gene. According to a recent review article by Lemos and Thakker (2008) a total of 1,133 germline mutations and 203 somatic mutations of the MEN1 gene have been published. They consist of 459 different germline and 167 different somatic mutations. A total of 61 of the germline mutations were also found to occur as somatic mutations, thus the total number of different MEN1 mutations was 565. The 1,133 germline mutations consisted of 41% frameshift deletions or insertions, 23% nonsense mutations, 20% missense mutations, 9% splice site mutations, 6% in-frame deletions or insertions, and 1% whole or partial gene deletions (Lemos & Thakker 2008). Mutations at four sites accounted for 12.3% of all mutations (c.249_252delGTCT, deletion at codons 83–84; c.1546_1547insC, insertion at codon 516; c.1378C>T (Arg460Ter); and c.628_631delACAG, deletion at codons 210–211), thereby indicating potential mutational hotspots (Lemos & Thakker 2008). In addition to the mutational
hotspots, some of the recurrent mutations are due to a founder effect, which has
been reported e.g. from Tasmania, Newfoundland and Northern Finland (Olufemi
et al. 1998, Burgess et al. 2000, Kytölä et al. 2001). In Northern Finland, two
different founder mutations (c.1356_1367del12 and c.1546insC, originally
designated as 1466del12 and 1657insC, respectively) have been found (Kytölä et
al. 2001).

Nonsense and frameshift mutations would presumably cause truncated MEN1
proteins that would lack at least one of the NLSs and other functional domains, or
they could also result in loss of the translated protein because of nonsense-medi-
ated mRNA decay (NMD) (see Lemos & Thakker 2008). Missense mutations may
affect functionally critical amino acid residues involved in protein interactions,
thus leading to inactivation of menin. In addition, some missense mutations have
been found to alter the capacity of menin to regulate the target promoters (La et al.
2006), or to result in the reduction of protein stability and enhanced proteolytic
degradation (Yaguchi et al. 2004). The splice-site mutations are predicted to lead
to accumulation of unspliced or incompletely spliced precursor mRNA, or the
appearance of aberrantly processed mRNA from the use of alternative normally
occurring splice sites, or novel, or cryptic splice sites (see Lemos & Thakker
2008). There are examples of splice site mutations leading to frameshift and result-
ing in a premature termination codon (Turner et al. 2002, Lemos et al. 2007).

Genotype-phenotype correlation

Most reports doubt the existence of any kind of genotype-phenotype correlation in
the MEN1 syndrome, implying that the underlying mutation would not predict the
clinical picture of affected heterozygote. Neither the location of the mutation along
the gene nor the mutation type seems to have any effect upon the phenotype
There are some exceptions or variants that argue against the statement of lacking
genotype-phenotype correlation. First of all, MEN1 mutations have been reported
in 42 families with isolated hyperparathyroidism (FIHP), and 38% of these are
missense mutations that are less likely to result in a truncated, inactivated protein.
This proportion of missense mutations among FIHP families contrasts
significantly (P<0.01) with the situation in MEN1 patients in whom 20% are
missense mutations (Lemos & Thakker 2008). Second, there seems to be an
overrepresentation of mutations leading to truncated menin among patients with
thymic carcinoids (Lim et al. 2006). In a review of 22 separate MEN1 families
with thymic carcinoids, all but two (91%) had mutations coding for a truncated protein, even though, when compared with the prevalence of truncating mutations in all reported \textit{MEN1} mutations, it was not statistically significant (P=0.39) (Lim \textit{et al.} 2006). Third, there is also a prolactinoma variant of \textit{MEN1} called Burin (Newfoundland) variant of \textit{MEN1}, which is characterized by a high prevalence of prolactinoma and a low prevalence of gastrinoma (Olufemi \textit{et al.} 1998). The reported families with prolactinoma variant of \textit{MEN1} have been associated with truncating mutations, if a mutation has been found (Olufemi \textit{et al.} 1998, Hao \textit{et al.} 2001, Kong \textit{et al.} 2001). Nevertheless, in a large Tasmanian \textit{MEN1} pedigree with a splice-site mutation c.446–3C>G, prolactinoma was found commonly (50%) in two branches but uncommonly in others, indicating that there are other genetic determinants inherited separately from the \textit{MEN1} gene that modify the clinical features (Burgess \textit{et al.} 1998a, Burgess \textit{et al.} 2000). Fourth, there is a report by Bartsch \textit{et al.} (2000) including 21 \textit{MEN1} patients that claims that patients with truncating mutations in the N-or C-terminal region (exons 2, 9 or 10) of the \textit{MEN1} gene had a significantly higher rate of malignant pancreatic endocrine tumours (PET) (55% vs 10%; P<0.05) and tended to have shorter disease-free intervals (26 vs 92 months, P=0.11) than patients with other mutations. Fifth, Kouvaraki \textit{et al.} (2002) found that all (100%) of the 21 patients with known PET and \textit{MEN1} mutation status who had frameshift mutations had PETs, whereas 22 (79%) of 28 patients with all other types of \textit{MEN1} mutations had PETs (P=0.03). In addition, 14 of the 29 patients with pituitary tumours had frameshift mutations in exon 2 (representing 2 of 9 kindreds); frameshift mutations in exon 2 were not found in any of the 20 patients without pituitary tumours (P<0.001). In addition, all 4 glucagonomas were associated with frameshift mutation in exon 2 (P=0.004), while bronchial and thymic carcinoids were more frequently associated with mutations in exon 10 (Kouvaraki \textit{et al.} 2002).

\subsection*{2.2 Other disorders with inherited predisposition to primary hyperparathyroidism (PHPT) and/or anterior pituitary adenomas}

Table 5 shows disorders of genetic tumour predisposition featuring endocrine gland involvement. Apart from \textit{MEN1} that has already been discussed, entities involving predisposition to primary hyperparathyroidism and/or anterior pituitary adenomas are reviewed in more detail in this chapter. It is estimated that familial forms constitute approximately 5% of PHPT showing autosomal dominant mode of inheritance. An inherited condition should be suspected in younger patients
with PHPT (<40 years old), in patients with multiple parathyroid adenomas or hyperplasia at surgery (indicative of MEN1 or familial hypocalciuric hypercalcaemia), atypical parathyroid adenomas or parathyroid carcinoma (indicative of hyperparathyroidism-jaw tumour syndrome), and with a family or past medical history pointing to any of the syndromes which can cause PHPT (MEN1 and 2A, hyperparathyroidism-jaw tumour syndrome, familial hypocalciuric hypercalcaemia) (Miedlich et al. 2003, Table 5). Similarly to parathyroid tumours, most pituitary adenomas occur sporadically. It is suggested that hereditary tumour syndromes may be associated with approximately 5% of pituitary tumours (Daly et al. 2006). A hereditary form of pituitary adenoma may be suspected in patients with features of any of the known syndromes associated with pituitary adenoma (MEN1, Carney complex, isolated familial somatotropinoma, familial pituitary adenoma; Table 5) or a family history indicative of these. As with familial forms of PHPT, young age has been associated with GH-secreting tumours in the setting of isolated familial somatotropinoma (Soares & Frohman 2004).
<table>
<thead>
<tr>
<th>Disorder (OMIM)</th>
<th>Genetic background</th>
<th>Major manifestations and endocrine gland involvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckwith-Wiedemann syndrome, BWS (130650)</td>
<td>CDKN1C (11p15.5), NSD1 (5q35), KCNQ10T1 (11p15.5), H19 (11p15.5), 11p15 locus alterations</td>
<td>Overgrowth of various organs, Exomphalos, Wilms tumour, Adrenocortical cytomegaly, carcinoma, Neuroblastoma, Hepatoblastoma</td>
</tr>
<tr>
<td>Carney complex 1, CNC1 (160860)</td>
<td>PRKAR1A (17q23-q24)</td>
<td>Spotty skin pigmentation, PPNAD, Myxoma(tosis), various sites, Acromegaly, pituitary adenoma, LCCSCT, Thyroid nodules or carcinoma, Psammomatous melanotic schwannoma, Blue (epitheloid) nevus, Breast ductal adenoma, Osteochondromyxoma</td>
</tr>
<tr>
<td>Carney complex 2, CNC2 (605244)</td>
<td>gene? (2p16)</td>
<td>Same as CNC1</td>
</tr>
<tr>
<td>Carney-Stratakis syndrome, CSS (606864)</td>
<td>SDHB (1p36.1-p35), SDHC (1q21), SDHD (11q23)</td>
<td>Paraganglioma, multicentric, GIST</td>
</tr>
<tr>
<td>Carney triad, CT (604287)</td>
<td>not known</td>
<td>Paragangliomas, GIST, Pulmonary chondromas, Adrenocortical adenomas</td>
</tr>
<tr>
<td>Cowden syndrome, CS (158350)</td>
<td>PTEN (10q23.31)</td>
<td>Lhermitte-Duclos disease, Mucocutaneous lesions, Breast cancer (Follicular) thyroid cancer, Medullary thyroid cancer, Endometrial carcinoma</td>
</tr>
<tr>
<td>Familial isolated hyperparathyroidism, FIHP (145100)</td>
<td>gene? (2p13.3-14), MEN1 (11q13), HRPT2 (1q31.2), CASR (3q13.3-q21)</td>
<td>Parathyroid tumours</td>
</tr>
<tr>
<td>Familial isolated pituitary adenoma, FIPA (102200)</td>
<td>not identified</td>
<td>Various types of pituitary adenomas, functional and nonfunctional</td>
</tr>
<tr>
<td>Familial medullary thyroid cancer, FMTC (155240)</td>
<td>RET (10q11)</td>
<td>Medullary thyroid cancer</td>
</tr>
<tr>
<td>Familial papillary thyroid cancer, papillary renal cancer, FPTC/PRN1 (605642)</td>
<td>gene? (1q21)</td>
<td>Papillary thyroid cancer, Papillary renal neoplasia, Multinodular goitre</td>
</tr>
<tr>
<td>Hyperparathyroidism-jaw tumour syndrome, HPT-JT (145001)</td>
<td>HRPT2 (1q31.2)</td>
<td>Parathyroid adenoma/carcinoma, Ossifying fibromas, Kidney cysts and tumours</td>
</tr>
<tr>
<td>Disorder (OMIM)</td>
<td>Genetic background (chromosome location)</td>
<td>Major manifestations and endocrine gland involvement</td>
</tr>
<tr>
<td>----------------</td>
<td>----------------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>Isolated familial somatotropinomas, IFS (102200)</td>
<td>gene? (11q13.1-q13.3)</td>
<td>Growth hormone producing pituitary adenoma (acromegaly, gigantism)</td>
</tr>
<tr>
<td>Li-Fraumeni syndrome, LFS (151623, 609265, 609266)</td>
<td>TP53 (17p13.1) CHK2 (22q12.1) gene? (1q23)</td>
<td>Bone and soft-tissue sarcomas Breast carcinoma Brain tumours Adrenocortical carcinoma Leukeamias</td>
</tr>
<tr>
<td>McCune-Albright, MAS (174800)</td>
<td>GNAS1* (20q13.2)</td>
<td>Polystotic fibrous dysplasia Café-au-lait spots Precoceous puberty Thyrotoxicosis Plitary gigantism with or without an adenoma Hyperprolactemia Cushing's syndrome</td>
</tr>
<tr>
<td>Multinodular goitre, MNG1* (138800)</td>
<td>gene? (14q)</td>
<td>Multinodular goitre Papillary thyroid cancer</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type 1, MEN1 (131100)</td>
<td>MEN1 (11q13)</td>
<td>Parathyroid adenoma Neuroendocrine gastroenteropancreatic tumours Plitary adenoma Adrenal lesions</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type 2A MEN2A (171400)</td>
<td>RET (10q11)</td>
<td>Medullary thyroid cancer Pheochromocytoma Parathyroid adenoma</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type 2B MEN2B (162300)</td>
<td>RET (10q11)</td>
<td>Medullary thyroid cancer Pheochromocytoma Characteristic faces Thickening of the corneal nerves Hyperplasia of the intrinsic autonomic ganglia in the wall of the intestine</td>
</tr>
<tr>
<td>Multiple endocrine neoplasia type 4, MEN4 (610755)</td>
<td>CDKN1B (12p13)</td>
<td>Pituitary adenoma Parathyroid tumours</td>
</tr>
<tr>
<td>Neurofibromatosis type 1, NF1 (162200)</td>
<td>NFT (17q11.2)</td>
<td>Café-au-lait spots Neurofibromas Axillary and inguinal freckling Pseudoarthrosis Lisch nodules Scoliosis Neoplasia, mainly CNS (rarely duodenal carci-noid, somatostatinoma, pheochromocytoma)</td>
</tr>
<tr>
<td>Nonmedullary thyroid cancer 1, NMTC1* (606240)</td>
<td>gene? (2q21)</td>
<td>Papillary thyroid cancer, especially follicular variant</td>
</tr>
<tr>
<td>Primary pigmented nodular adrenocortical disease 2, PPNA2 (610475)</td>
<td>PDE11A (2q31)</td>
<td>Adrenocortical hyperplasia, adenoma Cushing’s syndrome</td>
</tr>
<tr>
<td>Paragangliomas 1, PGL1 (168000)</td>
<td>SDHD (11q23)</td>
<td>Head and neck paragangliomas Pheochromocytoma</td>
</tr>
<tr>
<td>Paragangliomas 2, PGL 2 (601650)</td>
<td>gene? (11q13.1)</td>
<td>Head and neck paragangliomas</td>
</tr>
</tbody>
</table>
Multiple endocrine neoplasia type 2 (MEN2) is a rare autosomal dominant disorder of tumour formation and developmental abnormalities affecting approximately 1 out of 30,000 individuals (Ponder 2001). MEN2 can be divided further into three distinct phenotypes; MEN2A, MEN2B (formerly MEN3) and FMTC with genetic defects in the RET gene in all of them (Table 5, Ponder 2001). The most common type is MEN2A, accounting for 65% of the families (Eng et al. 1996), whereas MEN2B is probably the least common form of MEN2 (Ponder 2001). The latter is also the most distinctive variant of MEN2 (Table 5, Ponder 2001). All variants of MEN2 show a high and early penetrance for medullary
thyroid carcinoma, but PHPT is present only in MEN2A with a prevalence of multigland parathyroid tumours in 20–30% of the patients (Brandi et al. 2001). Thyroidectomy during childhood is recommended to prevent medullary thyroid cancer in all MEN2 variants. In addition, pheochromocytoma is found in 50% of the MEN2 patients (Brandi et al. 2001). Therefore, clinically MEN2A is readily differentiated from familial isolated hyperparathyroidism or other forms of familial PHPT.

Unlike most other genes underlying tumour predisposition conditions, RET is not a tumour-suppressor gene. Instead, it acts as an oncogene through gain of function in the context of MEN2. Interestingly, loss of activity mutations in RET result in Hirschsprung disease of the colon and rectum (Ponder 2001). In addition, RET mutations show clear correlations with the variant of the MEN2 phenotype (Marx 2005). The majority (90%) of the RET mutations in MEN2A occur in 6 cysteines in a small 25-amino-acid domain in the extracellular region, whereas almost all RET mutations in MEN2B are confined to one cytoplasmic amino acid Met918Thr (Marx 2005). Instead, in FMTC most RET mutations overlap with those in MEN2A, although there are other mutations seemingly specific to FMTC in amino acid 533 of the extracellular domain and in amino acids 791–891 of the cytoplasmic domain (Marx 2005).

2.3.1 Hyperparathyroidism-jaw tumour syndrome (HPT-JT)

The hyperparathyroidism-jaw tumour (HPT-JT) syndrome is a rare autosomal dominant disorder characterized by the occurrence of parathyroid tumours and ossifying fibromas usually affecting the mandible or the maxilla (Bradley et al. 2006, Table 5). Some HPT-JT patients may also develop uterine tumours and renal lesions including Wilms tumours, hamartomas and polycystic disease (Teh et al. 1996, Bradley et al. 2005, Bradley et al. 2006). In HPT-JT, PHPT is the most common and usually the first manifestation with a prevalence of approximately 90%. PHPT in HPT-JT is usually due to a solitary adenoma, although multigland disease may also occur and approximately 15% of the parathyroid tumours are malignant (Simonds et al. 2002, Bradley et al. 2005, Bradley et al. 2006). The prevalence of jaw tumours is approximately 35%, and these may appear as early as 13 years of age (Cavaco et al. 2001). In addition, other tumours, including Hurter cell thyroid adenomas, pancreatic adenocarcinomas and testicular mixed germ cell tumours have also been observed in one or two patients with HPT-JT (Haven et al. 2000). Mutations in HRPT2 are detected in the majority of patients with HPT-JT
(Carpten et al. 2002). It is of note that germline mutations in HRPT2 can be found in patients with apparently sporadic parathyroid carcinoma (Shattuck et al. 2003).

2.3.2 Familial hypocalciuric hypercalcaemia (FHH)

Unlike other disorders predisposing to PHPT, familial hypocalciuric hypercalcaemia (FHH) is not a hereditary tumour syndrome. Instead, it is an autosomal dominant condition with an underlying loss-of-function mutation in the CASR (calcium-sensing receptor) gene in the majority of the cases (Pollak et al. 1993, Thakker 2004). However, there is evidence that FHH is a heterogenetic condition with two other loci identified with yet undiscovered genes at chromosome 19p (OMIM 145981) and 19q (OMIM 600740) (Heath et al. 1993, Lloyd et al. 1999). Secretion of PTH is regulated by extracellular calcium through a G protein-coupled calcium-sensing receptor (Miedlich et al. 2003). An allelic disorder to FHH is neonatal severe primary hyperparathyroidism (NSHPT) that is a life-threatening disorder characterized by severe neonatal hypercalcaemia, failure to thrive, bony undermineralization, multiple fractures and ribcage deformity (Thakker 2004). NSHPT results from homozygous loss-of-function mutations in CASR, although patients with sporadic NSHPT have been reported to be associated with de novo heterozygous CASR mutations (Pearce et al. 1995).

Normally, PTH secretion is inhibited by small increments in extracellular calcium levels above normal. Subsequently, calcium uptake from the intestine, renal absorption and release of calcium from bone are reduced to re-establish the normal value (Miedlich et al. 2003). In case of heterozygous CASR mutation, inactivation of the calcium-sensing receptor results in half-maximal inhibition of PTH secretion only at increased concentrations of serum calcium (calcium set-point) (Miedlich et al. 2003, Thakker 2004).

The clinical features of FHH include mild to moderate hypercalcaemia, normal or mildly elevated serum PTH concentrations, mild hypermagnesaemia and inappropriately low urinary calcium excretion (calcium clearance to creatinine clearance ratio <0.01) (Thakker 2004). Hypercalcaemia in FHH is highly penetrant at all ages, even in the perinatal period (Marx et al. 1981, Simonds et al. 2002). It is also typical that hypercalcaemia is not cured by parathyroid surgery, which in fact is almost always contraindicated in FHH (Miedlich et al. 2003). At surgery, hyperplasia of the parathyroid glands can be found (Thorgeirsson et al. 1981), although somewhat conflicting findings do exist (Law et al. 1984). There is one kindred reported with a highly atypical phenotype including family members with
hypercalciuria, hyperphosphaturia, inappropriately high serum PTH levels and renal stones resembling mild PHPT (Carling et al. 2000). In addition, normocalcaemia after parathyroidectomy and the presence of nodular hyperplasia or adenoma in some of the enlarged parathyroid glands were features not typically seen in FHH (Carling et al. 2000). Even though patients with FHH are usually asymptomatic, chondocalcinosis, pancreatitis and gallstones may be rare associations (Davies et al. 1981, Heath 1989, Thakker 2004).

2.3.3 Familial isolated hyperparathyroidism (FIHP)

Familial isolated hyperparathyroidism (FIHP) is a subgroup of familial hyperparathyroidism that can result from the incomplete expression of a syndromic form of familial hyperparathyroidism, or from full expression of other, genetically yet undetermined entities (Simonds et al. 2002; table 5). The diagnosis of FIHP is made in kindreds with PHPT in the absence of clinical, biochemical, and radiological evidence of MEN1, HPT-JT or FHH (Cetani et al. 2006). It is suggested that the distinction between FIHP and the syndromic categories arbitrarily depends on the sensitivity of diagnostic tests used to detect the syndrome (Simonds et al. 2002). Cetani et al. (2006) reviewed the overall results of genetic studies in FIHP families, which showed that in total 18% (range, 0–57%) of the kindreds showed mutations in MEN1, 12% (range, 0–18%) in CASR, and 5% (range, 0–29%) in HRPT2. In the majority of the FIHP kindreds the genetic cause remains unknown (Teh et al. 1998b, Kassem et al. 2000, Simonds et al. 2004, Cetani et al. 2006). Warner et al. (2006) has provided evidence for another locus for familial isolated hyperparathyroidism finding linkage to a 1.7 Mb region on chromosome 2p13.3-14. The gene has not yet been identified.

2.3.4 Carney complex (CNC)

Carney complex (CNC) is a rare autosomal dominant condition that has been described in about 500 people to date. In more than 60% of the patients it is caused by inactivating mutations in the gene encoding for the protein kinase A (PKA) type 1A regulatory (R1) subunit (Kirschner et al. 2000, Boikos & Stratakis 2007, Table 5). Another locus on chromosome 2 (2p16) has been reported, but the gene possibly underlying CNC2 has not yet been identified (Stratakis et al. 1996). The diagnosis of CNC is made if two of the main manifestations (Table 5) of the syndrome are present, confirmed by histology, biochemical testing or imaging.
Supplemental criteria include affected first-degree relative and inactivating mutation of the PRKAR1A gene (Boikos & Stratakis 2007). The median age at diagnosis of CNC is 20 years (Stratakis et al. 2001). It is suggested that patients with CNC and/or germline PRKAR1A mutations should be followed up for the manifestations of the disease annually to improve their prognosis (Boikos & Stratakis 2007).

The spotty pigmentation or lentigines in CNC appear as small, brown to black macules that have a typical distribution including the lips, conjunctiva and inner or outer canthi, vaginal and penile mucosa. The characteristic pigmentation typically develops shortly before, or during puberty, although it may also be the first sign at birth (Stratakis et al. 2001). Other pigmented lesions such as blue or other nevi, café-au-lait spots and depigmented lesions may appear at different ages (Carney et al. 1998).

Primary pigmented nodular adrenocortical disease (PPNAD) and cardiac myxomas seem to have a bimodal distribution among CNC patients; the majority of patients present with PPNAD or cardiac myxomas in the second and third decade of life, but a second group of patients may be diagnosed with either type of tumour (or rarely, both) in the first 2–3 years of life (Boikos & Stratakis 2007). Overall, Cushing’s syndrome due to PPNAD is observed in 25–30% of patients with CNC. Since CNC associated Cushing’s disease is often cyclical and atypical, developing progressively over the years, it is difficult to diagnose (Stratakis et al. 1998, Stratakis et al. 2001). Cardiac myxomas are the most common among the noncutaneous lesions found in CNC patients (Stratakis et al. 2001). They are responsible for more than 50% of the disease-specific mortality among CNC patients (Stratakis et al. 2000). Skin myxomas are found especially in the eyelid, external ear canal, breast and nipples. Other possible sites to find myxomas include the oropharynx, the female genital tract and the pelvis. Breast myxomatosis may be found through fat-suppressed magnetic resonance imaging (Boikos & Stratakis 2007).

Large-cell calcifying Sertoli cell tumours (LCCSCT) and thyroid nodules often appear during the first decade of life and progress gradually to replace the normal testicular and thyroid follicular tissue, respectively. In addition, malignant growth in later years is possible (Boikos & Stratakis, 2007). LCCSCTs are almost always found by ultrasonography in postpubertal male patients in CNC, therefore detection of testicular microcalcifications by this method can be used as a diagnostic tool in CNC (Boikos & Stratakis, 2007). LCCSCTs are usually benign in CNC, and they rarely have any endocrine consequences due to increased P-450 ar-
matase expression leading to gynaecomastia and precocious puberty (Boikos & Stratakis 2007). Thyroid gland nodules are common among patients with CNC; by ultrasonography up to 75% of patients have cystic or multinodular disease (Stratakis et al. 2000). Follicular or thyroid cancer may develop in up to 10% of CNC patients with pre-existing thyroid pathology (Stratakis et al. 1997, Boikos & Stratakis 2007).

Biochemical acromegaly with elevation of growth hormone and IGF-I levels and subtle hyperprolactinaemia may be present in up to 75% of patients, although clinically evident acromegaly is a relatively infrequent manifestation of CNC (Boikos & Stratakis 2006). Most CNC patients do not have any tumorous findings in pituitary imaging studies (Boikos & Stratakis 2006). Similar to patients with Mc-Cune Albright disease, pituitary hyperplasia appears to precede tumour development in CNC. Also, most patients in CNC have mild hypersomatotropinaemia starting in adolescence (Boikos & Stratakis 2006). Acromegaly is characterized by a slow, progressive course in CNC, with pituitary tumours being detected from the third decade of life. Also, in many cases acromegaly has not become apparent until after operation for Cushing’s syndrome (Boikos & Stratakis 2007). There is also evidence that pituitary tumours may be multicentric in CNC, supporting the hypothesis of hyperplasia developing into an adenoma (Pack et al. 2000).

Psammomatous melanotic schwannoma (PMS) is very rare as a sporadic, isolated tumour. In association with CNC it may become malignant. PMS may be found anywhere in the central and peripheral nervous system, but its most frequent site has been the gastrointestinal tract and the paraspinal sympathetic chain in patients with CNC (Boikos & Stratakis 2007). An unusual mammary tumour, breast adenoma, and osteochondromyxoma of bone are additional tumours associated with CNC (Boikos & Stratakis 2007).

2.3.5 Familial isolated pituitary adenoma (FIPA)

Pituitary adenomas of all types can occur in a familial setting in the absence of MEN1 and CNC, the phenotype being termed as familial isolated pituitary adenoma (FIPA, Table 5). Before an international and multicentric study reported by Daly et al. (2006), there were only a few reports on other familial isolated pituitary adenomas apart from isolated familial somatotropinoma (IFS) (Gardner et al. 1989, Links et al. 1993, Berezin & Karasik 1995).
In the report by Daly et al. (2006) there were 64 FIPA families including 138 affected individuals with 55 prolactinomas, 47 somatotropinomas, 28 nonsecreting adenomas and 8 ACTH-secreting tumours. A first-degree relative of 76% of the affected individuals was affected. A single tumour phenotype occurred in 30 families (homogenous families), whereas different tumour types occurred in the rest (heterogeneous families). FIPA cases were younger at diagnosis than sporadic cases (38.4±16.3 vs. 41.9±15.1 years, respectively; P=0.015). Prolactinomas from heterogeneous families were larger and had more frequent suprasellar extension than their sporadic or homogenous counterparts. Instead, prolactinomas in homogenous families were similar to sporadic prolactinomas in respect to the finding that 71% of the cases were females with microprolactinomas. One of the male patients from a heterogeneous FIPA family developed a malignant prolactinoma described earlier by Petrossians et al. 2000 (Daly et al. 2006). Familial cases with non-functioning tumours were also younger at diagnosis (P=0.03) and had more frequently invasive tumours (P=0.024) than sporadic ones. The demographic and clinical characteristics of the familial and sporadic Cushing’s disease groups did not differ significantly from one another (Daly et al. 2006).

2.3.6 Isolated familial somatotropinoma (IFS)

Isolated familial somatotropinoma (IFS) is defined as the occurrence of at least two cases of acromegaly/gigantism in a single family in the absence of CNC or MEN1 (Daly et al. 2006, Table 5). Approximately 100 cases of IFS in more than 40 different families have been reported (Luccio-Camelo et al. 2004, Soares & Frohman 2004).

According to a recent review (Soares & Frohman 2004) the clinical manifestations of IFS were similar to those in patients with sporadic somatotropinomas. The incidence of macroadenomas in patients with sporadic acromegaly varies from 60–90%, while it was 88% in IFS. Gigantism was present in 21 of the 96 cases (12%) on which information was available. Immunohistochemical studies were performed on 35 tumours, of which 20 (57%) exhibited prolactin immunostaining. Many of these patients were mildly hyperprolactinaemic (Soares & Frohman 2004). The histological types of the adenoma vary from somatotroph (most cases) to mammosomatotroph even within the same family (Verloes et al. 1999). It was also noted that there was a preponderance of invasive macroadenomas at the time of diagnosis among the familial cases of somatotropinoma (Verloes et al. 1999, De Menis & Prezant 2002). In addition, IFS patients (n=26, 12 families) representing
19% of the FIPA cases and families analysed by Daly et al. (2006), had more aggressive tumours, with extrasellar (P=0.023) and suprasellar extension (P=0.043) occurring more frequently than in heterogeneous somatotropinoma families. The median age at diagnosis was 26 years in IFS cases, whereas in sporadic cases of acromegaly the average age at onset is 40–50 years (Soares & Frohman 2004). IFS patients analysed by Daly et al. (2006) were diagnosed at a mean age of 33.8±18.9 years, which was more than 10 years younger than somatotropinoma patients from either heterozygous familial isolated pituitary adenoma families (49.3±16.3, P= 0.002) or sporadic cases (44.1±13.5, P=0.0023). In addition, all five patients with gigantism belonged to IFS families (Daly et al. 2006). The suboptimal results of surgical treatment in reported IFS patients compared to those of sporadic GH-secreting tumours were pointed out by De Menis & Prezant (2002).

Most IFS pedigrees are small, including only two affected relatives (Verloes et al. 1999), and the disease was expressed in only a single generation in 60% of the families and across multiple generations in the others reviewed by Soares & Frohman (2004). It has been suggested that IFS is inherited as an autosomal dominant disease with reduced or incomplete penetrance (Verloes et al. 1999, Soares & Frohman 2004, Soares et al. 2005). Loss of heterozygosity at chromosome 11q13, the locus of the MEN1 gene, has been reported in both sporadic and familial somatotropinomas, although the MEN1 sequence and expression has appeared normal in most cases (Gadelha et al. 2000). In 2000, Gadelha et al. provided evidence for a linkage of the IFS locus to chromosome 11q13.1-q13.3 and for a potential second locus at 2p16-12. The latter was excluded in subsequent studies in several different families (Soares & Frohman 2004), but the hypothesis of the chromosome region 11q13 as harbouring a potential IFS locus was supported by further studies (Luccio-Camelo et al. 2004, Soares et al. 2005).

2.3.7 Multiple endocrine neoplasia type 4 (MEN4)

Multiple endocrine neoplasia type 4 was only recently brought to the OMIM database on the basis of a single report on humans (Table 5). Pellegata et al. (2006) reported a 48-year-old female patient who developed acromegaly and had a 3-cm pituitary tumour removed when she was 30. Histologically the tumour was an invasive pituitary adenoma with growth hormone hyperproduction, high mitotic activity, and cell atypia. Sixteen years later she was diagnosed with PHPT. At the time of the study she had not yet undergone surgery, although the authors suggested that PHPT in this patient was likely to be caused by parathyroid
hyperplasia or adenoma. The patient was found to carry a germline heterozygous nonsense mutation (c.G692A) in \textit{CDKN1B} (\textit{cyclin-dependent kinase inhibitor 1B}) encoding the cyclin-dependent kinase inhibitor p27\textsuperscript{kip1}. The proband’s older sister, also a mutant carrier, was diagnosed at age 55 with renal angiomyolipoma. Her son developed testicular cancer at age 28, but his mutational status, as well as that of the proband’s father with acromegaly, is unknown (Pellegata \textit{et al.} 2006).

Ozawa \textit{et al.} (2007) analysed the \textit{CDKN1B} gene in 16 cases with sporadic tumours both in the parathyroids and the pituitary, and another 18 cases with related features of familial tumours (an index case with parathyroid and/or pituitary tumours and one first-degree relative with the other in that tumour pair). They found no pathogenic alterations in the \textit{CDKN1B} gene in any of the patients, which led to the conclusion that the MEN1 variant with sporadic parathyroid tumours, sporadic pituitary tumours, and no identified \textit{MEN1} mutation is usually not caused by \textit{CDKN1B} germline mutations (Ozawa \textit{et al.} 2007).
3 Purpose of the present study

1. To study the clinical features of Northern Finnish MEN1 mutation carriers and to evaluate whether there is genotype-phenotype correlation in relation to a particular mutation and the resulting phenotype.

2. To study whether MEN1 mutation carriers are at increased risk of premature mortality.

3. To study the familial cluster of pituitary adenoma presumably due to founder mutation effect of an unknown gene and to describe the clinical features and genetic background of this new clinical entity.

4. To study the possible role of different genetic factors in the aetiology of primary hyperparathyroidism in patients with features of hereditary predisposition.
4 Subjects and methods

4.1 Subjects and families

4.1.1 Clinical features and genotype-phenotype correlation of MEN1 in Northern Finland (I)

*MEN1* mutation carriers, identified at the Department of Clinical Genetics in Oulu University Hospital (OUH) during 1982–2001 and belonging to the MEN1 families originating from Northern Finland were included in the study. Initially, 25 index patients received genetic counselling for MEN1, which was also offered to their relatives at risk. After genetic counselling, a total of 159 subjects opted for further studies. Prior to 1997, the ascertainment of heterozygotes was based on both biochemical and radiological information, combined later with *MEN1* linkage analysis. All these cases were confirmed by direct mutation analysis when it became possible in 1997. From then on all the new cases have been directly analysed by *MEN1* mutation testing. Among the 159 individuals studied, the *MEN1* gene analysis revealed 87 heterozygotes including 25 index patients and 62 newly diagnosed family members. Of the newly diagnosed heterozygotes, five were excluded from the analysis for various reasons (two were tested as newborns, two did not enter the screening programme, and one was living outside Finland). Thus, the study population consisted of 82 *MEN1* mutation carriers.

4.1.2 Study on Premature Mortality in MEN1 founder mutation carriers (II)

All the families with one or more MEN1 probands with the two founder mutations (1466del12 and 1657insC) were included. There were altogether 15 probands in 13 families with the MEN1 syndrome. In the families, 40 cases have been documented to have the 1466del12 mutation (9 families) and 30 the 1657insC mutation (4 families). The pedigrees of probands made during the initial visits at the department of Clinical Genetics at OUH were further extended by genealogical studies. The names, dates, and places of birth of the ancestors and the dates of death were traced using Finnish church records. Practically all the roots going back to 1700 were evaluated to find common ancestors and many even further. The lines of inheritance back to the MEN1 patients were studied to evaluate the
obligatory carriers and their spouses with respect to lifespan, including all individuals born before 1929.

**4.1.3 Pituitary adenoma predisposition (III)**

Three small clusters of familial pituitary adenoma in Northern Finland were included in the study. The first cluster displayed two cases of acromegaly and one case of gigantism. The second cluster consisted of two patients, one diagnosed with acromegaly, and the other with prolactinoma. The third cluster consisted of two distantly related patients with gigantism. Genealogy data reaching back to the the 18th century had been generated by family members based on publicly available official population registries. The first two clusters could be linked by genealogy to a large pedigree (Figure 1, family 1), and the third appeared as separate (Figure 2, family 2). Additional pituitary adenoma patients belonging to the pedigree were identified by using data on a previously characterized population-based cohort of 54 patients diagnosed with GH-secreting pituitary adenoma between 1980 and 1999 at OUH (Kauppinen-Mäkelin et al. 2005), patient interviews, and a computerized search for all cases with archived samples of pituitary adenomas at OUH from 1978 to 2000.

Samples of 13 affected individuals and 7 obligatory carriers as well as 4 key unaffected relatives (parents) were obtained from families 1 and 2 (Figure 1 and 2). The sample material was either blood or paraffin-embedded tissue. For the population-based study 45 blood or paraffin-embedded samples were obtained from OUH. These patients with sporadic GH-secreting pituitary adenoma were diagnosed between 1980 and 1999. Genomic normal DNA from one German individual and one Turkish individual, as well as from one Italian sibling pair with somatotropinoma, was included in this study. In addition, DNA from 10 unselected Finnish sporadic acromegaly patients was available. For the controls of the Finnish AIP mutations, 219 anonymous Finnish blood donors from the Oulu region were used. For the Italian AIP mutation 52 anonymous Italian blood donors, 94 UK Caucasians (Human Random Control DNA panel, Porton Down, Salisbury, Wiltshire, UK), and 109 unrelated Caucasian CEPH (Centre d'Etude du Polymorphisme Humain) individuals were used as controls.
4.1.4 Primary hyperparathyroidism patients with features of genetic predisposition (IV)

Medical records of 286 patients treated for PHPT during 1974–2001 in Oulu University Hospital were reviewed. The patient was included in the study when at least one of the following inclusion criteria was met: multiglandular, recurrent or persistent PHPT, another MEN1-related manifestation (enteropancreatic, pituitary, adrenal or foregut neuroendocrine tumour), age at onset of PHPT 40 years or under, or PHPT/MEN1-related manifestation(s) in the family. The first documented finding of hypercalcaemia or a urinary tract stone were used to determine the age at onset of PHPT. Twenty of the patients were excluded since they had already been diagnosed as having MEN1 based on genetic or clinical evidence. Altogether, 56 patients meeting the inclusion criteria were invited to the study by a written invitation, and 29 of them were willing to take part in the study.

4.2 Biochemical and imaging studies of MEN1 mutation carriers and PHPT patients with features of genetic predisposition (I, IV)

All MEN1 mutation carriers had regular follow-ups at the closest endocrinology unit for screening of the MEN1-related manifestations. The basic follow-up visit every 1 to 3 years consisted of biochemical screening of serum sample (ionized calcium, PTH, pancreatic polypeptide (PP), gastrin, chromogranin A (CGA) and prolactin). Radiological imaging (ultrasound (US), computed tomography (CT), magnetic resonance imaging (MRI)) of the abdomen was performed every 1–3 years and of the pituitary (CT, MRI) every 5–10 years. In cases of active disease, the patients were seen more frequently and a wide range of different diagnostic methods was used, including somatostatin-receptor scintigraphy to detect neuroendocrine tumours of various sites. SPSS 12.0.1 (SPSS Inc., Chigaco, IL, USA) was used for statistical analyses.

PHPT patients with features of genetic predisposition were screened for ionized calcium, PTH, PP and prolactin in order to identify MEN1 suspected patients since approximately 10–20% of cases are missed in MEN1 mutation analysis (Brandi et al. 2001). A clinical evaluation by an endocrinologist and more detailed biochemical screening was offered to patients with marked elevation of serum PP and/or prolactin, or evidence of recurrent PHPT. The prolactin concentration was analysed by automated chemiluminescence system (Advia Centaur, Bayer Corporation, NY, USA) and PTH (Nichols Institute Diagnostics, San Clemente, CA,
USA) and PP (Euro-Diagnostica, Arnhem, the Netherlands) by radioimmunoassay following the instructions of the manufacturers. Testing of serum cortisol, gastrin, ionized calcium, FSH, LH, testosterone, free thyroxine (FT4), TSH, and plasma CGA, was done by standard automated techniques on clinical service basis for those with marked elevation of serum PP and/or prolactin, or evidence of recurrent PHPT.

4.3 Obtaining clinical data and causes of death (I)

The medical records of the MEN1 mutation carriers were reviewed for the presence and timing of the MEN1-related manifestations, any other tumours and deaths. In addition, the death certificates and the autopsy reports were studied, if available, to evaluate the causes of death.

The onset and diagnosis of PHPT was defined as the first incidence of hypercalcemia with an inappropriately elevated serum PTH level. The classification of GEP tumours was based on clinical evidence of excess hormone secretion or the lack thereof, and in operated patients it was confirmed by histology using the WHO criteria (Kloppel et al. 2004). For nonfunctional pancreatic tumours (NFPT), the diagnosis was based on either constantly elevated tumour markers (S-PP and/or P-CGA) or visualization of the tumours by imaging examinations in the absence of other pancreatic hormone oversecretion and clinical syndrome. The diagnosis of gastrinoma was based on elevated serum gastrin levels, in most cases confirmed by an appropriate rise during secretin test/or increased gastric acid output. Gastroscopy and measurement of parietal cell autoantibodies were used to rule out the possibility of atrophic gastritis. Pituitary adenomas were also classified according to the presence of hormonal activity or the lack thereof on clinical and biochemical basis. They were considered microadenomas when less than 10 mm and macroadenomas when equal to or greater than 10 mm in diameter (Hardy 1973). Adrenal glands were registered as affected when evidence of hyperplasia or tumours was seen in imaging studies, and as hormonally active if indicated by biochemical testing (1.5 mg overnight dexamethasone test, measurement of 24-h urine normetanephrines and metanephrines, and in hypertensive patients measurement of plasma renin and serum aldosterone). The follow-up time was counted from the first visit to a health care institution for MEN1 suspicion in the patient or family. The last point of observation period was counted as the last information concerning MEN1-associated documentation available by the end of August 2003 or the patient’s death by that time.
Mean ages were reported as mean ± 1 S.D. Binary logistic regression analysis was used for analysing the covariates affecting the likelihood of having different MEN1 manifestations. The study cohort was analysed in two groups containing either all the ascertained heterozygotes or just the founder mutation carriers. Covariates included in all of the analyses were age at the end of the follow-up, gender and mutational background (mutation class when analysing all the cases, and the exact mutation when analysing the founder mutation carriers). Confidence intervals (CI) of 95% are reported in parenthesis after odds ratio (OR) values. P<0.05 was considered significant. Penetrance of the major manifestations by age was calculated by Kaplan-Meier analysis.

4.4 Comparing mean values of age at death (II)

SPSS for Windows (Release 10.0.7) was used for statistical analyses. For comparing mean values of ages in the groups, Student’s t test was used, and Levene’s homogeneity-of-variance test was used to ascertain appropriateness of the analysis. Using revised (1881–1990) and abridged (1751–1880) life tables for Finland, an estimate of expected lifetime was applied for each obligatory MEN1 carrier and their spouses (Kannisto & Nieminen 1996, Turpeinen & Kannisto 1997). These tables give sex- and birth-year category-specific life expectancy values in years at a certain age. In this survey, life expectancy at the age of 25 years was used. Birth-year categories in earlier years were in 10-year and later in 5-year stratification periods. The significance of the differences in causes of death was tested by Person χ² test and Fisher’s exact test.

4.5 Histopathology and immunohistochemistry (III)

Immunohistochemistry of familial and sporadic adenomas for hypophyseal hormones (ACTH, GH, FSH, LH, TSH, and PRL) was performed according to standard laboratory procedures using LabVision Autostainer 480 (LabVision Corp., Fremont CA).

4.6 SNP array and linkage analyses (III)

To identify the predisposing locus for pituitary adenoma, genomic DNA was isolated from blood by the PureGene DNA isolation kit (Puregene, gentra Systems, Minneapolis, MN). SNP genotyping was performed for 16 individuals
from family 1 using Human Mapping 50K Xba 240 SNP array (Affymetrix, Santa Clara, CA). Signal intensity data was analysed using the GeneChip DNA analysis software v. 3.0.2.8 (GDAS) (Affymetrix). The genotyping data were converted into appropriate linkage format by the ALOHOMORA software (Ruschendorf & Nurnberg 2005). Because current linkage programmes are not suitable for analysing large numbers of markers in large pedigrees (Lingaas 2003), family 1 had to be divided into 3 separate branches for the genome-wide parametric and non-parametric multipoint calculations with the Allegro software (Gudbjartsson et al. 2000, Gudbjartsson et al. 2005). In these analyses LOD scores for three branches were calculated separately and added together by loci.

In order to obtain maximum information from the linkage data, the entire family 1 data was reanalysed as one pedigree in regions displaying LOD scores over 3 with the SimWalk2 2.91 software (Sobel & Lange 1996, Sobel et al. 2001) using sets of 10–20 markers per analysis. Suitable analysis files for SimWalk2 were created with the Mega2 data handling programme (Mukhopadhyay et al. 2005). In order to evaluate the effect of linkage disequilibrium (LD) in regions with LOD scores over 3, the data were further analysed with reduced marker density (<0.1 cM) using SimWalk2 software.

The most likely haplotypes were constructed with the Allegro and SimWalk2 software and the results were visualized with HaploPainter V.024beta (Thiele & Nurnberg 2005). Fine mapping studies were performed on genomic DNA isolated from blood or paraffin-embedded normal tissue. Thirty published and novel microsatellite markers from chromosomal region 11q12.2-11q13.3 (physical location 61.4–69.0 Mb), as well as the six most informative SNP-markers from Human Mapping 50K Xba 240 SNP array mapping to the region of interest were utilized. Analyses of microsatellites and SNP-markers were carried out using ABI3730 sequencer (Applied Biosystems, Foster City, CA). Microsatellite data were analysed with Genemapper software v. 3.7 (Applied Biosystems). The most informative markers in the shared region were used for the overall parametric linkage calculation in families 1 and 2 by the SimWalk2 software with default general parameters. The disease model parameters were as follows: phenocopies for acromegaly or gigantism 0.0002, and for any pituitary adenoma 0.01, penetrancies for homozygous and heterozygous disease genotypes 0.1 and the disease allele population frequency 0.001. All the other individuals except the affected ones were considered unknown phenotype.
4.7 Expression profiling (III)

Expression profiles were obtained for 16 individuals, 9 affected/obligatory carriers and 7 controls. As controls, 5 unrelated spouses from families 1 and 2 were used. Total RNA was extracted from whole blood with the PAXgene Blood RNA Kit (PreAnalytiX, Hombrechtikon, Switzerland) and concentrated with RNeasy MinElute Cleanup columns (Qiagen, Valencia, CA). The quality of RNA was analysed using a spectrophotometer and an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). Biotin-labelled and fragmented cRNA was prepared from 5 µg of total RNA with procedures recommended for the Human Genome U133 Plus 2.0 array expression analysis (Affymetrix). The HG-U133 Plus 2.0 Chips were hybridized according to manufacturer’s instructions, and scanned with GeneChip® scanner 3000 (Affymetrix). GeneChip Operating Software (GCOS) (Affymetrix) was used to obtain quantitative expression information for the probe sets. The quantitative expression data were normalized by scaling all chips to the average gene expression data of all 16 chips, and the expression of each probe set was divided by the mean expression of that probe across all the samples so that the resulting mean expression for every probe was 1. The normalized expression data were filtered to remove expression values below detectable levels using the Affymetrix Detection Algorithm assigned flag cells. Subsequent analyses were restricted to probe sets with detectable expression in a sufficient number of samples to allow a Student’s \( t \)-test between the affected and control groups. Data processing and analyses were performed with GeneSpring 7.0 software (silicon Genetics, Redwood City, CA).

4.8 Mutation screening and search for loss of heterozygosity (LOH) (III, IV)

Mutation screening of the candidate gene for pituitary adenoma predisposition, \( AIP \), was done from genomic DNA isolated either from blood of paraffin-embedded normal tissues. Primers (Original article III, Supported online material, Table S4) were designated to amplify the coding region and exon-intron boundaries of \( AIP \). PCR protocols are available upon request. Sequencing was performed using the Big Dye 3.1 termination chemistry and ABI3730 DNA sequencer (Applied Biosystems). LOH analyses were done from paraffin-embedded tumour samples by sequencing the corresponding mutation position.
In the study including PHPT patients with features of genetic predisposition, peripheral blood samples were used as a source of constitutional DNA in the genetic analyses. Mutation screening of the MEN1, HRPT2, CASR, CDKN1B and AIP genes was performed by sequencing the entire coding region and exon-intron boundaries. For MEN1, HRPT2, CASR and CDKN1B the primers used for amplification and sequencing, as well as the annealing temperatures for the amplifications, have been published previously (Villablanca et al. 2002, Villablanca et al. 2004, Georgitsi et al. 2007) or are available upon request, and for AIP as mentioned above. For MEN1, HRPT2 and CASR the sequencing was done with the Big Dye Terminator cycle sequencing kit (Perkin Elmer) and run in an automated sequencer ABI377 (Perkin Elmer / Applied Biosystems, Foster City, CA). For CDKN1B the sequencing was done with the Big Dye 3.1 termination chemistry and an ABI3730 DNA sequencer (Applied Biosystems, Foster City, CA).

4.9 Ethical issues

Appropriate research permissions have been obtained from the Ethical Board of the Northern Ostrobothnia Health Care District and the Ethical Board of Helsinki University Hospital. All patients gave their informed consent for blood specimens. Tumour samples were studied under the authorization of the National Authority for Medicolegal Affairs if the patient’s authorization was not available. Relatives of patients were only contacted through patients. Genetic counselling was offered to the patients and relatives if a mutation was detected in any of the genes studied.
Fig. 1. Pedigree of the family 1 with pituitary adenoma. Individuals available for the study are indicated by A2, A5, etc. Numbers within diamonds indicate numbers of children. Circles, females; squares, males; slashes through symbols, deceased. Blackened symbols, somatotropinoma; horizontal lines, prolactinoma; blackened symbols and horizontal lines, mixed pituitary adenoma. Generations are indicated by Roman numerals on the left. Generation I is from the 18th century (Original publication III).
Fig. 2. Pedigree of the family 2 with pituitary adenoma. Individuals available for the study are indicated by A8 and A34. Numbers within diamonds indicate numbers of children. Circles, females; squares, males; slashes through symbols, deceased. Blackened symbols, somatotropinoma. Generations are indicated by Roman numerals on the left (Original publication III).
5 Results

5.1 MEN1 in Northern Finland; clinical features and genotype-phenotype correlation (I)

Out of the 82 individuals (35 men, 47 women) found to be MEN1 heterozygous carriers 68 (83%) were found to carry either the mutation 1466del12 or 1657insC, which are the founder mutations in Northern Finland as described earlier (Kytölä et al. 2001). The mutation 1466del112 was found in 39 individuals belonging to 9 families, and 1657insC was found in 29 individuals from 4 families. Three additional mutations D418N, G156R and R527X were found in 9, 3 and 2 individuals, respectively, from three different families. For the 82 mutation-positive individuals, the mean age at onset of the follow-up was 40.8 ± 14.6 yr (range, 11–70 yr) and the mean age at the end of the observation period was 48.1 ± 15.4 yr (range, 18–78 yr). The mean duration of the follow-up time was 7.3 ± 4.5 yr (range, 0–18 yr). Age-dependent penetrance of the major manifestations according to Kaplan-Meier analysis is shown in Figure 1 in the original publication I. The prevalence, mean age at diagnosis and risk factors of the major MEN1 manifestations among the 82 MEN1 heterozygotes are shown in Table 6.

PHPT was detected in 31 males and 45 females (n=76; 93%) (Table 6). Only six patients (7%) remained normocalcaemic during the follow-up. For these six patients without PHPT the mean age at onset of the follow-up was 40.8 ± 14.6 yr (range, 11–70 yr) and the mean age at the end of the observation period was 48.1 ± 15.4 yr (range, 18–78 yr). The mean duration of the follow-up time was 7.3 ± 4.5 yr (range, 0–18 yr). Age-dependent penetrance of the major manifestations according to Kaplan-Meier analysis is shown in Figure 1 in the original publication I. The prevalence, mean age at diagnosis and risk factors of the major MEN1 manifestations among the 82 MEN1 heterozygotes are shown in Table 6.

PHPT was detected in 31 males and 45 females (n=76; 93%) (Table 6). Only six patients (7%) remained normocalcaemic during the follow-up. For these six patients without PHPT the mean age at onset of the follow-up was 40.8 ± 14.6 yr (range, 11–70 yr) and the mean age at the end of the observation period was 48.1 ± 15.4 yr (range, 18–78 yr). The mean duration of the follow-up time was 7.3 ± 4.5 yr (range, 0–18 yr). Age-dependent penetrance of the major manifestations according to Kaplan-Meier analysis is shown in Figure 1 in the original publication I. The prevalence, mean age at diagnosis and risk factors of the major MEN1 manifestations among the 82 MEN1 heterozygotes are shown in Table 6.

PHPT was detected in 31 males and 45 females (n=76; 93%) (Table 6). Only six patients (7%) remained normocalcaemic during the follow-up. For these six patients without PHPT the mean age at onset of the follow-up was 40.8 ± 14.6 yr (range, 11–70 yr) and the mean age at the end of the observation period was 48.1 ± 15.4 yr (range, 18–78 yr). The mean duration of the follow-up time was 7.3 ± 4.5 yr (range, 0–18 yr). Age-dependent penetrance of the major manifestations according to Kaplan-Meier analysis is shown in Figure 1 in the original publication I. The prevalence, mean age at diagnosis and risk factors of the major MEN1 manifestations among the 82 MEN1 heterozygotes are shown in Table 6.

In this patient cohort, four types of GEP tumours could be found: nonfunctional pancreatic tumours, gastrinomas, other hormonally active pancreatic tumours and neuroendocrine tumours of the gastrointestinal tract. GEP tumours were found in 26 males and 34 females (n=60 or 73%) (Table 6). Mean age at the end of the follow-up for the patients not having any signs of GEP tumours was 34.0 ± 12.4 yr (range, 18–65 yr). Of the patients with GEP tumours, 18 (30%) had undergone an operation to remove or reduce the size of the tumour. For the whole group of GEPs, no genotype-phenotype correlation was found.
Table 6. Prevalence, mean age at detection and risk factors of the major multiple endocrine neoplasia type 1 (MEN1) manifestations among the 82 heterozygotes in relation to different mutations found. Binary logistic regression analysis was used for analysing the covariates affecting the likelihood of having different MEN1 manifestations. The study cohort was analysed in two groups containing either all the ascertained heterozygotes (n=82) or just the founder mutation carriers (n=68). Covariates included in all of the analyses were age at the end of the follow-up, gender and mutational background (mutation class when analysing all the cases, and the exact mutation when analysing the founder mutation carriers). Confidence intervals (CI) of 95% are reported in parenthesis after odds ratio (OR) values. P<0.05 was considered significant. Mutation class 1 (MC 1) (n= 51) includes in-frame/missense mutations (1466del12, D418N, G156R) and mutation class 2 (MC 2) (n= 31) includes frameshift and nonsense mutations (1657insC, R527X). Mutation 1 (M 1) is 1466del12, mutation 2 (M 2) is 1657insC.

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Mean age of detection ± 1 SD, range</th>
<th>1466del12 (n= 39)</th>
<th>1657insC (n= 29)</th>
<th>D418N (n= 9)</th>
<th>G156R (n= 3)</th>
<th>R527X (n= 2)</th>
<th>Total (n= 82)</th>
<th>Risk factor, OR (CI), P</th>
<th>Founder mutation carriers (n= 68)</th>
<th>Risk factor, OR (CI), P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHPT</td>
<td>39.8 ± 13.5 (16–69)</td>
<td>95</td>
<td>90</td>
<td>89</td>
<td>100</td>
<td>100</td>
<td>93</td>
<td>Age, 1.08 (1.01–1.17), 0.031</td>
<td>No risk factors</td>
<td></td>
</tr>
<tr>
<td>GEP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all</td>
<td>46.8 ± 12.8 (21–69)</td>
<td>74</td>
<td>72</td>
<td>67</td>
<td>67</td>
<td>100</td>
<td>73</td>
<td>Age, 1.12 (1.06–1.19), 0.000</td>
<td>Age, 1.11 (1.05–1.17), 0.000</td>
<td></td>
</tr>
<tr>
<td>metastasis</td>
<td>55.1 ± 12.4 (28–70)</td>
<td>23</td>
<td>14</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>16</td>
<td>Age, 1.07 (1.02–1.12), 0.008</td>
<td>Age, 1.08 (1.03–1.15), 0.005</td>
<td></td>
</tr>
<tr>
<td>NF</td>
<td>45.3 ± 12.5 (23–69)</td>
<td>39</td>
<td>69</td>
<td>44</td>
<td>33</td>
<td>50</td>
<td>50</td>
<td>Age, 1.09 (1.04–1.15), 0.001</td>
<td>Age, 1.08 (1.03–1.14), 0.004</td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>51.6 ± 11.4 (34–70)</td>
<td>39</td>
<td>3</td>
<td>22</td>
<td>0</td>
<td>50</td>
<td>23</td>
<td>Age, 1.09 (1.04–1.15), 0.001</td>
<td>Age, 1.08 (1.03–1.14), 0.004</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>NA</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>33</td>
<td>0</td>
<td>2</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>GI</td>
<td>55.4 ± 9.9 (44–68)</td>
<td>5</td>
<td>7</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Pituitary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all</td>
<td>40.2 ± 14.4 (12–62)</td>
<td>26</td>
<td>31</td>
<td>22</td>
<td>67</td>
<td>50</td>
<td>29</td>
<td>No risk factors, No risk factors</td>
<td>No risk factors</td>
<td></td>
</tr>
<tr>
<td>PRL</td>
<td>40.9 ± 14.5 (22–62)</td>
<td>18</td>
<td>21</td>
<td>22</td>
<td>67</td>
<td>0</td>
<td>21</td>
<td>No risk factors, No risk factors</td>
<td>No risk factors</td>
<td></td>
</tr>
<tr>
<td>NF</td>
<td>41.1 ± 14.8 (12–54)</td>
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<td>7</td>
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<td>0</td>
<td>50</td>
<td>7</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Mixed</td>
<td>NA</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Thymic</td>
<td>51.3 ± 5.0 (45–55)</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0</td>
<td>50</td>
<td>4</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Adrenal</td>
<td>53.7 ± 10.6 (32–70)</td>
<td>39</td>
<td>24</td>
<td>44</td>
<td>33</td>
<td>100</td>
<td>35</td>
<td>Age, 1.09 (1.05–1.14), 0.000</td>
<td>Age, 1.09 (1.04–1.14), 0.000</td>
<td></td>
</tr>
</tbody>
</table>

PHPT, primary hyperparathyroidism is considered as a sign of parathyroid tumour (hyperplasia or adenoma); GEP, gastroenteropancreatic tumour; NF, nonfunctional tumour, G, gastrinoma; Other GEP, insulinoma (1657insC), growth hormone-releasing-hormone secreting tumour GHRHoma (G156R); GI, gastrointestinal tract tumours (other than duodenal gastrinoma); PRL, prolactin secreting pituitary adenoma, The mixed adenoma (in 1657insC mutation carrier) secreted both prolactin and TSH, NA, not applicable due to small number of subjects.
The most prevalent type of GEP tumours was a nonfunctional pancreatic tumour (NFPT) present in 17 males and 24 females (n= 41 or 50%). Of these, 20 (49%) had the 1657insC, 15 (37%) the 1466del12, 4 (10%) the D418N, 1 (2%) the R527X and G156R mutation. In the whole patient cohort, the mutation class including the frameshift and nonsense mutations was found to increase the risk of having a NFPT with an OR of 3.26 (CI, 1.27–8.33), P= 0.014. In the founder mutations group, the 1657insC mutation gave an OR of 3.56 (CI, 1.29–9.83), P= 0.015.

Gastrinomas were suspected clinically and biochemically in 8 males and 11 females (n=19 or 23%). Of these patients, 15 (79%) had the 1466del12 founder mutation. Two other patients (11%) had the D418N mutation, one patient (5%) had the 1657insC founder mutation, and another one (5%) the R527X mutation. Gastrinoma patients were found in 7/9 (78%) of the families carrying the 1466del12 mutation. In the whole study group, the mutation class including the in-frame deletion and missense mutations predicted the risk for gastrinoma with an 6.77 (CI, 1.31–35.0), P=0.022, and in the founder mutations group the 1466del12 mutation gave an OR of 15.1 (CI, 1.73–131.9), P=0.014.

An insulinoma was found in one female patient (age 31 years), and a pancreatic growth-hormone-releasing-hormone secreting tumour resulting in acromegaly was seen in one male patient (age 21 years). Neuroendocrine tumours of the gastrointestinal tract (other than duodenal gastrinomas) were found in 5 female patients, associating with hypergastrinemia in 3. All of these five patients were also affected by pancreatic endocrine lesions. All the patients with metastasized GEP tumours had either the 1466del12 (9 cases) or the 1657insC (4 cases) mutation. Lymph node metastases were found in 3 males and 3 females, and liver metastases in 5 males and 2 females.

Pituitary adenomas were found in 24 (29%) patients (7 males, 17 females) (Table 6). Mean age for those without a tumour was 47.5 ± 16.0 yr (range 18–78 yr) at the end of the follow-up. Among the patients with pituitary adenomas, 17 (71%) had prolactinoma, 6 (25%) had a nonfunctional tumour, and one patient had a mixed adenoma secreting both prolactin and TSH. At imaging studies, 12 (50%) were classified as macroadenomas, and 11 (46%) were microadenomas. One prolactinoma could not be visualized. Of the patients with pituitary adenomas, 12 (50%) had received some therapy. Dopamine agonist treatment had been given to 10 (42%), and 5 had also been operated on. Another patient with prolactinoma received only operative treatment, and one further patient with a nonfunctional adenoma had received radiation therapy. In addition to the patients with adenomas,
there was one further patient with sellar tumour diagnosed at 9 years of age. According to the histology report, the removed tumour was angiosarcoma. The tumour sample was not available for review. The patient received radiation therapy and was followed up by an endocrinologist for panhypopituitarism. At 37 years of age, he was found to be a MEN1 heterozygote through family screening. Two years after entering the follow-up he died of metastasized parathyroid cancer. Genotype had no effect on the risk of pituitary adenoma.

Neuroendocrine tumours beyond the gastrointestinal tract were all thymic in origin (thymic carcinoids or neuroendocrine carcinoma of thymus) and they were found in three male patients (Table 6). The patients were two brothers with the 1657insC mutation at ages 45 and 52 years, and another male patient with the R527X mutation at 55 years. By the end of the follow-up the brothers had developed local recurrences at 47 and 55 years of age, and the former was also found to have skeletal metastases in the spine before his death at 51 years of age. The third patient did not show any signs of thymic neuroendocrine carcinoma six months after the operation.

Adrenal lesions were present in 29 (35%) of the patients (12 males, 17 females) (Table 6). All the adrenal lesions were considered benign and hormonally inactive, except for two patients, who had subclinical hypercortisolism. Of the patients with adrenal lesions, 28 (97%) were also affected by one or more GEP-tumours including 15 nonfunctional pancreatic tumours (52%), 12 gastrinomas (41%) and 1 with both (3%). The likelihood of adrenal lesions was not associated with a certain genotype.

Other tumours found in the study population included 5 cases of thyroid carcinoma (4 papillary, 1 medullary type), 4 cases of renal tumours (2 angiomyolipomas, 2 carcinomas; multifocal papillary carcinoma and clear cell carcinoma), 2 breast carcinomas (both invasive ductal carcinoma), 3 uterine tumours (leiomyoma and leiomyolipoma in one patient, mesodermal mixed tumour in another). One patient was diagnosed with prostate adenocarcinoma and transitional cell carcinoma of bladder. In addition to uterus, leiomyomas were found in the ileum, ventricle and epididymis. Systematic search for cutaneous tumours or uterine leiomyomas was not performed. Meningioma was found in two patients. Four of the thyroid cancers appeared in two families carrying either 1657insC or D418N mutation (2 cases in each). The fifth was found in a 1466del12 mutation carrier who had also had clear cell renal cancer and breast cancer.
During the observation period 9 men and 6 women died at a mean age of 59.0 ± 12.7 yr (range 39–72 yr) and 62.9 ± 15.0 yr (range, 36–79 yr), respectively. MEN1 was considered to be a direct cause of death in 7 cases. Of these, 6 patients’ death was associated with MEN1-related malignancy. A metastasized GEP tumour was the direct cause of death in 5 patients, including two pancreatic gastrinomas with liver metastases, two NFPTs with liver and the other one with local lymph node metastases, and one neuroendocrine carcinoma of the stomach and the duodenum with multiple metastases. One of the patients died of parathyroid cancer with multiple widely spread metastases. In addition, for one patient diagnosed as having gastrinomas 15 years earlier, the cause of death was haemorrhage from a duodenal ulcer. He had refused to attend the follow-up visits 8 years earlier. MEN1 was a contributing factor to death in 3 cases presenting as a metastasized pancreatic neuroendocrine tumour in 2 patients and as a thymic neuroendocrine carcinoma in one patient. Five patients died of non-MEN1-related causes.

5.2 Effect of MEN1 founder mutations on premature mortality (II)

With one exception, common ancestors for all the MEN1 patients were found. The pedigrees of the founder mutations are illustrated in Figures 3 and 4. The initial group consisted of 34 obligatory gene carriers, 16 males and 18 females, (MEN1 positive in later text) and of 33 spouses, 18 males and 15 females, born between 1728 and 1929. Two male spouses were excluded from the analyses because of insufficient information (IX:11, IX:17 in Figure 3). Thus, the final study group consisted of 34 heterozygotes and 31 spouses. Four female subjects were still alive, two gene positive (IX:12 born 1927 and IX:16 born 1924 in Figure 3) and two spouses (IX:3 born 1922 in Figure 3 and III:8 born 1928 in Figure 4). In the 1466del12 mutation pedigree, there were 28 heterozygotes (15 males and 13 females) and 25 spouses (11 males and 14 females), and in the 1657insC mutation pedigree, six heterozygotes (one male and five females) and six spouses (five males and one female). The living subjects were excluded from true lifespan analysis, but a subanalysis using their age as lifespan was made.

MEN1 obligate carrier males (n=16) died at a mean age of 61.1 ± 12.0 years (range, 39–89) and MEN1 obligate carrier females (n= 16) at a mean age of 67.2 ± 10.7 years (range, 42–79). Table 7 summarizes the results of comparative lifespan analyses, which showed no significant differences between any of the groups analysed.
Fig. 3. Pedigree of 1466del12 mutation-positive families with MEN1 case(s) originating from common ancestors. Only obligatory gene carriers (affected, closed symbols) and their spouses are illustrated in descending generations. Subjects born 1930 or later are excluded. Birth years of ancestors are shown. Subjects are numbered by generation (Roman numbers) and by order in the generation (Arabic numbers). Subjects IX:3, IX:12, and IX:16 were alive as of December 14, 2001. Subject IX:9 was not married (Original publication II, © The Endocrine Society, 2004).
Fig. 4. Pedigree of 1657insC mutation-positive families with MEN1 case(s) originating from common ancestors. Only obligatory gene carriers (affected, closed symbols) and their spouses are illustrated in descending generations. Subjects born 1930 or later are excluded. Birth years of ancestors are shown. Subjects are numbered by generation (Roman numbers) and by order in the generation (Arabic numbers). Subject III:8 was alive as of December 14, 2001 (Original publication II, © The Endocrine Society, 2004).

Table 7. Mean ages of death (years) of 32 obligatory MEN1 founder mutation carriers (1466del12 and 1657insC) compared to the mean ages of death of 29 unaffected spouses in sex groups. The comparison was also done between life expectancy and mean age of death in each group (affected and unaffected subjects in sex groups). Lifespan analysis included deceased subjects from the founder mutation pedigrees born between 1728 and 1929. Subjects from the 1466del12 mutation pedigree included 26 affected/heterozygous (15 males and 11 females) and 24 unaffected spouses (11 males and 13 females). Subjects from the 1657insC mutation pedigree included 6 affected/heterozygous (1 male and 5 females) and 5 unaffected male spouses.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean age (range)</th>
<th>Comparison of mean age at death, P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected (m) (n=16)</td>
<td>61.1 ± 12.0 (39–89)</td>
<td>affected (m) vs. life expectancy, 0.841 (NS)</td>
</tr>
<tr>
<td>Life expectancy</td>
<td>61.7 ± 3.1</td>
<td></td>
</tr>
<tr>
<td>Unaffected (m) (n=16)</td>
<td>65.8 ± 15.3 (34–84)</td>
<td>affected (m) vs. unaffected (m), 0.342 (NS)</td>
</tr>
<tr>
<td>Life expectancy</td>
<td>62.2 ± 1.7</td>
<td>unaffected (m) vs. life expectancy, 0.377 (NS)</td>
</tr>
<tr>
<td>Affected (f) (n=16)</td>
<td>67.2 ± 10.7 (42–79)</td>
<td>affected (f) vs. life expectancy, 0.806 (NS)</td>
</tr>
<tr>
<td>Life expectancy</td>
<td>66.5 ± 3.3</td>
<td></td>
</tr>
<tr>
<td>Unaffected (f) (n=13)</td>
<td>67.7 ± 14.7 (46–87)</td>
<td>affected (f) vs. unaffected (f), 0.917, (NS)</td>
</tr>
<tr>
<td>Life expectancy</td>
<td>63.7 ± 3.5</td>
<td>unaffected (f) vs. life expectancy, 0.272 (NS)</td>
</tr>
</tbody>
</table>

M, male; f, female; vs., versus; NS, nonsignificant, P-value <0.05 was considered significant.
In addition, in linear regression analysis with the age of death as a dependent variable and pedigree group, MEN1 trait, and sex as explaining variables and expected lifespan and birth year as covariates, no significant associations explaining death age were encountered.

The causes of death of 55 out of 61 subjects were found, and causes of death of six subjects were not attained (one proband and five spouses). Reliability of the causes was classified by the investigator as certain (36%), quite certain (13%), and as only based on church records (51%). The main causes of death were cardiovascular disease (41.8%), infection (16.4%, mainly tuberculosis), ageing (12.7%), trauma (5.5%), withering (5.5%), malignancy not classically associated with MEN1 (5.5%), MEN1-associated causes of death (9.1%, often classified as cancer in the abdomen) and MEN1 causes (3.6%, such as gastroenteropancreatic tumor). Withering (synonyms wilting, fading, and wasting away) as a cause of death is a descriptive term and could imply a chronic disease, often malignancy, causing death. When the last four (withering, malignancy, MEN1-associated cause of death, and MEN1 cause) were classified as possible MEN1 causes and others as not MEN1 causes, a statistically significant difference was found between probands and spouses in females (Fisher’s exact test, \( P = 0.003 \)) but not in males (Fisher’s exact test, \( P = 0.355 \)). In female subjects, out of 15 probands, eight (53%) died of a possible MEN1 cause, whereas of the 12 female spouses, none died of a possible MEN1 cause. Excluding 1657insC mutation from the analyses did not abolish significance, but in the subgroup of 1657insC mutation families, statistical significance could not be estimated because of small numbers.

5.3 Characterization of clinical and genetic aspects of pituitary adenoma predisposition (PAP) (III)

In the genome-wide search, the highest parametric LOD score of 3.9 in chromosome 11 at 68.4–73.6 cM (Xba240deCode genetic map, Haldane sex-average cM) was detected for the high-stringency phenotype (acromegaly or gigantism, 6 individuals). The haplotype construction of family 1 individuals placed the PAP locus between SNPs rs174449 and rs 1938685 (chromosome 11, 61.7–69.0 Mb, Ensembl, version 36, December 2005), harbouring 295 genes. Families 1 and 2 shared the linked haplotype, which segregated perfectly with acromegaly. The added maximum LOD score for these two families was 7.1 with high-stringency (acromegaly or gigantism) criteria (6.3 for family 1 and 0.8 for family 2) and 8.3 (7.5 for family 1 and 0.8 for family 2) with the low-stringency criteria.
(any pituitary adenoma) criteria. Two individuals with prolactinoma appeared to represent phenocopies (A9 and A10).

Expression profiling revealed 172 probe sets that mapped in the linked region. The two lowest $P$ values were obtained for the two separate probe sets representing $AIP$ (also known as $XAP2$ and $ARA9$, Genbank no U78521.1) ($P= 0.00026$ and $P= 0.00114$). Thus $AIP$ was chosen as the prime candidate for mutation analysis. One other gene, $galectin-12$ ($LGALS12$) was also chosen on the basis of decreased expression and an association of $galectin-3$ to pituitary tumorigenesis (Riss et al. 2003). No difference was detected in $MEN1$ expression. The coding region of $AIP$ was sequenced, and a nonsense mutation Q14X (where Q is Gln), perfectly segregating with the GH-secreting adenoma phenotype in families 1 and 2, was identified (Figure 5). The mutation was absent in 209 local blood donors. $LGALS12$ and $MEN1$ analyses were negative. In the population-based material, including 4 cases of families 1 and 2, six displayed Q14X, and one displayed IVS3-1G>A, affecting the splice acceptor site of exon 4. The latter change was screened among 219 local blood donors with negative results. The clinical characteristics of affected $AIP$ mutation carriers are shown in Table 8. The age at diagnosis, sex, and size of adenoma were compared between PAP ($n=7$) and $AIP$ mutation-negative ($n=38$) patients. Differences in tumour size or sex distribution were not observed. PAP patients were significantly younger than mutation negative patients (24.7 ± 10.7 versus 43.6 ± 11.9 years, $P= 0.0003$). In addition, 10 unselected Finnish sporadic acromegaly patients from whom DNA and appropriate authorization was available were screened for $AIP$ mutations. Two patients were found to carry the Q14X mutation (Table 8). Three families with two affected individuals from other countries were also studied. While no mutation was detected in the German and Turkish sample, the Italian siblings displayed a nonsense mutation R304X (where R is Arg) in exon 6 (Table 8). The change was absent in 203 Caucasian controls from the United Kingdom and the CEPH, as well as in 52 local (Trevisio) blood donors. The phenotype in the siblings resembled that seen in Finns; young age at onset and no visible evidence of autosomal dominant transmission.

LOH-analysis was possible in eight tumours from mutation-positive individuals, including five somatotropinomas, one mixed-type tumour, and two prolactinomas; loss of the wild-type allele was detected in all cases, showing that these tumours were null with respect to $AIP$. 
5.4 Mutation analysis of MEN1, HRPT2, CASR, CDKN1B and AIP genes in PHPT patients with features of genetic predisposition (IV)

The mean age of onset of PHPT in the 29 patients (20 females, 9 males) was $38.4 \pm 10.6$ yr (range, 19–56 yr) and mean age at diagnosis of PHPT $42.0 \pm 10.2$ (range, 20–61 yr). More than one of the inclusion criteria was met by 7 (24%) patients. Eighteen patients (13 females, 5 males) met the inclusion criterion of age being 40 years or less at the time of onset of PHPT and their mean age was $31.3 \pm 5.2$ yr (range, 19–39 yr) at onset, and $36.5 \pm 8.1$ yr (range, 20–55 yr) at diagnosis. Eleven patients (38%) had multiglandular disease; 9 with two affected glands and 2 with three hyperplastic glands. Histology of removed parathyroid gland(s) showed hyperplasia in 14 patients (48%), adenoma in 12 patients (41%), both hyperplasia and adenoma in two (7%) and hyperplasia or adenoma in one (3%). Four patients (14%) met the inclusion criterion of recurrent or persistent PHPT. One patient was regarded as having another MEN1 lesion in addition to PHPT; patient 1617 had had bilateral adrenal enlargements diagnosed as incidentalomas and pancreatic cysts. Family history of PHPT was present in 3 patients (1143; a sister, diagnosed at 72 years, 1237; mother at 73 years and a sister at 38 years, 2037; aunt at 51 years). Three patients had MEN1 suspected lesions in the family (1211; mother, liver metastasis of a carcinoid tumour at 79 years, 1241; several relatives at mother’s side affected by abdominal cancer, 1605; mother, pulmonary carcinoid
<table>
<thead>
<tr>
<th>Patient</th>
<th>AIP Mutation</th>
<th>Sex</th>
<th>Clinical Diagnosis</th>
<th>Age at Diagnosis (years)</th>
<th>Histopathological Diagnosis</th>
<th>Elevated Pituitary Hormone(s)</th>
<th>IHC of adenoma</th>
<th>Adenoma Diameter (mm)</th>
<th>Surgery on Adenoma</th>
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<td>GH++, PRL+, c</td>
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*Belong to the population-based cohort, #belong to unselected acromegaly patients. Adrenocorticotropic hormone (ACTH), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH) and luteinizing hormone (LH) negative, NA not available; GH, growth hormone; PRL, prolactin; IHC, immunohistochemistry.
tumour). In addition, two patients (1370, 1276) who were invited to the study for their young age at onset were found to be cousins. Furthermore, the mother and sister of patient 1156, and the daughter of patient 1198 have been found to have PHPT after starting this study.

Patient 1237 was found to have the 1466del12 (c. 1356_1367del12) founder mutation in the \textit{MEN1} gene (Kytölä \textit{et al.} 2001). No other disease causing mutations were detected in the \textit{MEN1}, \textit{HRPT2} or \textit{CASR} genes in the rest of the patients (n= 28). \textit{CDKN1B} and \textit{AIP} genes were analysed in 27/28 patients (patient 1609 withdrew her consent at this point) with no pathogenic alterations found. Common polymorphic sequence variations were detected in all genes studied. According to biochemical screening, 5 patients had hypercalcaemia (1142, 1153, 1155, 1198, 1237), 4 of which (all but 1142) were found to have recurrent PHPT on a subsequent evaluation by endocrinologist. They were also rescreened biochemically for MEN1 manifestations with no positive findings, except for the patient with the \textit{MEN1} mutation. The level of PP was markedly elevated in five patients (1211, 1216, 1237, 1377, 1617). Three of them were evaluated and examined by endocrinologist with no further evidence of pancreatic tumours. The level of serum PP in the patient with the \textit{MEN1} mutation has varied from normal range to approximately 2-fold to normal in control screenings, whereas serum gastrin level has not been elevated. Imaging studies have not been performed yet. One of the patients (1617) was not willing to undergo further examinations. None of the patients (n=29) had elevated levels of prolactin.
6 Discussion

6.1 Clinical features of MEN1 and genotype-phenotype correlation

Two founder mutations enriched in Northern Finland and resulting in a cluster of MEN1 patients (Kytölä et al. 2001), as well as a well-organized health care system offer a good opportunity to study this disease within an unbiased material on population basis. In the present study, we found 15 (38%) patients with gastrinoma among 39 individuals carrying the 1466del12 mutation, and only one (3%) patient with gastrinoma among 29 individuals carrying the 1657insC mutation. This seems to indicate clearly that the 1466del12 carrier patients are more prone to develop gastrinomas. On the other hand, the prevalence of nonfunctional pancreatic tumours among the 1466del12 mutation carriers was significantly lower, 15 (38%) vs. 20 (69%), when compared to 1657insC mutation carriers. Resembling our findings, enteropancreatic lesions showed intrafamilial homogeneity in a 10-year prospective screening study published from Sweden (Skogseid et al. 1991). In their study, insulin-proinsulin excess was significantly overrepresented in a family with a pronounced malignant profile of their pancreatic tumours, whereas in another family all pancreatic lesions were benign, and only the Zollinger-Ellison syndrome was displayed. Contrary to the study by Skogseid et al. (1991), we could not find any difference between families according to malignant behaviour of GEP tumours. Instead, male gender seemed to be a risk factor for developing metastasized GEP tumours.

Hao et al. (2004) described two kindreds (A and B) with a low prevalence of gastrinoma (10%) and high prevalence of prolactinoma (40%). The authors pointed out that the difference from typical MEN1 was more striking for gastrinoma than for prolactinoma. Patients from kindred B harboured a nonsense mutation Y312X, while no MEN1 mutation was identified in kindred A (Hao et al. 2004). Patients with prolactinoma variant from Newfoundland had an R460X nonsense mutation (Olufemi et al. 1998). A common finding between cases with prolactinoma variant and 1657insC mutation carriers is the low prevalence of gastrinoma. In addition, all three mutations led to truncated proteins, like most of the MEN1 mutations (Chandrasekharappa & Teh 2003). In the present study the mutation class leading to truncated proteins (frameshift insertion/nonsense mutation) was found to be a risk factor for nonfunctional pancreatic tumour, while mutation class leading to presumably lesser changes in the menin protein (in-frame
deletion/missense mutations) predicted the risk for gastrinomas. In a paper of
genotype/phenotype correlation of \textit{MEN1} mutations in sporadic gastrinomas
(Goebel \textit{et al.} 2000) the authors combined their data with results taken from other
cases of sporadic gastrinomas (Wang \textit{et al.} 1998). They stated that 40% (11/28) of
the mutations described (8 missense and 3 in-frame deletions) altered the amino
acid sequence of menin, while 60% (17/28) resulted in a truncated protein (Goebel
\textit{et al.} 2000). In familial MEN1 the majority (73%) of the mutations led to a

The 1466del12 mutation, located in exon 10, is an in-frame deletion that is
predicted to shorten the menin protein by 4 amino acids at codons 453–456. The
deleted region locates in the area that is required for interaction with PEM
(polymorphic epithelial mucin), NM23H1 (nonmetastatic protein 23, homolog 1)
and CHES1 (checkpoint suppressor 1) proteins (Chandrasekharappa \& Teh 2003,
Lemos \& Thakker 2008). The frameshift mutation 1657insC at codon 516 is
predicted to cause a premature stop codon 14 codons upstream (Turner \textit{et al.}
2002). This would affect the binding site with SMAD3 (SMA- and MAD-related
protein 3), ASK (activator of s-phase kinase) and CHES1, and two of the nuclear
localization signals (NLSa, NLS2) would be cut off. It is also possible that the
frameshift mutation results in loss of the translated protein because of
nonsense-mediated mRNA decay (NMD). (Chandrasekharappa \& Teh 2003,
Lemos \& Thakker 2008). It is not known whether these changes in interaction due
to \textit{MEN1} mutations have any role in the tumorigenesis of gastrinomas or
nonfunctional pancreatic tumours.

While the issue of genotype-phenotype correlation in MEN1 is under debate,
it is a widely recognized feature in von Hippel-Lindau disease (VHL) (Table 5).
The association of pheochromocytoma with missense mutations in the \textit{VHL}
gene was discovered more than ten years ago (Crossey \textit{et al.} 1994, Chen \textit{et al.}
1995). Moreover, Ong \textit{et al.} (2007) studied 537 individuals with VHL disease, and they
discovered that missense mutations resulting in substitution of a surface amino
acid conferred a higher pheochromocytoma risk than those that resulted in the
disruption of structural integrity. Currently, it is not known what the key factors are
for pheochromocytoma development in VHL disease. However, increased JunB
expression secondary to altered atypical protein kinase C signalling has been
linked to failure of developmental apoptosis in adrenal medullary progenitor cells
in VHL disease as well as other causes of familial pheochromocytomas. (Lee \textit{et al.}
2005, Ong \textit{et al.} 2007). Ong \textit{et al.} (2007) also pointed out that even though VHL
disease has been considered to demonstrate a classical “two-hit” model of
tumorigenesis, phenotypically normal renal cells from VHL patients demonstrate altered gene expression patterns compared to normal control kidney cells (Stoyanova et al. 2004). These findings suggest that the presence and nature of the germline mutation ("first-hit") may influence cell function and susceptibility to tumorigenesis.

In the present study, the likelihood of having a GEP tumour was increased with advancing age with an OR of 1.12 in the entire group, and 1.11 in the subgroup of the founder mutation carriers. According to Kaplan-Meier analysis the penetrance of GEP tumours is 100% by 70 years of age (Original publication I, Figure 1). Similarly to the findings in a Tasmanian kindred study (Burgess et al. 1998), however, when nonfunctional pancreatic tumours were analysed alone, they did not associate with advancing age. Our findings of age-related penetrance of other various MEN1 lesions are in agreement with previous studies (Skogseid et al. 1991, Burgess et al. 1998, Geerdink et al. 2003, Hao et al. 2004). In the present study, the peak of the prevalence of pituitary tumours was reached around 60 years of age (Original publication I, Figure 1), and advancing age was not found to be a risk factor for pituitary tumours. A similar finding with prolactinoma was also seen in the Tasmanian kindred (Burgess et al. 1998). In addition, in our study, mutational background or gender did not predict the risk for pituitary adenoma or prolactinoma. In a large multicentre study, Vergès et al. (2002) found that 85% of the tumours were macroadenomas, whereas in our study the proportion of macroadenomas was 50%. This difference may reflect the higher proportion of newly diagnosed heterozygotes with early diagnoses in our study. Furthermore, in the study of Vergès et al. (2002), there were tumours secreting GH or ACTH in 4% and 2%, and cosecreting and nonsecreting tumours in 4% and 6% of the cases. The almost total lack of other functioning pituitary adenomas than prolactinomas in our study may be due to a lower overall number of pituitary tumours as compared to the number of pituitary adenomas (n= 136) in the big multicentre study (Vergès et al. 2002). Nevertheless, the lack of GH-secreting adenomas has also been shown in the Tasmanian kindred where 124 patients were reviewed (Burgess et al. 1996b).

The only identifiable risk factor for developing PHPT was advancing age with an OR of 1.08 per year. According to Kaplan-Meier analysis the penetrance of PHPT would be 100% by 70 years of age (Original publication I, Figure 1). One patient was found to have multiglandular parathyroid cancer, which is extremely rare either in sporadic form or associated with MEN1. It is not known whether the past radiation therapy on presumed sellar angiosarcoma, or peculiar family history
of different tumours (mother; thyroid papillary carcinoma, renal clear cell carcinoma, breast cancer, leiomyoma and leiomyolipoma of uterus, mother’s sister; mesodermal mixed tumour of uterus) had any aetiological impact on the development of parathyroid cancer.

The prevalence of adrenal tumours (35%) in this study is one of the highest reported in the literature (Table 1). This could be due to the high frequency of imaging studies we used. There are also reports including adrenal carcinoma and pheochromocytoma in varying numbers of patients (Table 2). We were only able to find benign lesions, and only two were associated with subclinical hypercortisolism. Similarly, an indolent course of adrenal lesions has also been reported elsewhere (Burgess et al. 1996d, Barzon et al. 2001).

The occurrence of neuroendocrine gastrointestinal tumours, earlier known as carcinoids, only in women in the present study may be a coincidence. Nevertheless, we noted thymic neuroendocrine tumours in this study only in men in accordance with previous reports (Teh et al. 1998a,c, Ferolla et al. 2005). Although we did not find any bronchial neuroendocrine tumours in our study, they are usually seen more frequently in women (Sachithanandan et al. 2005). Regarding to these data, it is apparent that there exists some clinical variation in MEN1 according to the gender of the affected heterozygote.

There were five cases of thyroid cancer in this study. The aetiology of these and other tumours outside the classical MEN1 tumour spectrum noted in our study is not known. However, others have shown by LOH studies that leiomyomas of the oesophagus and uterus, meningioma and lipoma may be part of the MEN1 syndrome (Pack et al. 1998, McKeeby et al. 2001, Asgharian et al. 2004). It is also of note that medullary thyroid carcinoma is typically associated with MEN2 and its occurrence in our patient with MEN1 is probably a coincidence.

6.2 Mortality in MEN1 (I, II)

Of the deaths caused directly by MEN1 among the 82 heterozygotes, six out of seven (86%) were caused by metastasized tumours associated with MEN1 (5 patients with a GEP tumour, one with parathyroid cancer), and only one was caused by complications of peptic ulcer disease. In three other cases MEN1 was a contributing factor with a malignant tumour (two patients with GEP, one with a neuroendocrine tumour of the thymus). This is in accordance with some other recent studies showing that malignant islet cell tumours and malignant carcinoids are the major causes of MEN1-associated death, whereas in older studies peptic
ulcer disease was the most common MEN1-associated cause of death (Table 3). Due to the small overall number of deaths in this patient cohort no further analysis on mortality was undertaken. Instead, the main result of our genetically established genealogical survey was that the lifespan of the subjects considered to be MEN1 heterozygotes did not show significant differences in comparison with the group of their spouses in sex groups or general life expectancy estimates.

We have had an opportunity to observe two family trees with a founder mutation. To ascertain that the true common ancestors are found, practically all the pedigree roots to the 18th century were evaluated, and many were evaluated even further. Nonpaternity is a possibility always worth bearing in mind, and this is probably the case in the 1466del12 mutation-positive family, which could not be connected to ancestors despite the same mutation. Haplotype analysis around the mutant $MEN1$ allele in our families has shown that the area is quite conserved, and thus recent common ancestors within 10 generations could be expected to be found (Kytölä, S, unpublished data). One of the advantages in our retrospective survey of $MEN1$ heterozygotes and their spouses is that gene carriers can also be included, thus completing the picture. In addition, the evaluation period is almost 300 years, a feature unsurpassed by modern prospective studies for centuries. One of the drawbacks in the present genealogical survey is the assumption that the $MEN1$ gene does not cause mortality before the age of 25 years. According to the literature, this is quite rare (Brandi et al. 2001), also according to our own unpublished data. Thus, the use of life expectancy tables for 25-year-olds can be regarded as acceptable. Another selection bias in our study is that these persons were practically all ancestors in the study group, and fertility is obligatory to be an ancestor. $MEN1$ positivity can cause infertility, for example through hyperprolactinaemia, and thus omit subjects from this survey (Schuppe et al. 1999). Pituitary adenomas, most of them prolactinomas, are present in approximately 22±4% of patients with MEN1 (Hao et al. 2004). The family trees of the two MEN1 pedigrees imply that heterozygosity has not, at least markedly, reduced fertility and the number of children. Heterozygote advantage cannot be excluded when family trees of expanding MEN1 families are looked at. The genetic fitness is close to 100%. Including siblings was not generally possible because MEN1 status could not be judged in them.

The birth and death dates are reliable, grounded on a sound tradition of church records kept by clergy and initially legislated by the king of Sweden. The causes of death have also often been recorded by the clergy, but up until the 20th century they
were quite descriptive because of unprofessional classification and lack of autopsies.

When evaluating whether excess mortality exists, the death rate of the whole population to be compared with is a crucially important factor. In the years 1700–1880, death rates of the population of Finland were remarkably high, resembling those of present-day underdeveloped countries, until a sustained decline in mortality began in the decade 1870–1880 (Turpeinen & Kannisto 1997). Life expectancy was much higher at age 15 than at birth, demonstrating a high mortality in childhood. This is why the life expectancy value changes in adults were minor or moderate compared with major changes in children’s life expectancy when approaching the year 1900 (Turpeinen & Kannisto 1997). Birth year and life expectancy showed a strong positive mutual correlation in the whole group and subgroups of men and women as can be expected, because they represent population value estimates. Between the years 1750 and 1885, the life expectancy of 25-year-olds was very variable and did not show any consistent trends, but was rather a plateau. During that period, the life expectancy mean of 25-year-old males was 59.5 yr (range, 55.2–62.7 yr) and that of females 61.0 yr (range, 57.2–64.5 yr). After 1885, there was a clear, strong positive linear correlation between life expectancy and birth year. During that phase, life expectancy reached modern lifespan levels, and the rise from 1885 was 13 yr in males and 18 yr in females.

When comparing the lifespan of the obligatory gene carriers to the lifespan of imaginary reference subjects with the same sex and birth year in the same 5- to 10-yr period, the results confirmed the data obtained from the comparison of gene carrier group to spouse group. Assumptions that genetic screening will reduce morbidity as well as mortality in MEN1 in the future have been postulated in scientific literature (Clerici et al. 2001). The findings here, which are in contrast with previous studies, could be related to differences in genotypes or gene-environment interactions. Still, the most probable reason that our genealogical survey did not show excess premature mortality in MEN1 patients can be mainly explained by high mortality in the whole population because of periods of famine, infectious diseases, and wars. Presymptomatic diagnosis has not been shown to yield clear clinical benefit in patients with MEN1 (Arnold 2008). Our retrospective view did not show a harmful effect of MEN1 gene trait positivity to life expectancy, but it can be speculated whether the situation is the same nowadays, when life expectancy is from 13 yr (in men) to 18 yr (in women) higher. The historical study population did not show any significant associations
with the age of death when pedigree group, MEN1 trait, and sex were used as explaining variables, and expected lifespan and birth year as covariates. The trend of increasing lifespan did not come up when subjects born in 1860 or later were analysed in subgroups of sex and probands and spouses separately, perhaps due to the small number of subjects. All four subjects who were excluded from the main analysis because they were still living had already exceeded the expected lifespan [two MEN1 subjects (+1.1 and 6.1 yr) and two spouses (+8.2 and 0.8 yr)], and including them in the analyses with age as lifespan did not change results.

A large multigenerational MEN1 family with clinical expression suggestive of anticipation was reported by Giraud et al. (1997). Anticipation is a phenomenon in which severity increases and age of onset decreases in successive generations. It is most commonly found in neuropsychiatric disorders and associated with trinucleotide expansions. In the family described by Giraud et al. (1997) two obligate gene carriers died at the ages of 85 and 76 without the history of MEN1, whereas two other living gene carriers above the age of 65 had had no clinical evidence of MEN1. In the fourth generations four out of eight affected members had severe MEN1-related and atypical malignancies. In the fifth generation, all five patients were below the age of 22 when the disease was detected. Repeated expansion detection studies were carried out but failed to detect any expansion. The authors suggested that anticipation is uncommon in MEN1, and that cosegregation of other modifying genes might be involved, resulting in more severe phenotypes in subsequent generations (Giraud et al. 1997). The hypothesis of anticipation could theoretically explain our finding that the lifespan of the MEN1 obligate heterozygotes did not show significant differences in comparison with the controls, but further studies are needed to explore the issue.

In our genealogically formed study group, there was a significant difference in causes of death between female probands and female spouses ($P =0.003$), but the same was not true in males. In female subjects, out of 15 probands, eight (53%) died of a possible MEN1 cause, whereas of the 12 female spouses, none died of a possible MEN1 cause. This means that female obligatory gene carriers showed a different pattern of causes of death from female controls. The difference in significance between females and males could partly be explained by lower life expectancy in males, and thus competing death causes could have intervened before MEN1 causes. A retrospective classification of old causes of death into MEN1-related and other causes yields only a crude estimate for the evaluation of the effect of MEN1 gene positivity, as MEN1-related causes of death included mainly causes that could be regarded as resulting from a malignancy. Reliability of
the initial causes of death in the documents was classified by the investigator as certain in 36% of the cases.

6.3 Pituitary adenoma predisposition and AIP (III)

Combining chip-based technologies with genealogy data we identified germline mutations in the aryl hydrocarbon receptor interacting protein (AIP) gene in individuals with pituitary adenoma predisposition (PAP). A nonsense mutation Q14X was found in affected members of families 1 and 2. In addition, we had DNA samples from 45 of the 54 acromegaly patients belonging to the population-based study cohort, including four cases from families 1 and 2. Out of 45 patients from the population-based cohort, 6 displayed Q14X, and one displayed IVS3-1G>A. These alterations were absent in more than 200 local blood donors. It is of note that six out of the fifteen patients diagnosed under 35 years of age in the population-based series had AIP mutation. Thus, in a population-based series from Northern Finland, two AIP mutations account for 16% of all patients diagnosed with pituitary adenomas secreting growth hormone and for 40% of the subset of patients who were diagnosed when they were younger than 35 years of age. Another series of 10 unselected Finnish sporadic acromegaly patients were screened and Q14X was found in two of them, which is compatible with findings in the population-based cohort. The possible role of AIP in pituitary adenoma predisposition in other populations was confirmed by our finding that Italian siblings with somatotroph adenoma displayed a nonsense mutation R304X.

The clinical diagnosis of the PAP patients identified during this study includes acromegaly (n= 10), gigantism (n= 5), and prolactinoma (n= 3). Histopathological diagnosis of the GH-secreting adenomas was either mammosomatotroph adenoma or somatotroph adenoma. Loss of the wild-type allele was detected in all of these tumour types, strengthening the notion that PAP is associated with predisposition to both prolactinomas and GH-secreting pituitary adenomas. Mammosomatotroph adenomas are bihormonal tumours secreting both GH and prolactin (Thapar et al. 1995). In accordance with this, three out of five patients diagnosed with mammosomatotroph adenoma had had elevated levels of GH and prolactin. In at least one of the patients (A34) serum prolactin was not measured before surgery on pituitary adenoma. Young age at onset seems to be a characteristic finding in patients with PAP; AIP mutation carriers (n=7) were significantly younger than mutation negative patients (24.7 ± 10.7 versus 43.6 ± 11.9 years, P = 0.0003) in the population-based acromegaly cohort. Thus, for identification of PAP patients,
young age at onset is a useful indicator. Differences in tumour size or sex distribution were not observed.

The PAP phenotype with very-low-penetrance susceptibility to GH-secreting pituitary adenoma and prolactinoma does not fit well to any of the known familial pituitary adenoma syndromes, including MEN1, Carney complex, familial isolated pituitary adenoma and isolated familial somatotropinoma (Frohman & Eguchi 2004, Heaney & Melmed 2004, Daly et al. 2006). These syndromes are familial, and the low penetrance of PAP appeared unique. However, there are some similarities when comparing the PAP phenotype to all of these syndromes. For instance, in addition to PAP, prolactinoma is found commonly in MEN1 and FIPA patients (Table 1). Similar biorhonal overproduction of GH and prolactin may be seen especially in patients with Carney complex, IFS and PAP (Daly et al. 2006, Soares & Frohman 2004). Younger age at diagnosis of pituitary adenoma has also been found in FIPA patients compared to sporadic cases (38.4 vs. 41.9 years) in a study by Daly et al. (2006). Also in the same study, IFS patients were diagnosed at a mean age of 33.8 years, which was more than 10 years younger than somatotropinoma patients from either heterozygous FIPA families (49.3 years) or sporadic cases (44.1 years). Even younger age at onset, 26 years, was found by Soares and Frohman (2004) in a review of 46 IFS families reported in the literature. Also, in CNC, biochemical findings of mammosomatotroph hyperplasia can be found in most patients starting from adolescence (Boikos & Stratakis 2007). A common finding between IFS and PAP is also that both show reduced or incomplete penetrance, although this phenomenon seems to be even more striking in our PAP patients with only one affected sibling pair found in the Finnish patients. Other AIP mutation positive cases were more distant relatives or did not have any family history suggestive of pituitary adenoma. Some of the IFS families may share the same aetiology as PAP patients, since the locus for IFS has been linked to 11q13.1-q13.3 (Gadelha et al. 2000, Luccio-Camelo et al. 2004, Soares et al. 2005). Following our initial discovery of pituitary adenoma predisposition with AIP gene germline mutations (Original paper III), it has been estimated that 15% of FIPA patients and 50% of IFS patients carry AIP mutations (Beckers & Daly 2007).

It has not been previously realized that genetic predisposition to pituitary adenoma, in particular the GH-oversecreting type, can account for a substantial proportion of cases. Our study not only reveals this aspect of the disease, but also provides molecular tools for efficient identification of predisposed individuals. Without preexisting risk awareness, the patients are typically diagnosed after years
of delay, leading to substantial morbidity. Simple tools for efficient clinical follow-up of predisposed individuals are available, underlining the importance of our findings. Our results suggest that inherited tumor susceptibility may be more common than previously thought. The identification of the PAP gene indicates that it is possible to identify the causative genetic defects in low-penetrance conditions even in the absence of a strong family history.

AIP (OMIM 605555) was originally identified by its interaction with the hepatitis B virus X protein and thus designated XAP2 (hepatitis B virus X-associated protein) (Kuzhandaivelu et al. 1996) (Figure 5). AIP consists of 330 amino acids with regions of amino acid sequence similarity to the 52-kDa FK506-binding protein known to be associated with the glucocorticoid receptor (Figure 5). AIP forms a complex with the aryl hydrocarbon receptor (AHR) and two 90-kD heat-shock proteins (HSP90). (Carver & Bradfield 1997.) The R304X mutation removes the AHR binding region (Bell & Poland 2000, Petrulis & Perdew 2002). ARH is a ligand-activated transcription factor that regulates a variety of xenobiotic metabolizing enzymes (Meyer & Perdew 1999). Dioxin-like chemicals display high affinity to AHR, which mediates most of the toxic responses of these agents. AHR also participates in cellular signalling pathways (Marlowe & Puga 2005). AIP modulates the subcellular localization of AHR and prevents the AHR from undergoing nucleocytoplasmic shuttling (Pollenz & Dougherty 2005). Nuclear translocation of AHR is also regulated by a complex formed by AIP and PDEA2 (phosphodiesterase type 2A) (de Oliveira et al. 2007). AIP also binds to and attenuates the activity of PDE4A5 (cyclic AMP-specific phosphodiesterase type 4 isoform A5) as well as PPAR (peroxisome proliferator-activated receptor alpha) (Bolger et al. 2003, Sumanasekera et al. 2003). It has also been found recently by Kang and Altiere (2006) that AIP associates directly with survivin, which is a multifunctional member of the IAP (inhibitor of apoptosis) family, thus preserving protein stability and a cytosolic anti-apoptotic threshold. Survivin is over-expressed in foetal tissues and human cancers, but it is almost undetectable in normal tissues. Overexpression of survivin has also been observed in benign central nervous system tumours including pituitary adenomas. (Hassounah et al. 2005, Wasko et al. 2005). It has also been reported that AIP functions as a cytosolic factor that mediates preprotein import into mitochondria by binding to both translocases of the outer membrane of mitochondria (Tom) 20 and preproteins (Yano et al. 2003). Recently, it was also shown by Froidevaux et al. (2006) that AIP is necessary for T3-independent activation of TRH transcription mediated by TR1 (thyroid hormone receptor 1).
The mechanism by which AIP exerts its tumour-suppressive action in the pituitary remains to be determined. Further work on the functional role of AIP should prove informative in revealing key cellular processes involved in the genesis of pituitary adenomas, including potential drug targets.

### 6.4 PHPT patients with features of genetic predisposition (IV)

This is the first study where the five major genes (MEN1, HRPT2, CASR, CDKN1B and AIP) known to cause PHPT or related disorders have been systematically studied in a well-defined cohort of patients. We have studied extensively 29 selected patients in order to estimate the aetiological role of germline mutations in these genes in PHPT predisposition. Only one patient (3%) with a germline mutation (MEN1, 1466del12) was found. However, this finding is in line with our hypothesis that due to the two founder mutations in the district of Oulu University Hospital, there may be more undiscovered MEN1 patients in the area. We were not able to study all the potential cases of PHPT, since only 52% of the invited patients participated in the study. In addition, incomplete family history in the medical records may have hampered identification of some of the familial cases.

The inclusion criteria were chosen based on known features of hereditary parathyroid tumour predisposition (Simonds et al. 2002). With the exception of the repeatedly high level of S-PP in patient 1237, not a single patient in this cohort had endocrine features that would have met the definition of MEN1 syndrome, which requires the existence of 2 of the 3 main MEN1-related tumours (parathyroid adenoma, entero-pancreatic neuroendocrine tumour, and pituitary adenoma) (Brandi et al. 2001). The clinical features of patient 1617 (adrenal incidentalomas, pancreatic cysts) were suggestive of MEN1, but she refused further clinical evaluation. The elevated level of PP of other patients was not regarded as a sign of pancreatic neuroendocrine tumour in subsequent evaluations by endocrinologists. It is recognized, however, that these patients with elevated levels of PP may require surveillance for pancreatic tumour development. In addition, patient 1235 had had pheochromocytoma which could be suggestive of MEN2, but the absence of medullary thyroid carcinoma at the age of 66 years makes this diagnosis unlikely. Even though it has also been suggested that atypical presentations of MEN1 may include pheochromocytoma (Dackiw et al. 1999), the absence of evidence of other major tumours prevents setting the diagnosis of MEN1. Even though MEN1 features were dominating in our inclusion criteria, obvious cases of
other familial forms of PHPT should have been picked up since family history of PHPT was one of the inclusion criteria. It should also be noticed that the mutation detecting methods used in this study do not necessarily find all possible pathogenic alterations in the studied genes, such as large deletions or mutations in promoter or other regulatory regions of the genes.

In the entire PHPT patient population reviewed at least 7% (21/286) of the cases were affected by MEN1. The total number of PHPT patients examined in the Oulu University Hospital during 1978–2001 was approximately 350, leading to a prevalence of 6% of MEN1 in this patient group. Earlier, it had been estimated that the fraction of MEN1 in PHPT would be 2–3% (Marx 2001). By the year 2001 there were more than 20 known MEN1 mutation carriers with PHPT in the area. Not all of them were caught in the computerized hospital records search for PHPT, probably because presymptomatically identified MEN1 carriers usually have mild forms of PHPT, and thus clinicians may choose not to use the respective diagnosis code used as a tag in the search. Since it is likely that many of the presymptomatically diagnosed carriers of MEN1 are not included in the examined cohort of PHPT the present work also represents more accurately the natural setting of PHPT patients in general.

The frequency of somatic MEN1 mutations has previously been studied in sporadic parathyroid tumours with mutations found in 12–21% of the tumours (Heppner et al.1997, Carling et al. 1998, Farnebo et al. 1998). In these studies no germline mutations have been found. In contrast, Uchino et al. (2000) studied parathyroid tumours of 112 unselected patients and peripheral blood leukocytes from 64 of the patients. MEN1 mutation was found in 25/112 (22%) of the tumour specimens, and three of the mutations could also be found in the corresponding blood sample DNA (Uchino et al. 2000). In addition, several studies on selected (increased risk of being a MEN1 mutation carrier) groups of PHPT patients have been reported, with varying MEN1 mutation frequencies. In five different studies including patients with sporadic PHPT with no other MEN1-related lesions, MEN1 mutation was detected in 0 to 40% of cases (mean 14%) (Cupisti et al. 2000, Langer et al. 2003, Cardinal et al. 2005, Ellard et al. 2005, Tham et al. 2007). In four studies including patients with sporadic PHPT and at least one other MEN1-related manifestation, the MEN1 mutation detection rate varied between 0 and 50% (mean 21%) (Rojiers et al. 2000, Cardinal et al. 2005, Ellard et al. 2005, Tham et al. 2007). Family history of hyperparathyroidism alone (FIHP) was associated with a 22 to 57% (mean 38%) detection rate of MEN1 mutation, and family history of MEN1-related lesions with a 73 to 100% (mean 83%) detection
rate of MEN1 mutation (Cardinal et al. 2005, Ellard et al. 2005, Tham et al. 2007). If our patients are divided into corresponding groups, the figures for MEN1 mutation detection rate are as follows: 0% (0/19) in sporadic PHPT patients, 0% (0/1) in sporadic PHPT patients with at least one MEN-related manifestation, 14% (1/7) in familial PHPT, and 0% (0/3) in PHPT patients with family history of MEN1-related lesions. Our low MEN1 mutation rate in the patients examined may at least partly be due to the fact that we deliberately excluded known patients with MEN1.

In two of the studies reviewed above the fraction of multiglandular disease among MEN1 mutation negative and positive could be estimated. In the study by Cupisti et al. (2000) two of the five patients with germline mutations had multiglandular disease, whereas one of the patients with double adenoma had a mutation in the tumour tissue, but DNA from healthy tissue was not available for further studies. Two of the mutation negative patients diagnosed at 13 and 18 years had single adenomas. In the study by Langer et al. (2003) all the 15 PHPT patients were diagnosed at 40 years or less, and two (13%) of the patients with multiglandular disease were found to have MEN1 mutation, whereas the rest (n=13, 87%) with solitary adenoma were MEN1 mutation negative. Of the studies reviewed above, three concluded that patients with young age (<35 or 40 years) and/or multiglandular disease (Cupisti et al. 2000, Roijers et al. 2000) or hyperplasia (Tham et al. 2007) would be suitable for MEN1 mutation screening, whereas two of the studies (Langer et al. 2003, Cardinal et al. 2005) recommended patients with young age (<30 or 40 years) and multiglandular disease for MEN1 mutation screening. In our study, there were three patients with multiglandular disease and young age at onset, including the patient with the 1466del12 MEN1 mutation; therefore, according to the above-mentioned criteria she would have been recommended for mutation testing even without the knowledge of family history. This is an important issue since approximately 10% of MEN1 mutations arise de novo, the affected patients having no remarkable family history.

Somatic HRPT2 mutations are found in the majority of parathyroid carcinomas, but very rarely in sporadic adenomas (Howell et al. 2003, Cetani et al. 2004). In addition, germline mutations of HRPT2 can be found in patients with apparently sporadic parathyroid carcinoma (Shattuck et al. 2003). Interestingly, germline HRPT2 mutations are found on average in 5% (range 0–29%) of FIHP kindreds (Cetani et al. 2006). Similarly, the rate of CASR germline mutations in FIHP families has been on average 12%, ranging from 0 to 18% (Cetani et al. 2006). Probably due to the low number of proven familial cases in this study and
the overall low frequency of HRPT2 and CASR mutations in FIHP kindreds, it is not surprising that we did not find any mutations in these genes.

Pellegata et al. (2006) reported the first case with a germline CDKN1B mutation. She was a 48-year-old Caucasian woman with acromegaly diagnosed at 30 years. At age 46, she was diagnosed with PHPT, but she had not yet been operated on (Pellegata et al. 2006). The second reported patient with CDKN1B mutation and MEN1-like phenotype was diagnosed with PHPT at the age of 47 years (Georgitsi et al. 2007). She had been diagnosed with small-cell neuroendocrine cervical carcinoma at the age of 45 years and ACTH-secreting pituitary adenoma at the age of 46 years. The prevalence of CDKN1B mutations in PHPT patients without prominent syndromic features has not been studied earlier. Similar to our findings, the absence of pathogenic alterations in CDKN1B gene was found in a study by Ozawa et al. (2007), where they examined patients with a MEN1 variant with sporadic parathyroid and pituitary tumours and no identified MEN1 germline mutation.

AIP gene defects were not detected in PHPT patients in this study. This is in accordance with our recent study where somatic AIP mutations were not found in 79 sporadic endocrine (non-pituitary) tumours including 6 parathyroid adenomas and 2 carcinomas (Raitila et al. 2007). Instead, 2 prolactinomas out of 9 were found to harbour the Northern Finnish founder mutation Q14X (Raitila et al. 2007). The PHPT study population in this study originates from the same area as the PAP patients with the Q14X mutation. Therefore, the absence of Q14X mutation carriers in this study leads to the conclusion that AIP gene defects may not have a role in the pathogenesis of PHPT.

Based on our study and those reviewed above, it appears that sporadic and familial PHPT patients with multiglandular hyperplasia and young age at onset (below 40 years) are good candidates for MEN1 mutation screening. It also seems that CDKN1B and AIP may not have a significant contribution to PHPT predisposition.
7 Conclusion and future prospects

The major observations and conclusions of this study are as follows:

1. Clinical features in Northern Finnish MEN1 patients were similar to those reported by other studies (Table 1 and 6). PHPT was present in 93%, GEP tumours in 73%, pituitary adenomas in 29% and adrenal lesions in 35% of the MEN1 mutation carriers. Subgroups of different GEP tumours were unevenly distributed among carriers of the two MEN1 founder mutations: gastrinoma was present in 38% of the 1466del12 mutation carriers and in 3% of the 1657insC mutation carriers. Instead, NFPT was found in 39% of the 1466del12 carriers, and in 69% of the 1657insC mutation carriers. In the founder mutations group (n= 68) the OR for gastrinoma was 15.1 (CI, 1.73–131.9; P= 0.014) in the 1466del12 mutation carriers compared to the 1657insC mutation carriers. In the whole study group (n= 82) the OR for gastrinoma was 6.77 (CI, 1.31–35.0; P= 0.022) in the mutation class including in-frame and missense changes compared to the mutation class including frameshift and nonsense mutations. The 1657insC mutation carriers appeared to be at risk for NFPT with an OR of 3.56 (CI, 1.29–9.83, P= 0.015), whereas the mutation class including the frameshift and nonsense mutations predicted the risk for NFPT with an OR of 3.26 (CI, 1.27–8.33; P= 0.014). Our study indicates that there is some variation in the risks for certain types of GEP tumours between patients with different MEN1 mutations. Whether this phenomenon is caused by mutation per se and subsequent variations of mutated menin products in different transcriptional interactions or some other factor, such as a modifier gene cosegregating with MEN1 gene, needs further studying. Furthermore, more research is needed before extending these findings from our Northern Finnish families to other families carrying the same MEN1 gene mutations. It is also important to update the data concerning the Northern Finnish MEN1 patient cohort and test whether our findings in this study can be repeated.

2. The MEN1 pedigree study population of subjects born between 1728–1929 gives information on the survival of gene heterozygotes in a historical context. The lifespan of MEN1 positive subjects was equal to their spouse group and also similar to the life expectancy estimates derived from Finnish national statistics. Thus, the MEN1 gene did not show any harmful effect on survival in our analyses. More prospective studies are needed to assess if the MEN1 gene
has a statistically significant negative effect on survival in modern times, when life expectancy has generally increased in the population.

3. The clinical features and genetic background of previously uncharacterized pituitary adenoma predisposition, PAP, were defined in this study. Linkage analysis in families 1 and 2 placed the locus for PAP in chromosome 11q12-11q13, a region previously implicated in IFS and including the *MEN1* gene. On the basis of gene expression data in the linked area, *AIP* was chosen for further analysis. A nonsense mutation Q14X was found perfectly segregating with the acromegaly/gigantism phenotype. Out of 45 patients from the population-based acromegaly cohort 6 had Q14X mutation and one had a nucleotide substitution IVS3-1G>A affecting the splice acceptor site of exon 4. In the population-based cohort of patients with GH-secreting adenoma, PAP patients (*n* = 7) were significantly younger than mutation negative patients (*n* = 38) (24.7 ± 10.7 versus 43.6 ± 11.9 years, *P* = 0.0003), but no differences in tumour size or sex distribution were observed. In the population-based series, six of the fifteen patients diagnosed under 35 years of age had *AIP* mutation. Thus, in a population-based series from Northern Finland, two *AIP* mutations account for 16% of all patients diagnosed with pituitary adenomas secreting growth hormone and for 40% of the subset of patients who were diagnosed when they were younger than 35 years of age. Another series of 10 unselected Finnish sporadic acromegaly patients were screened and Q14X was found in two of them, which is compatible with findings in the population-based cohort. In addition, germline *AIP* mutation (R304X) was also found in Italian siblings with GH-secreting pituitary adenoma. Loss of the wild-type allele was detected in eight tumours (five GH-secreting, one secreting both GH and prolactin, two prolactinomas) from mutation-positive individuals available for LOH-studies. This finding strengthened the notion that PAP is associated with predisposition to prolactinoma, GH-secreting adenoma and bihormonal GH and prolactin secreting tumours. The results of LOH analyses also indicated that *AIP* is likely to act as a tumour suppressor.

Further work on the functional role of AIP should prove informative in revealing key cellular processes involved in the genesis of pituitary adenomas, including potential drug targets. Future goals also include studying the phenotypic variation and penetrance of PAP, and the role of *AIP* mutations in patients with both sporadic and familial pituitary adenomas outside Northern
Finland. Predictive testing of AIP mutation carrier status will also need further studying.

4. We examined 29 PHPT patients with features of genetic predisposition by mutation screening of 5 different genes including MEN1, HRPT2, CASR, CDKN1B and AIP and by serum screening of ionized calcium, PTH, prolactin and PP. Of the 29 patients, only one was found to carry a mutation in one of these genes. A female patient diagnosed with PHPT due to multiglandular disease at 38 years of age and having recurrent PHPT and a family history of PHPT displayed the founder mutation 1466del12 of MEN1. Based on our study and those reported by others it appears that sporadic and familial PHPT patients with multiglandular hyperplasia and young age at onset (below 40 years) are good candidates for MEN1 mutation screening. It also seems that CDKN1B and AIP may not have significant contribution to PHPT predisposition. Our future work will focus on expanding our familial PHPT material with subsequent genealogical and molecular analyses for predisposition gene identification, and this and similar efforts elsewhere should shed new light on the molecular basis of PHPT susceptibility.
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MULTIPLE ENDOCRINE NEOPLASIA TYPE 1 (MEN1) AND PITUITARY ADENOMA PREDISPOSITION (PAP) IN NORTHERN FINLAND

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