Long-term outcome of Multiple Endocrine Neoplasia type 1 related manifestations

Results from the DutchMEN1 Study Group

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Long-term outcome of Multiple Endocrine Neoplasia Type 1 related manifestations
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Lange termijn uitkomsten van Multipele Endocriene Neoplasie Type 1 gerelateerde manifestaties
Resultaten van de DutchMEN1 Study Group
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op
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door

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Promotoren: Prof. dr. G.D. Valk
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General introduction

Chapter 1 General introduction

Multiple Endocrine Neoplasia type 1

Multiple Endocrine Neoplasia type 1 (MEN1) is an inherited endocrine tumour predisposition syndrome. The syndrome was first recognized by the Austrian pathologist Erdheim in the beginning of the 20th century; named by Mayo Clinic endocrinologist Underdahl and coworkers in the nineteen fifties; its inherited nature noted by Austrian born New York physician Wermer (hence the alternative name Wermer syndrome for MEN1) and finally the *MEN1* gene on chromosome 11q13 was simultaneously discovered in 1997 by American and European research groups[1-5].

Primary hyperparathyroidism (pHPT), duodenopancreatic neuroendocrine tumours (dpNET) and pituitary adenomas are the three main manifestations of MEN1. At the age of 80 the penetrance of pHPT is almost 100%, the penetrance of dpNETs is over 80% and that of pituitary adenomas approaches 80%[6,7]. Other less prevalent endocrine manifestations are neuroendocrine tumours of the stomach, lung and thymus and adrenal adenomas. In addition to these endocrine manifestations, it was recently shown that MEN1 is also a breast cancer susceptibility syndrome[8,9]. Nowadays, in the Netherlands the life expectancy of patients with MEN1 is still 10 years shorter when compared with the general population, mostly caused by metastasized neuroendocrine tumours[6].

Challenges and opportunities of a rare genetic disease

The prevalence of MEN1 is estimated at one to ten in hundred thousand[1]. MEN1 is therefore a rare or so-called "orphan" disease. In Europe, a disease is considered rare when it occurs in less than one in two thousand citizens, a definition originating from the EU Regulation on orphan drugs (No. 141/2000).

A rare (genetic) disease poses special challenges to patients and their treating physicians. Diagnostic delay may occur due to unfamiliarity of physicians with the disease. When a diagnosis is established, finding a physician with special expertise and experience in the disorder can be difficult. Physicians treating patients with rare diseases are hampered in their decisions as robust clinical guidelines based on high quality scientific research are unavailable. Funding for research into rare diseases is difficult to obtain. Research itself is challenging as randomized controlled trials (RCTs) are rarely possible due to the low number of inclusions as well as the low event rate. Observational studies can provide valuable evidence in rare diseases but are prone to different forms of bias such as selection bias, information bias, and confounding[10]. On the other hand valuable information can be gathered from rare diseases on the pathogenesis of more common conditions, which may lead to the development of new treatment strategies. An example from an entirely different area is the development of PCSK9 inhibitors, which started with the discovery of the *PCSK9* gene in familial hypercholesterolemia[11].

Societal impact and patient involvement

Next to the scientific quality of research, the societal impact of research is considered more and more important. This is the ability of scientific research to answer social questions and to provide practical solutions or policy implications either directly or in the future [KNAW The societal impact of applied health research Council for Medical Sciences Amsterdam, 2002 Towards a quality assessment system]. Patient involvement in research can help enhance societal impact. In rare diseases, involving patients in different stages of scientific research can contribute to the prioritizing of the research agenda, the recruitment of patients, formulating patient oriented research outcomes and distributing research results[12]. However, while shared decision making has made its way into the consulting room, patient involvement in scientific research is far less common. Fortunately, this is changing. One example of this is the establishment in 2010 in the United States of the Patient-Centered Outcomes Research Institute (PCORI). Its goal is "to fund research that can help patients and those who care for them make better-informed decisions about the healthcare choices they face every day, guided by those who will use that information."

DutchMEN1 Study Group

Even before this was widely advocated, the need for patient involvement was recognized by our research group. Led by the University Medical Center (UMC) Utrecht, the DutchMEN1 Study Group (DMSG) was established in 2008. The DMSG is a collaboration between all Dutch UMCs and the Dutch MEN patient organisation (Belangengroep MEN). The mission statement of the DMSG is to improve the care of MEN1 patients by performing high quality research addressing clinically important questions. Research questions were formulated before data collection in close collaboration with the patient group, to ensure relevance of the results to those living with MEN1 and their treating physicians.

As the Netherlands is a small country and medical care for patients with MEN1 is provided almost exclusively by the UMCs (tertiary referral centres), a unique opportunity emerged to create a nationwide unselected MEN1 database. This DMSG database is a retrospective longitudinal database, in which all adult MEN1 patients treated in Dutch UMCs from 1990 onwards are included. Patients are identified by a standard procedure using the hospital diagnosis registries. Clinical data are collected for every quarter of every year. To minimize observer bias, the retrospective DMSG database contains only raw data. Database structure and variables included are dictated by the clinical questions formulated.

This thesis

The research described in this thesis focuses on the natural course of MEN1 in general and the most prevalent MEN1-related manifestations leading to the highest morbidity for patients e.g. neuroendocrine tumours and primary hyperparathyroidism. Based on determinants of the natural course and genotype-phenotype correlations the optimal follow-up and treatment is sought.

Clinical questions

Since the discovery of the *MEN1* gene in 1997 a presymptomatic genetic diagnosis of *MEN1* mutation carriers is possible[1]. The 2001 Consensus guidelines for diagnosis and therapy of MEN1 and MEN2 - which were the active guidelines when we started the work on this thesis - stated that genetic testing can be offered to relatives of MEN1 patients[13]. The updated 2012 clinical practice guidelines for MEN1 take this advice further and germline mutation testing of asymptomatic relatives is recommended at the earliest opportunity[14]. Consequentially, MEN1 will be diagnosed much earlier in the course of the disease. The phenotypical landscape of MEN1 will therefore change. It will also include families with a low penetrance of the disease and manifestations that might have been clinically silent for months or even years will be detected by periodic screening as recommended in the guidelines[14, 15]. Early genetic diagnosis might increase life expectancy and improve quality of life[16]. In **Part I** we aim to describe the clinical phenotype of MEN1 in the era of genetic diagnosis and we analyse if a diagnosis by genetic screening leads to a better outcome at the end of follow-up.

Primary hyperparathyroidism is the most prevalent manifestation of MEN1 and responsible for most MEN1-related surgeries[6,17,18]. As such, it has a significant impact on the quality of life in many ways. This may be through symptoms of hypercalcemia and sequelae such as renal stones and osteoporosis, through the necessity of repeated surgery because of persistent and/or recurrent disease, but also through surgical complications such as postoperative hypocalcaemia which can have an important impact on daily life. Controversy exist as to the optimal surgical strategy in MEN1-related pHPT and both subtotal parathyroidectomy as well as total parathyroidectomy with autotransplantation are advocated[19-21]. In **Part II** we aim to determine the optimal surgical strategy for MEN1-related pHPT and describe the course of postoperative hypoparathyroidism. We first performed a systematic review and meta-analysis including data from the UMC Utrecht. The next research article shows the first results from the DMSG database in answer to the clinical questions of the preferred surgical strategy and the course of postoperative hypoparathyroidism.

Neuroendocrine tumours are the most important determinants of MEN1-related survival. The phenotype of MEN1-related NETs, however, has changed importantly from the first description of the syndrome. Before the introduction of proton pump inhibitors, uncontrolled peptic ulcer disease was the most important cause of death, while presently, metastasized NETs are the most important cause of death[16, 22, 23]. In addition, due to advanced imaging techniques, early genetic diagnosis and a regular program of periodical screening advised in clinical guidelines, we see an increase in the detection of clinically silent and small NETs[14]. More insight into the natural course of MEN1-related NETs is therefore needed to be able to determine the optimal follow-up and treatment for individual patients. In **Part III** this subject is addressed. Firstly, we provide a comprehensive review on the natural history and genetic background of MEN1-related NETs. Secondly, in results from the DMSG database, we describe the long-term natural course of lung and thymus NETs and small NF-pancreatic NETs and its modifiers.

In Multiple Endocrine Neoplasia type 2, another inherited endocrine tumour susceptibility syndrome caused by mutations in the *RET* oncogene on chromosome 10, there is a clear genotype-phenotype correlation. The specific *RET* mutation determines whether the risk of aggressive medullary thyroid carcinoma is modest, high or highest and therapy advices differ accordingly[24]. In MEN1 such a genotype-phenotype correlation with clear clinical consequences is not known. There have been reports of a MEN1 variant with frequent prolactinoma and infrequent gastrinoma (MEN1 Burin) and variants with only primary hyperparathyroidism (familial isolated hyperparathyroidism). It is unclear however if these are true genotype-phenotype correlations as different mutations have been linked to the MEN1 Burin variant and mutations in familial isolated hyperparathyroidism are also found in MEN1 patients with the full syndrome[25,26]. Rather than a relationship between a specific mutation and clinical outcome, there has been research showing a relation between the type and location of the mutation and clinical outcome[27-31]. However, these studies all differ in their subdivision of type and location, making it hard to draw definitive conclusions. **In Part I, II and III** we have also looked into genotype-phenotype correlations, based on previously reported associations as well as based on the predicted effect on the menin protein.

In **chapter 9** lessons learned from this thesis are discussed with a special emphasis on their societal impact. Consequences for daily practice are discussed as well as directions for future care and research.

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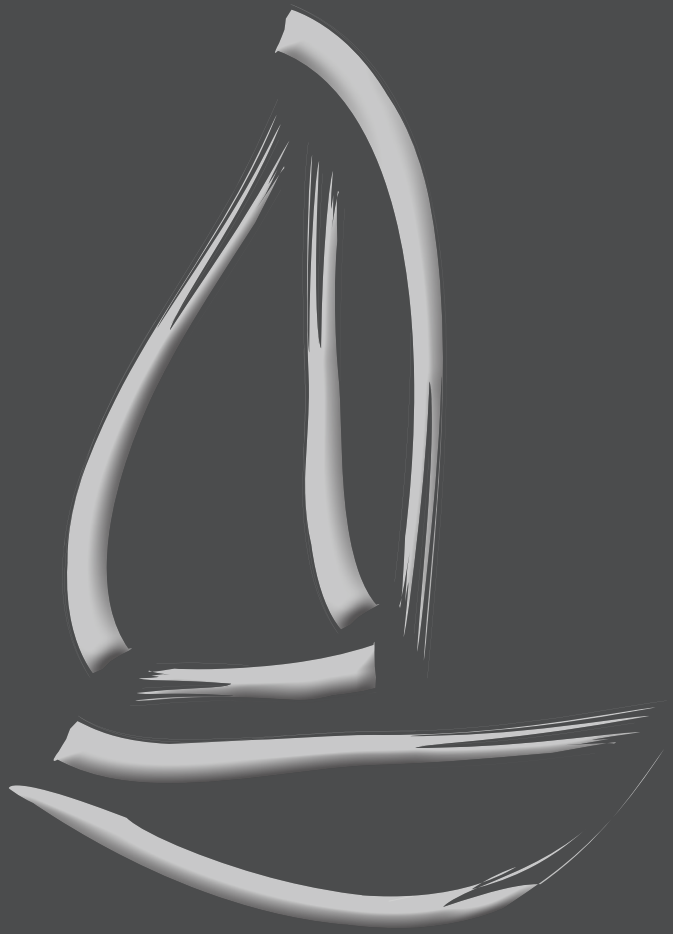
Part I



MEN1 in the era of genetic diagnosis

Chapter 2

Care for patients with multiple endocrine neoplasia type1: the current evidence base

Familial Cancer 2011;10:157-71

Care for patients with multiple endocrine neoplasia type1: the current evidence base

Pieterman CRC, Vriens MR, Dreijerink KMA, Van der Luijt RB, Valk GD

Abstract

Multiple endocrine neoplasia type 1 (MEN1) is a rare disease caused by mutations in the *MEN1* gene on chromosome 11. It is characterized by the occurrence of primary hyperparathyroidism (pHPT), duodenopancreatic neuroendocrine tumours (pNET), pituitary tumours (PIT), adrenal adenomas (ADR) and neuroendocrine tumours (NET) of the stomach, bronchus and thymus. MEN1 is a syndrome with high penetrance and high morbidity. Malignant NETs are the most important cause of MEN1-related death. Since 1997 the diagnosis can be made by genetic screening.

MEN1 is a complex syndrome and the endocrine manifestations cannot be viewed upon as coinciding sporadic tumours. Differences in epidemiology and pathology between MEN1-related tumours and their sporadic counterparts show that a unique approach is needed. Therefore the care for MEN1 patients should be provided by a centre of expertise. Early genetic diagnosis and periodical screening are important pillars of care. For primary hyperparathyroidism surgery is the most important treatment modality, with a subtotal parathyroid gland resection as the procedure of choice. In neuroendocrine tumours surgery also is the most important treatment modality. Selective tumour enucleation has no place in the surgical treatment of MEN1-related pNETs; the exact procedure depends on the functionality of the tumour. In MEN1-associated pituitary and adrenal adenomas, watchful waiting and medical therapy play more important roles.

In the twenty-first century new developments will impact the care for MEN1 patients. These developments should be critically evaluated in clinical research with the ultimate goal of optimizing the care for MEN1 patients on an evidence base.

Introduction

In 1903, Erdheim[1] reports a necropsy case of a pituitary adenoma and enlarged parathyroid glands; the first published case of multiple endocrine neoplasia type 1 (MEN1). In 1953, Underdahl *et al.*[2] introduce the term 'multiple endocrine adenomas' for the combined occurrence of parathyroid, pituitary and pancreatic islet adenomatosis and the first familial occurrence of this syndrome is reported[3]. In 1954, Wermer[4] recognises an inherited syndrome of multiple endocrine adenomas affecting the endocrine pancreas, parathyroid glands and anterior pituitary. He postulates that this syndrome is probably caused by a mutation in a single gene and inherited in an autosomal dominant fashion. In 2010, how do we care for patients with the autosomal dominant inherited disorder called MEN1, caused by mutations in the *MEN1* gene on chromosome 11?

Abbreviations

5-HIAA	5-hydroxyindolacetic acid	MEN2	multiple endocrine neoplasia type 2
ADR	adrenal adenoma	MLPA	multiplex ligation–dependent probe amplification
AIP	aryl hydrocarbon receptor-interacting protein	MRI	magnetic resonance imaging
CDK	cyclin-dependent kinase	mTOR	mammalian target of rapamycin
CDKN	cyclin-dependent kinase inhibitor	NET	neuroendocrine tumour
CgA	chromogranin A	NF	non-functioning
CT	computed tomography	PET	positron emission tomography
DHEAS	dehydroepiandrosterone sulphate	pHPT	primary hyperparathyroidism
DNA	deoxyribonucleic acid	PIT	pituitary tumour
DPR	distal pancreatic resection	pNET	duodenopancreatic neuroendocrine tumour
ECL	enterochromaffin-like	PTH	parathyroid hormone
EUS	endoscopic ultrasound	SF-36	short form 36
IGF-1	insulin-like growth factor 1	SRS	somatostatin receptor scintigraphy
LOH	loss of heterozygosity	TCT	transcervical thymectomy
MEN1	multiple endocrine neoplasia type 1	ZES	Zollinger-Ellison syndrome

MEN1 is a rare disease with an estimated prevalence of 1-10/100.000[5,6]. It is characterized by the occurrence of parathyroid hyperplasia or adenomas (primary hyperparathyroidism (pHPT)), neuroendocrine tumours of the pancreas and duodenum (pNETs), anterior pituitary tumours (PIT), gastric, bronchial and thymic neuroendocrine tumours (NET) and adrenal adenomas (ADR). Of the mutation carriers 82-99% has at least one manifestation of the disease at the age of 50[7-9]. In the last years, however, there have been reports of low penetrance in some families[10,11]. The burden of disease is high in MEN1, with signs and symptoms occurring from overproduction of hormones, tumour growth and malignancy, but also from complications of (surgical) treatment. Patients with MEN1 have a shorter life

expectancy than the general population[12-14]. Today, the most important causes of MEN1-related death are malignant pNETs and thymic NETs[12-15].
With the discovery of the *MEN1* gene, family members can be identified through presymptomatic genetic screening with subsequent periodical screening to detect manifestations when patients are still asymptomatic.
This review focuses on the clinical management of MEN1. The evidence was gathered using PubMed searches for each individual topic, in case of pNETs also combined with Embase and Cochrane searches.

Pathogenesis of MEN1

MEN1 is caused by inactivating germline mutations in the *MEN1* gene[15,16]. The *MEN1* gene is a classic tumour suppressor gene: in accordance with Knudson's "two-hit" hypothesis, both *MEN1* alleles need to be inactivated before a MEN1 tumour can develop. Loss of the wild-type *MEN1* allele (loss of heterozygosity (LOH)) is observed frequently in MEN1 tumours[17]. To date, more than 450 different germline *MEN1* gene mutations have been reported[18]. These mutations are found scattered over the entire gene. Some mutations occur in apparently unrelated families, which can point to possible mutational hotspots in the *MEN1* gene[18,19].
Reports regarding a genotype-phenotype correlation are inconsistent[20-23]. Within the MEN1 spectrum, two specific MEN1 variants have been described. Firstly, a variant with frequent prolactinoma and infrequent gastrinoma, also called MEN1 Burin [24]. Whether this represents a true genotype-phenotype correlation is unclear because different mutations have been linked to this variant[24]. Secondly, familial isolated hyperparathyroidism, which is more often associated with missense mutations[25]. Since mutations of patients with familial isolated hyperparathyroidism are also found in MEN1 patients a genotype-phenotype relation is difficult to establish[25]. Apart from mutations in the *MEN1* gene itself, mutations in other genes might modify the phenotypic expression and contribute to the diversity of the disorder. It has been shown in mice models that a different genetic background leads to a different MEN1 phenotype[26]. In humans, loss of heterozygosity at chromosomes 3p and 18q has been observed in MEN1-related pancreatic endocrine tumours[27]. Further identification of the genes involved will give more insight into the development of MEN1 manifestations.
The *MEN1* gene encodes the menin protein. Menin is a ubiquitous nuclear protein that is involved in several intracellular processes including the regulation of gene transcription. Menin is a component of a histone methyltransferase protein complex which regulates transcription of target genes[28]. Aberrant function of this complex leads to changes in gene expression that could lead to MEN1 tumorigenesis[29].

Diagnosis and periodical screening

MEN1 can be diagnosed clinically if a patient has two of the three main MEN1 related tumours (pHPT, pNET, PIT)[30]. Familial MEN1 is defined as one MEN1 patient with at least one first degree relative with one of the three main MEN1 related tumours[30].

At present the diagnosis can be established by direct mutation testing. The DNA-sequence of the coding region of a patient's *MEN1* gene is compared to a normal reference sequence. In addition to sequencing, recently it became possible to search for large deletions or duplications in the *MEN1* gene by using the Multiplex Ligation-dependent Probe Amplification (MLPA) assay.

MEN1 patients, their relatives and patients suspected of MEN1 are eligible for mutation testing. In patients who have parathyroid, pancreatic and pituitary tumours a *MEN1* mutation is detected in 91-93% of the familial cases and 31-69% of the sporadic cases[31,32]. In patients who have two of these tumours the detection rate is 56-69% in familial and 2-23% in sporadic cases, falling to 29-57% and 0-4% in familial and sporadic cases with one tumour[31,32]. Recent studies have shown that in some families where diagnostic *MEN1* gene testing failed to detect a causal germline mutation, inherited mutations of other genes were identified. These genes include the aryl hydrocarbon receptor-interacting protein (AIP) gene and four cyclin-dependent kinase inhibitor genes: CDKN1B (p27, Kip1), CDKN1A (p21, Cip1, Waf1) CDKN2B (p15, CDK4I) and CDKN2C (p18, INK4C)[33-37]. Although these were rare, investigation of these genes may be useful in *MEN1* mutation-negative families with a strong suspicion of a hereditary endocrine tumour syndrome.

MEN1 patients and mutation carriers should be subjected to periodic screening in order to detect manifestations in an early stage[30]. Table 1 shows our screening protocol based on the international consensus statement regarding diagnosis and therapy of MEN1 and MEN2[30]. Early genetic diagnosis and subsequent periodical screening is associated with less morbidity and mortality at follow-up[38,39]. However, it remains debatable if periodic screening in its present extensive form is really necessary. Waldmann *et al.* prospectively evaluated an extensive annual screening protocol in 35 *MEN1* mutation carriers which consisted of annual computed tomography (CT) of chest and abdomen, magnetic resonance imaging (MRI) of the pituitary, somatostatin-recepter scintigraphy (SRS), endoscopic ultrasound (EUS) and extensive biochemical screening[40]. The costs of this protocol were €2,100 per year per patient. Based on prospectively diagnosed lesions and growth rates, the authors suggested a less extensive screening protocol after an extensive first examination, consisting of EUS and biochemical screening (measurements of serum calcium, gastrin, pancreatic polypeptide and prolactine) at 3-year intervals. The costs of this protocol would be €700 per patient per year. For patients after pancreatic resection, with a bronchial or thymic neuroendocrine tumour and patients with newly diagnosed lesions a more close follow-up schedule is suggested[40]. Whether this is adequate should be confirmed in larger, multicenter trials.

Primary hyperparathyroidism

Primary hyperparathyroidism is the most prevalent manifestation of MEN1, occurring in 78-94% of patients and it is often the first clinical manifestation of the disease [8, 15, 22, 38-41]. The majority of the surgeries performed on MEN1 patients is also because of pHPT[12,42,43].

Signs and symptoms of hypercalcaemia can be summarized as stones (renal stones, nephrocalcinosis, polyuria, polydipsia, eventually leading to renal failure) bones (osteoporosis, osteitis fibrosa), abdominal groans (constipation, nausea, peptic ulcer, pancreatitis) and psychic moans (e.g. lethargy, fatigue, depression, memory loss, psychoses, personality change).

Characteristics of MEN1-associated pHPT – in contrast to sporadic pHPT – are (1) a younger age (2) an even gender distribution (3) multiglandular disease (4) more severe bone involvement and (5) lower levels of parathyroid hormone (PTH) [44]. pHPT also seems to be more severe in the presence of a Zollinger-Ellison Syndrome (ZES) and biochemical parameters of ZES are worse in the presence of hypercalcaemia[45]. Treatment of pHPT results in improvement of biochemical parameters of the ZES[45].

Surgery is the preferred treatment of pHPT and in MEN1 patients the preferred surgical procedure is a subtotal parathyroid gland resection, leaving a portion of the most normal appearing gland in site. Any surgery less than subtotal resection is associated with a high risk of persistent (0-43%) and a very high risk of recurrent disease (0-92%)[45-53]. Total parathyroidectomy with autotransplantation of parathyroid tissue leads to a higher rate of postoperative hypoparathyroidism (22-100%) compared to subtotal resection (9-60%)[45-55]. Although persistent disease does not occur with total resection, recurrence rates are comparable with subtotal resection (0-56% vs 5-45%)[45-55].

A routine thymectomy during primary surgery is aimed at removing ectopic parathyroid tissue and preventing the development of thymic NETs. Routine thymectomy does indeed seem to increase the likelihood of obtaining normocalcaemia, mostly if <4 glands are found during neck exploration[56,57].

Since recurrence rates up to 56% are reported even after total parathyroidectomy, secondary surgery is not infrequent[45-55]. Recurrence can occur from supernumerary or ectopic parathyroid glands, remnant tissue after previous subtotal resection or parathyroid grafts after previous total resection. During reoperative surgery the use of intraoperative PTH measurements is recommended[58-61].

Future medical therapy for hyperparathyroidism might be cinacalcet and/or somatostatin analogues. However, evidence for the use of these medications in MEN1-related pHPT is still anecdotal[62,63]. Another possible alternative for surgery is ethanol ablation therapy. There is one report of ethanol ablation therapy in patients with recurrent pHPT in MEN1[64]. Eighty-three percent of the patients had initial normalization of their serum calcium levels after treatment. Recurrence how-

Table 1. Protocol for periodical screening

	Starting age	Frequency	Content
Visit outpatients clinic	5 years	biannually	History and physical examination
Laboratory investigations	5 years	biannually	Ionized calcium, chloride, phosphate, parathyroid hormone, fasting glucose, fasting insulin, fasting c-peptide, glucagon, fasting gastrin, pancreatic polypeptide, prolactin, insulin-like growth factor 1, platelet serotonin, chromogranin A.
Imaging studies	15 years	every 2 yrs	MRI[a] of upper abdomen
		every 2-3 yrs	MRI[a] of pituitary (intravenous contrast with gadolinium)
		every 3-5 yrs	CT[b] of thorax

[a] Magnetic resonance imaging
[b] Computed tomography

ever, was common[64]. Currently, medical and ablative therapies have a limited role, mainly in recurrent pHPT or when contraindications to surgical therapy exist.

Neuroendocrine tumours

MEN1 patients are prone to develop NETs of foregut origin: thymus, bronchus, stomach, duodenum and pancreas[65]. Duodenopancreatic NETs are most frequently seen, whereas gastric, bronchial and thymic NETs occur less frequently.

Duodenopancreatic NETs (pNETs)

Epidemiology

Among MEN1 patients, the prevalence of clinically manifest pNETs is 35-75%[8,9,39,66-73]. pNETs are classified according to the secreted hormones. In patients with MEN1 non-functioning (NF) pNETs are most frequently seen, followed by gastrinomas, insulinomas and rare functioning tumours (eg vipomas, somatostatinomas and glucagonomas)[74]. Gastrinomas in MEN1 occur mostly in the duodenum [75,76]. Duodenal somatostatinomas and – anecdotally – glucagonomas have also been reported[77,78]. The other pNETs are exclusively pancreatic.

The penetrance of pNETs in MEN1 is 9% at the age of 20 rising to 84% at the age of 80[8,79]. Compared to sporadic pNETs, pNETs in MEN1 occur at a younger age, are more often multiple and are diagnosed at an earlier stage due to periodical screening[74,80]. With exception of insulinomas, MEN1 related pNETs seem to have a more indolent course than their sporadic counterparts, as evidenced by a lower rate of liver metastases[74].

However, pNETs are an important determinant of survival in MEN1 patients and the most important cause of MEN1-related death[12,13]. In a report from the French endocrine tumour registry, 10-year survival was 91% for MEN1 patients with insulinomas, 82% for gastrinomas, 62% for NF-pNETs and 54% for glucagonomas, vipomas and somatostatinomas[81].

Diagnosis

MEN1 patients are periodically screened for the presence of pNETs. Signs and symptoms can arise from the associated hormonal syndrome (table 2), tumour mass and/or metastases.

Biochemical markers used can be divided in general NET markers, specific pNET markers and provocation tests (table 3). None of these biochemical markers is validated for the use as screening tool in asymptomatic MEN1 patients. In these patients, test characteristics will undoubtedly differ from those described in literature, so further research in this area is needed.

As screening technique EUS is the most sensitive for detecting the small and multiple pancreatic tumours in MEN1[82-85]. CT and MRI are known to be less sensitive, but are important for the diagnosis of liver metastases and to provide preoperative information[86]. SRS [with single photon emission computed tomography (SPECT)] seems to be especially important in detecting occult tumours and metastases in MEN1 patients with raised biochemical markers[87-89]. Compared with SPECT, positron emission tomography (PET) has a higher sensitivity and special resolution, but classic FDG-PET is known to be of little value in pNETs[90]. A recent study also showed FDG-PET to be less sensitive than SRS in the detection of NETs, except for tumours with a proliferation index >15%, for which FDG-PET was superior[91]. In MEN1, such tumours represent a minority. However, new promising PET tracers are arising such as F-DOPA, Ga-DOTA-NOC and 5-HTP[92,93]. Little information is available regarding the use of these new tracers in the diagnosis of MEN1-related pNETs.

Angiography, either alone or combined with arterial stimulation venous sampling, can also be used in the diagnosis of insulinoma and gastrinoma[94]. With this technique the (functioning) tumour can be identified in patients with biochemically proven insulinoma/gastrinoma who have negative imaging or on the other hand have multiple tumours on imaging.

Treatment of the primary tumour

To date, surgery is the only curative treatment option for MEN1-related pNETs. However, surgical decision making is not easy. Goals of pancreatic surgery in MEN1-related pNETs are curing or alleviating the hormonal syndrome and preventing metastases, while preserving as much pancreatic tissue as possible. Not surprisingly much controversy exists on timing and extent of surgery in MEN1-related pNETs. Based on the available literature some remarks can be made to this subject.

Insulinomas in MEN1 should always be operated. Enucleating the tumour seems to be insufficient for MEN1-related insulinoma. Three studies show higher cure rates after a distal pancreatic resection (DPR) combined with enucleation of tumours from the head, compared with enucleation alone[95-98]. Due to new surgical techniques, a DPR is nowadays more likely to be a spleen preserving procedure. Patients with MEN1-related gastrinomas have satisfactory survival rates when the

Table 2. Hormonal syndromes in pNETs

	Signs and symptoms
Insulinoma	(1) Whipple's triad: neuroglycopenic symptoms (e.g. blurred vision, weakness, abnormal behaviour, seizures, coma) and hypoglycaemia, corrected by administration of glucose[167,168] (2) Symptoms typically occur before meals, during fasting and after exercise[167]
Gastrinoma	(1) Zollinger-Ellison Syndrome (ZES): hypersecretion of gastric acid caused by gastrin secretion from the gastrinoma leading to peptic ulcers, steatorrhoea and malabsorption (2) Symptoms: abdominal pain, diarrhoea, heartburn, nausea, vomiting, weight loss and gastro-intestinal bleeding[169]
VIPoma	WDHA syndrome: watery diarrhoea, hypokalaemia and achlorhydria[81]
Glucagonoma	Glucagonoma syndrome: necrolytic migratory erythema, diabetes mellitus and weight loss[81]
Somatostatinoma	Inhibitory syndrome: diabetes mellitus, diarrhoea/ steatorrhoea and cholelithiasis [81,170]

Table 3. Biochemical markers in pNETs

General NET markers	*serotonin-related markers: urinary 5-hydroxyindole acetic acid (5-HIAA), fasting plasma 5-HIAA, whole blood serotonin (5-HT) and platelet serotonin* (1) Less useful in foregut NETs which secrete less serotonin than midgut NETs [134] (2) When comparing the diagnostic value of urinary 5-HIAA, urinary serotonin and platelet serotonin, platelet serotonin had the best diagnostic value with an area under the curve of 0.81 (95% CI 0.67-0.95; data from sporadic NETs)[171] *Chromogranin A (CgA)* (1) In MEN1 related pNETs CgA is supposed to be a sensitive marker, although sensitivity reports range from 57-92%[172-175] (2) The sensitivity of CgA differs per tumour type. In a study of sporadic NETs CgA was raised in 100% of the gastrinomas, 69% of the NF-pNETs and only 10% of the insulinomas [176] *Neuron Specific Enolase (NSE)* As a NET tumour marker NSE seems to be less sensitive than CgA [176]
Specific pNET markers	*pancreatic polypeptide (PP), glucose, insulin, pro-insulin, c-peptide, gastrin, glugacon, vaso-active intestinal peptide, somatostatin, adrenocorticotropic hormone, growth hormone releasing hormone and calcitonin.* Used to determine if a functional syndrome exists.
Provocation tests	*Insulinoma: 72-hour fast* Considered the gold standard in the biochemical diagnosis of insulinoma[134] *Gastrinoma: secretin test* (1) Used in the diagnosis of gastrinoma, because in 67% of patients, serum gastrin is raised to the same levels as seen in more common conditions[134, 177,178] (2) Using a Δ serum gastrin of ≥ 120 pg/mL sensitivity and specificity are 94% and 100% (data from mixed sporadic and MEN1-related gastrinomas)[177]

indication for surgical excision is set at ≥ 2.5-3.0 cm[99,100]. However, in these series no biochemical cure of the gastrinoma is observed[99,100]. When operating patients if tumours surpass 1 cm, biochemical cure can be accomplished by performing extensive resections but not with local tumour excision alone[101-106]. Duodenal surgery is important for the treatment of gastrinomas, since duodenal tumours are almost invariably present[75,76, 99-108]. Patients with NF-pNETs > 2-3 cm appear to have a worse outcome compared to smaller tumours[79, 109]. Operating before tumours reach 2 cm in size seems logical, but this could not be confirmed in a study comparing surgery to watchful waiting in patients with tumours ≤ 2 cm[110]. Literature on rare functional pNETs in MEN1 is scarce, so no specific remarks to the surgical treatment of these tumours can be made.
Since preserving pancreatic tissue will almost always lead to recurrent pNETs, repeat surgery is sometimes necessary. Since morbidity and mortality are higher after reoperative surgery, these decisions should be tailor made for each patient by an experienced multidisciplinary team[111,112].

Gastric NETs

Pathogenesis and epidemiology

Gastric NETs are divided into three different types: type I and II are associated with elevated gastrin levels in atrophic gastritis (type I) and MEN1/ZES (type II); type III are sporadic tumours without known predisposing factors.
Type II (and type I) gastric NETs originate from the gastric enterochromaffin-like cells (ECL-cells) and are therefore also referred to as ECL-omas. These tumours occur almost exclusively in MEN1 patients with hypergastrinaemia, 21-37% of the patients with MEN1/ZES develop gastric NETs[113-116]. However, hypergastrinaemia alone is not enough for gastric NETs to occur and another factor, such as an *MEN1* mutation, is necessary[113, 117]. The importance of the *MEN1* mutation was underscored by Debelenko *et al.*[118], who found LOH at the 11q13 locus (which harbours the *MEN1* gene) in 75% of the type II gastric NETs. Thus gastric NETs are a true manifestation of the MEN1 syndrome, but hypergastrinaemia is necessary for their development. The importance of hypergastrinaemia is confirmed by the fact that a longer duration of ZES, higher levels of fasting serum gastrin and a more aggressive form of gastrinoma are associated with a higher risk of developing gastric NETs[113, 119].
Evidence on the prognosis of gastric NETs in MEN1 is scarce. At the moment – based on small studies – it is estimated that lymph node metastases occur in 30% and distant metastases in 10%[120-122]. Tumour related death is estimated to be less than 10%[120-122]. However reports on more aggressive behaviour also exist[114, 123].

Diagnosis

Type II gastric NETs are usually small (< 2 cm) and multiple and often asymptomatic[120-122]. In aggressive cases symptoms such as gastric bleeding, carcinoid syndrome, weight loss and gastric outlet obstruction have been reported[123].

Gastroscopy with biopsy samples is the most important diagnostic tool combined with EUS for tumours >1 cm[122]. Imaging techniques aimed at diagnosing distant metastases are only recommended in larger tumours or those demonstrating invasiveness at EUS[122]. In one study the use of SRS in the diagnosis of the primary tumour was evaluated. Among 162 ZES patients, a positive predictive value of 63% and a negative predictive value of 97% was found[124]. Gastrin and chromogranin A (CgA) are the only recommended laboratory tests[122].

Treatment of the primary tumour

For tumours <10 mm annual surveillance is recommended[122]. For larger tumours not involving the muscular layer of the stomach wall (with a maximum of six) endoscopic resection is recommended[122]. In other cases local resection should be performed, with partial or total gastrectomy with lymph nodes dissection being reserved for malignant tumours or recurrences after local resection[122].

Two case-reports have shown promising results for the use of somatostatin analogues in type II gastric NETs[125,126]. However, the exact indications for somatostatin therapy are not clear. Some have advocated its use as an alternative therapy for symptomatic or rapidly growing tumours and as a possible first-line therapy for large tumours and multiple tumours for which surgery is unfeasible[125]. Current guidelines do not recommend treatment with somatostatin analogues in non-metastatic cases[122].

Thymic NETs

Epidemiology

The prevalence of thymic NETs among MEN1 patients is 2.8-8%[39, 127-130].

Almost all patients are males, however some female patients have been reported [127-132]. Thymic NETs in MEN1 are rarely associated with hormonal syndromes [127-132]. Some series report that almost all patients with thymic NETs are heavy smokers[127, 130]. The mean age at diagnosis is around the fifth decade[127-130, 132].

Malignant thymic NETs are an important cause of death in MEN1 patients[15, 39, 133]. A recent study showed that a thymic NET was associated with the highest risk of death in MEN1 patients (hazard ratio 4.64 95% CI 1.7-12.4)[15]. Although thymic NETs are reported to have a poor prognosis, the course of the disease can be protracted. The most recent study reports a median survival of 9 years with a 10-year probability of survival of 36% (11.5-62%)[129].

Diagnosis

Thymic NETs are often asymptomatic until late in their natural course. In the most recent series, most patients were asymptomatic at diagnosis probably reflecting

earlier detection by periodical screening[127-129]. This is supported by a mean survival of 4.5 years in an earlier report compared with a median survival of 9 years in the most recent series[129,130].
The various neuroendocrine tumour markers, especially 5-hydroxyindolacetic acid (5-HIAA) and CgA, are not thought to be useful in the diagnosis of thymic NET[127-129, 134]. In the diagnosis of a thymic NET imaging studies are the most important with CT, MRI and SRS-scintigraphy being the recommended modalities[135,136].

Treatment of the primary tumour
Localized thymic NETs are treated surgically. Evidence for the use of (neo) adjuvant radiotherapy and chemotherapy is scarce and not specific for MEN1-related tumours. Some authors advocate routine preventive transcervical thymectomy (TCT) during parathyroid surgery[45,46, 51, 56]. However, there are reports of malignant thymic NET occurring after TCT[137], indicating that a true total thymectomy can only be performed with thoracotomy.

Bronchopulmonary NETs
Epidemiology
Bronchopulmonary NETs can be divided into well differentiated (typical carcinoid and atypical carcinoid) and poorly differentiated (small cell carcinoma and large cell neuroendocrine carcinoma) forms.
Bronchopulmonary NETs have been reported in 1.4-9.5% of the MEN1 patients in different series [15, 39, 68, 138] and seem to be more frequent in women than in men [65, 139]. However, in a recent large series no gender difference was seen[15]. A wide variety of age at diagnosis is reported[139]. As other MEN1-related tumours, bronchopulmonary NETs can be multicentric[139]. The prognosis of bronchopulmonary NETs in MEN1 is good. In one series no mortality or metastatic disease was seen after a mean ten years of follow-up and in another bronchial NETs were not associated with an increased risk of death[15, 139]. However, mortality due to bronchopulmonary NETs in MEN1 is reported[15, 133, 138].

Diagnosis and treatment of the primary tumour
There is only one patient series focusing on MEN1-related bronchopulmonary NETs[139]. In this series, 12 out of 32 patients had pulmonary nodules on CT, four of which were confirmed NETs, two were other tumours and on six no histological diagnosis was made[139]. Most patients were asymptomatic at diagnosis and carcinoid syndrome was not seen[139].
CT-scan is the preferred diagnostic tool for bronchopulmonary NETs, SRS can also be performed[139]. Surgery is the primary treatment[139].

Metastatic NETs

Epidemiology of metastases

Metastatic NET is the most important MEN1-related cause of death[12,13]. Thymic NETs have the highest metastatic potential, with 29-60% of the patients having solid organ or bone metastases, rising to 42-80% when including lymph node metastases[127-130]. Of the pNETs, rare functional pNETs seem to have the highest metastatic potential (liver metastases 0-50%), followed by NF-pNETs (almost 20%), gastrinoma (11%) and insulinoma (0-20%)[74].

Most information on metastatic NET in MEN1 is available from metastatic pNET. In one study 10-year survival of patients with synchronous distant metastases from pNETs was 34% (95% CI 0-72%)[21]. Another study showed a 34% (95% CI 6-62%) 10-year survival of patients with distant metastases from NF-pNETs, and a 60% 10-year survival was reported for MEN1-gastrinoma patients with metachronous liver metastases[79, 140]. The prognostic significance of lymph node metastases in MEN1-related pNETs remains debatable [21, 140, 141].

Treatment of metastatic NET

There are no studies available with specific focus on the treatment of metastatic NET in MEN1. However, recent European Neuroendocrine Tumours Society guidelines deal in detail with the treatment of metastatic NET in general[142-145]. Up until today there is no reason to assume that the treatment of metastatic NET in MEN1 should be different from the treatment in sporadic cases.

For liver metastases, surgery is the most important treatment modality in patients without extrahepatic disease and without diffuse liver involvement, because this is the only curative therapy[145]. Other treatments directed at the liver are local ablative techniques such as radio frequent ablation, arterial (chemo)embolization and – a new treatment modality – radioembolization with yttrium microspheres[145, 146].

Somatostatin analogues are registered for symptom control in functioning pNETs and NETs associated with the carcinoid syndrome. However, recently it has been demonstrated that somatostatin analogues also have an antiproliferative effect [147-149].

Next to treatment with these so-called 'cold' somatostatin analogues, treatment with radiolabeled somatostatin analogues is also important in advanced NETs. Up to 80% of the patients can achieve stable disease or partial remission with this treatment[143].

Interfon-alfa has also demonstrated some antiproliferative effect in different types of NET and in addition chemotherapeutic agents are used in the treatment of metastatic NET[142].

Several new therapies are rising in the treatment of metastatic NETs, but no data are available on their efficacy yet. Examples are the use of inhibitors of mammalian target of rapamycin (mTOR), tyrosine kinase and angiogenesis.

Pituitary tumours

Epidemiology

Pituitary tumours have been reported in 20-65% of patients with MEN1[8-9, 22, 39, 68-69, 72-73, 150-153]. Most patients are diagnosed in the fourth decade, which is comparable with sporadic pituitary tumours[8, 69, 73, 153]. However, the earliest manifestation of the MEN1 syndrome ever reported was a five-year old boy with a pituitary macro-adenoma[154]. Pituitary adenomas in MEN1 seem to occur more often in men than in women[73]. In MEN1-related pituitary adenomas prolactinoma is the most frequent (48-71%), followed by non-functioning adenomas (15-38%), somatotropinomas (9-23%) and Cushing's disease (4%)[39, 22, 73, 153]. Thyrotropinoma and gonadotrophinoma have been anecdotally reported[155,156]. Macro-adenomas seem to occur more often in MEN1 patients than in patients with sporadic pituitary adenomas[73]. In a pathology series MEN1-related tumours were larger, more often invasive, multiple (although still only in 4%) and plurihormonal on immunohistochemistry, when compared to sporadic tumours[157].

Malignant pituitary tumours demonstrating metastases are extremely rare. In the literature one case of metastatic prolactinoma in MEN1 is reported[158].

Diagnosis

Symptoms of pituitary tumours may arise from hormonal syndromes (see table 4) or from compression of surrounding tissue by the tumour (visual field defects, headache and hypopituitarism). In a large series 50% of the patients presented with symptoms related to hormonal hypersecretion and 29% with symptoms related to tumour growth[73].

Current guidelines recommend screening for pituitary disease by determining plasma levels of insulin-like growth factor 1 (IGF-1) and prolactin every year and performing an MRI every three years[30]. On indication this can be extended with plasma levels of other pituitary hormones and pituitary function tests.

Treatment

Treatment of pituitary tumours in MEN1 is not different from that of sporadic pituitary tumours. Dopamine agonists are the first-line treatment in prolactinomas, with transsphenoidal surgery being reserved for dopamine resistant cases. Radiotherapy is sometimes used after non-curative surgery. In somatotropinomas transsphenoidal surgery is the first-line treatment, with somatostatin analogues, pegvisomant and radiotherapy as second-line treatments. Surgery is also the preferred treatment in Cushing's disease. Small non-functioning tumours can be followed by regular imaging, with surgical treatment when tumour growth is observed.

Although treatment strategy does not differ between MEN1 and sporadic cases, treatment success seems to do. When looking at functioning tumours, normalization of pituitary hypersecretion occurred in 42% of the MEN1 patients after treatment, compared with 90% of the patients with sporadic tumours[73].

Table 4. Hormonal syndromes in pituitary adenomas

	Signs and symptoms
Prolactinoma	Hypogonadism (1) premenopausal women: amenorrhoea, galactorrhoea, infertility and lack of libido (2) men: impotence, lack of libido and infertility
Somatotropinoma	Acromegaly (1) adults: soft tissue growth (2) children: accelerated linear growth
Corticotropinoma	Cushing's syndrome (1) outward appearance: central obesity, abdominal striae, "moon face", "buffalo hump", easy bruising (2) metabolic changes: hypertension, impaired glucose tolerance, acne (3) psychiatric disorders: e.g. depression, psychosis (4) other: menstrual disorders, impotence, proximal muscle weakness
Thyrotropinoma	Hyperthyroidism or overt thyrotoxicosis (1) general: fatigue, hyperactivity, heat intolerance, weight loss (2) cardiac: increased heart rate, palpitations and atrial fibrillation (3) GI: increased appetite and frequent defecation (4) other: muscle weakness, excessive sweating, oligomenorhoea
Gonadotropinoma	women: ovarial hyperstimulation

Adrenal tumours

Epidemiology

In the early clinical series adrenal involvement in MEN1 was described in 5% of the patients[9, 68], rising to 11-35% in more recent series[8, 22, 39]. However, in studies focussing specifically on adrenal involvement in MEN1 this was seen in 27-73%[67, 159-163]. Adrenal involvement is usually diagnosed in the fifth decade[22, 159, 162, 163].

Adrenal lesions in MEN1 are mostly cortical although pheochromocytomas are sporadically reported[8, 160, 163]. A recent study of adrenal lesions in MEN1 patients evaluated with EUS showed the following distribution: 12% "plump adrenals", 35% nodular hyperplasia, 24% adenomas and 2% cysts. Bilateral involvement was seen in 47% of the patients[161]. Most adrenocortical lesion in MEN1 are non-functional, benign and stable during follow-up[159-163]. If a cortical functional syndrome is observed, this is mostly Cushing's syndrome, although anecdotal reports exist on aldosterone producing tumours[160, 163, 164]. Adrenal cortical carcinoma can also be seen in MEN1 patients, and in two series even comprised 17 and 22% of all adrenal lesions[160, 162].

Diagnosis

Adrenal lesions in MEN1 are usually identified through periodical screening with CT, MRI or EUS. Biochemical screening is not recommended[30], functionality should be tested when an adrenal lesion is visible. In an asymptomatic patient the first screening can consist of an overnight 1 mg dexamethasone suppression

test and measurements of free cortisol, fractionated catecholamines and metanephrines (normetanephrines and metanephrines) in a 24-h urine specimen. If available, measurements of plasma free metanephrines (metanephrines an normetanephrines) can also be used. On indication this can be extended with midnight salivary cortisol and measurement of plasma aldosterone concentration and plasma renin activity[165, 166].

Treatment

For most adrenal lesions in MEN1 regular follow-up with imaging is sufficient. Indications for surgery are in concordance with those in adrenal incidentalomas: size 40-60 mm, signs of malignancy or a functional syndrome. However, some groups advocate surgery in MEN1 related adrenal tumours >30 mm, because of the observed frequency of adrenal cortical carcinoma[163].

Quality of Life and ethical considerations

Living with MEN1 poses a threat to the quality of life. Patients live with a disease that is incurable by nature and might lead to malignant disease: one manifestation may appear to be cured, but a recurrence or another possible malignant manifestation will almost certainly occur. They undergo periodical screening with the accompanying uncertainty and anxiety, although periodical screening can also provide a feeling of safety. In addition they have to undergo many medical treatments: in one small series the mean number of surgeries per patients was 3.2, with 61% of the patients having had 3-7 surgeries[42].

Only one small study is available on the quality of life of patients with MEN1[42]. According to the Life Orientation Test used for assessing optimism, 70% of the MEN1 patients were pessimists[42]. When comparing short form 36 (SF-36) results of the MEN1 patients with gender- and age-matched population based controls, patients with MEN1 reported significantly lower levels of General Health and Social Functioning[42].

Ethical considerations are also important in the treatment of patients with the MEN1 syndrome, such as decisions around prenatal and preimplantation diagnosis of MEN1. Also important in this respect is the identification and screening of family members at risk of MEN1. How to advance when family members do not want to be screened for the mutation, families are not on speaking terms, when one hears of the existence of illegitimate children and what to do when parents don't want to subject their children to periodical screening. What is the extent of the responsibility of the physician and how far can you go? No easy answers exists. But it is important to be aware that these questions might arise if treating patients with MEN1.

Conclusion and challenges for the future

In conclusion we state that the care for MEN1 patients is complex and should be provided by a centre of expertise. With the endocrinologist as primary caregiver, all important decisions should be made in a regular meeting of a multidisciplinary team, comprising of an endocrinologist, endocrine surgeon, radiologist, specialist nuclear medicine and paediatrician; if necessary expanded with a neurosurgeon, (radiation) oncologist, pathologist and clinical geneticist.

Early genetic diagnosis and subsequent periodic screening are important pillars of the care for MEN1 patients. For primary hyperparathyroidism and neuroendocrine tumours surgery is the most important treatment modality, whereas watchful waiting and medical therapy play more important roles in MEN1-associated pituitary and adrenal adenomas.

The endocrine manifestations of MEN1 cannot be viewed upon as coinciding sporadic tumours. The differences in epidemiology and pathology between MEN1-related tumours and their sporadic counterparts show that a unique approach is needed. Therefore solid clinical research on MEN1 is necessary in order to come to evidence based guidelines for the care of these patients.

At the start of the twenty-first century we see new challenges in the care for MEN1 patients. Through sound epidemiologic research we need to reliably establish if a genotype-phenotype correlation exists and what other genes might play a role in the MEN1 phenotype. This may lead to individualized patient-care taking into account genetic profiles and the expression of the disease in individual patients. The most important challenge in imaging will be the early detection and subsequent follow-up of duodenopancreatic neuroendocrine tumours.

In therapy, the key-words for the twenty-first century will be individualized and targeted (medical and surgical) therapy. The use of medical therapy in pHPT needs to be further investigated in randomised trials. For pNET the value of medical therapy, possibly in an early stage of the disease, needs to be investigated in randomised trials. The usefulness of minimal invasive surgical techniques and radiologically guided therapy needs to be studied and subsequently tested in clinical trials.

Because MEN1 is a rare syndrome, it is often difficult to perform sound epidemiologic research and to conduct (randomised) clinical trials. Still, this research is necessary to take the care for MEN1 patients into the next century. Therefore, national (between institutions) and international collaboration of MEN1 research is of utmost importance.

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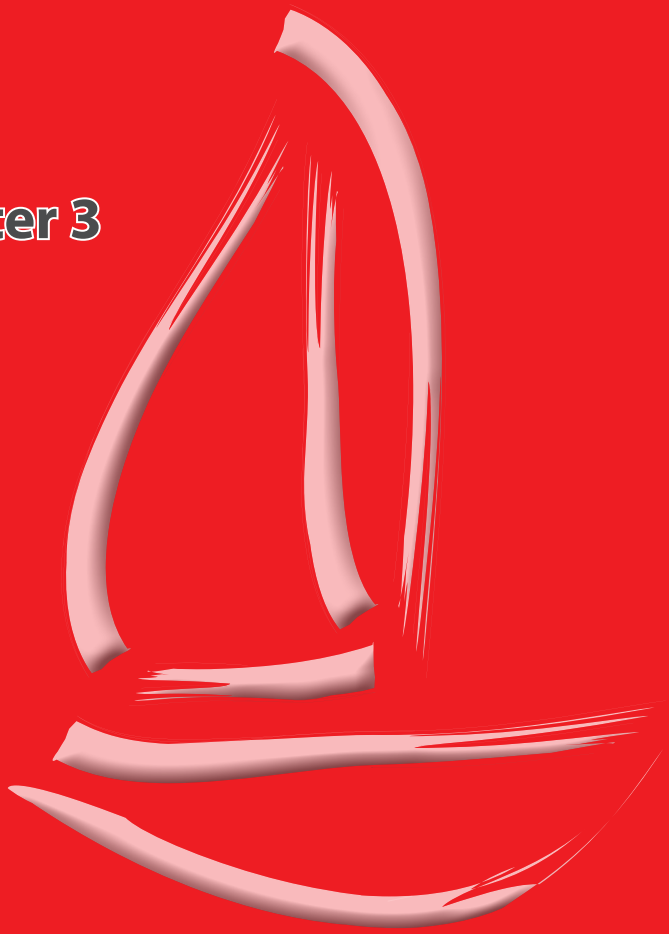
Chapter 3



Multiple endocrine neoplasia type 1 (MEN1): its manifestations and effect of genetic screening on clinical outcome

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Multiple endocrine neoplasia type 1 (MEN1): its manifestations and effect of genetic screening on clinical outcome

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Abstract

Objective

Effect of genetic screening on outcome in multiple endocrine neoplasia type 1 (MEN1) remains unclear. Expression of MEN1 is described using currently available diagnostic techniques. Manifestations and outcome are compared in patients diagnosed because of clinical expression with those diagnosed by genetic screening.

Design

Retrospective cohort study. Patients are divided into two groups: patients with a (1) clinical MEN1 diagnosis and (2) MEN1 diagnosis by genetic screening.

Patients and measurements

Demographic and clinical data were collected on MEN1 patients treated in the UMCU up to January 1st 2008. Results of mutation analysis were obtained from the Department of Medical Genetics.

Results

A total of 74 patients was included (median follow-up 5.5 year); 78% had hyperparathyroidism, 46% a pancreatic neuroendocrine tumour (NET), 38% a pituitary abnormality, 8% a NET of other origin and 16% an adrenal adenoma at the end of follow-up. Of the patients 18% had no manifestation. All five MEN1-related tumours were seen as first manifestation. Compared with patients identified by genetic screening, patients with a clinical MEN1 diagnosis had significantly more manifestations at diagnosis ($P<0.001$) and at end of follow-up ($P=0.002$). Eleven of 30 patients with a genetic MEN1 diagnosis (mean age at diagnosis 30.0 years) already had manifestations at diagnosis. No malignancy or death was seen in genetically diagnosed patients.

Conclusions

MEN1 is a syndrome with high morbidity. Genetic diagnosis is associated with less morbidity at diagnosis and at follow-up. Early genetic diagnosis might, therefore, lead to improvement of long-term outcome.

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal inherited disorder. Patients suffer from multiple tumours originating in different endocrine organs, sometimes even from a very young age [1]. This syndrome is characterised by the combined occurrence of (I) parathyroid hyperplasia or adenomas (II) neuroendocrine tumours of the pancreas (pancreatic endocrine tumour PET; III) neuroendocrine tumours (NET) of the gastro-intestinal tract, thymus or bronchus (IV) pituitary abnormalities and (V) adrenal hyperplasia or adenomas. Lipomas, leimyomas and skin disorders such as angiofibromas and collagenomas are also frequently seen. The reported prevalence of the principal manifestations among MEN1 patients varies[2-5]. In the Netherlands the prevalence of MEN1 syndrome is estimated to be 2-3/100.000[6]. MEN1 is caused by a germline mutation in the *MEN1* gene on chromosome 11, which is inherited in an autosomal dominant fashion[7]. Germline mutations are mutations in the DNA of germ cells and, with embryonic development, will be found in all cells of the body. The mutation is carried on from generation to generation. The *MEN1* gene, that was identified in 1997, encodes for the tumour suppressor protein menin[7]. This protein is involved in the regulation of cell proliferation. Absence of the menin protein leads to development of tumours. Almost everyone with a mutation in the *MEN1* gene will eventually develop the clinical MEN1 syndrome[1]. The clinical definition of MEN1, as is used in the Netherlands, is the combined occurrence of three out of the five manifestations mentioned above[6]. In a MEN1 family patients are considered to be affected if they have one out of five manifestations combined with MEN1 in a first degree relative. DNA-analysis is a powerful tool in the diagnosis of MEN1 syndrome. In a previously published Dutch study a *MEN1* mutation was found in all patients who fulfilled the clinical criteria for MEN1[8]. When the mutation is known in a MEN1 family, genetic (presymptomatic) screening of the family members is an option. Chances of having the MEN1 syndrome are 60% in patients from the general population if one of the following criteria is met: (I) age under 35 and one of the five principal tumours (II) more than one MEN1-associated lesion in one organ (III) two of the five principal tumours[8]. If a patient meets one of these criteria *MEN1* mutation analysis is justified.

Signs and symptoms of MEN1 occur as a consequence of overproduction of hormones, local mass effects or malignancy. Most MEN1-associated tumours are benign, but especially the NETs of the pancreas, the gastro-intestinal tract, the thymus and the bronchus might become malignant. MEN1 patients have a decreased life expectancy compared with the general population[9-11]. Today, malignancy – mostly from PETs – is the main MEN1 related cause of death[9, 10]. If a familial MEN1 syndrome is present, early genetic diagnosis subsequently followed by periodical monitoring for tumour manifestations if a mutation is confirmed, might lead to a better life expectancy and a better quality of life[11]. One small study in MEN1 patients showed a tendency towards better outcome in patients diag-

nosed by genetic screening[12]. However, it still remains unclear whether genetic screening does lead to a better outcome.
The aim of this study is to describe the sequence of manifestations in a Dutch MEN1 cohort using currently available diagnostic techniques. Second, to compare the prevalence of MEN1 manifestations in patients diagnosed clinically with those diagnosed by genetic screening. We assessed if presymptomatic screening for a *MEN1* mutation can identify patients before manifestations have occurred and if genetic screening leads to a better outcome at follow-up. The spectrum of manifestations within each identified mutation was also studied.

Patients and measurements
The hospital diagnosis registries (using data from 1978 to 2007) of the University Medical Center Utrecht for patients with MEN1 were used to identify the patients. All patients >16 years with a definite MEN1 diagnosis were included. Clinical and demographical information was collected from included patients and the medical records were reviewed for patient demographics, presence and timing of MEN1 related manifestations and death. The patients were divided into two groups. Group I consisted of patients with a clinical MEN1 diagnosis and group II consisted of patients diagnosed by genetic screening. Patients diagnosed by combined clinical and genetic findings were included in group I.
A clinical diagnosis of MEN1 was made when clinical criteria were met or when a patient had one of the five principal manifestations in combination with a positive family history. A genetic diagnosis was made if a patient was diagnosed by presymptomatic genetic screening. When the diagnosis of MEN1 was suspected, based on two of the five principal manifestations, in a patient with a negative family history which was subsequently genetically confirmed, it was considered to be a combined clinical and genetic diagnosis.
The protocol for periodical screening of MEN1 patients used, was based on the consensus guidelines published by Brandi *et al.* in 2001[13]. (Table 1) Before 2001 the usual care for all MEN 1 patients was in line with this protocol and based on the experience of the individual caregivers. Identical periodical screening was carried out in all MEN1 patients according to this protocol, regardless of the manner of diagnosis (clinical or genetic diagnosis).
Hyperparathyroidism was defined as the first reported episode of abnormal laboratory investigations (calcium, PTH).
A PET was confirmed when it was visible on imaging studies [magnetic resonance imaging (MRI) examination, CT-scan, endoscopic ultrasound (EUS) and/or somatostatin receptor scintigraphy (SRS)]. Functionality of PETs was determined by relevant laboratory investigations. Results of secretin tests were not included because reference values were lacking.
A pituitary abnormality was confirmed when this was identified on MRI-examination. Functionality was determined by laboratory investigations (PRL and IGF-1).
An adrenal tumour was identified using imaging studies (MRI-examination, CT-

Table 1. Protocol for periodical screening of MEN1 patients*

	Starting age	Frequency	Content
visit outpatients clinic	5 years	biannually	History and physical examination
laboratory investigations	5 years	biannually	Ionised calcium, chloride, phosphate, PTH, fasting glucose, fasting insulin, fasting c-peptide, glucagon, fasting gastrin, pancreatic polypeptide, PRL, IGF-1, platelet serotonin, chromogranin A.
imaging studies	15 years	every 2 years	MRI of upper abdomen MRI of pituitary (iv. contrast with gadolinium) MRI of mediastinum (in males)

* MEN1 patients, *MEN1* gene germline mutation carriers and MEN1 suspected patients without a confirmed mutation are eligible for periodical clinical monitoring.
MRI magnetic resonance imaging; iv. intravenous

scan or SRS) to show an adrenal lesion. Functionality of adrenal tumours (Cushing, pheocromocytoma, Conn) was determined by relevant laboratory investigations. NET of the gastro-intestinal tract, thymus or bronchus was identified when laboratory investigations (5-hydroxyindoleacetic acid and/or platelet serotonin) and imaging studies (MRI, CT-scan and/or SRS) were abnormal and the diagnosis was confirmed by biopsy.

Indications for interventions and subsequent interventions (e.g. medical and/or surgical) once a manifestation was diagnosed, did not differ between the patients diagnosed clinically and genetically.

Genetic analysis for MEN1 was initially performed by linkage analysis, using highly polymorphic DNA-markers near the *MEN1* locus on chromosome 11q13. Following the identification of the *MEN1* gene itself in 1997, direct mutation testing by DNA sequencing has become available. With direct mutation analysis the base-sequence of the *MEN1* gene in a symptomatic patient is determined and compared to a normal reference sequence. In this way the presence or absence of a specific mutation can be determined. Next to sequencing, large deletions or duplications in the *MEN1* gene are also sought for by using the multiplex ligation-dependent probe amplification assay.

The follow-up of the patients included in this study was part of regular medical care. The approaches described in this paper did not involve any randomization, experimental intervention or questionnaire and the anonymity of patients was not breached. As this article meets the conditions required under Dutch Law (WGBO) for making medical and/or personal data available for statistical or other scientific research, the Medical Ethics Review Committee of the University Medical Center Utrecht concluded that the Medical Research Involving Human Subjects Act (WMO) is not applicable.

Statistical analysis

SPSS 13.0 (SPSS Inc., Chicago, IL.) was used for statistical analyses. In describing the study population and outcomes the mean ± SD or median (IQR) was calculated, depending on the normal distribution. Differences between these variables were determined with respectively the student's *t*-test or the Mann-Whitney *U*-test. To detect significant differences in the number of manifestations between the two groups Pearson's χ^2 test was used. Differences were considered significant if $P<0.05$.

Results

Study population

According to the hospital registries 100 patients were initially identified. However, in 22 patients the diagnosis of MEN1 was uncertain and therefore they were excluded from the present study. These patients had attended the MEN1 screening program for several years because of a positive family history, but they neither developed any clinical manifestation, nor was mutation analysis performed. On four patients insufficient information was available and, therefore, they were also excluded from the present study. Subsequently, 74 patients were included in whom all tests and imaging procedures were performed.

General characteristics of the patients are shown in table 2. The study population consisted of 74 patients from 21 different MEN1 families. The median follow-up was 5.5 years (IQR 2.25-12.0; range 0-31). Forty-three (58%) patients were diag-

Table 2. Patient characteristics

Characteristics	Group I (*n*=43) Clinical MEN1 diagnosis	Group II (*n*=30) Genetic MEN1 diagnosis	Total (*n*=74)*
Sex M/ F (%)	22/21 (51/49)	12/18 (40/60)	35/39 (47/53)
Mean age at diagnosis years ± SD (range)†	34±14 (11-64)	30±14 (16-67)	32±13 (10-64)
Median year of diagnosis	1994	2002	1998
Mean age at end follow-up years ± SD (range)‡	47±14 (26-77)	36±14 (16-67)	42±15 (16-77)
Median follow-up yrs (IQR;range)§	11 (4.0-17.0; 0-31)	3 (2.0-6.0; 0-13)	5.5(2.25-12.0; 0-31)
Death/ alive	10/33	0/30	10/64
Death MEN1 related			
Yes	5		5
No	0		0
Unknown	5		5
Mean age death ± SD (range)	52±16 (26-72)	Not applicable	52±16 (26-72)

*In one patient the diagnostic method could not be determined; †age at diagnosis was not different between group I and II ($P=0.151$); ‡not including diseased patients; age at the end of follow-up differed significantly between group I and II ($P<0.0001$); §follow-up differed significantly between group I and II ($P=0.001$)

Table 3. Prevalence of manifestations in the study population

	Group I (*n*=43) Clinical MEN1 diagnosis		Group II (*n*=30) Genetic MEN1 diagnosis		Total (*n*=74)*	
Manifestation	n (%)	Mean age at diagnosis ±SD (range)	n (%)	Mean age at diagnosis ±SD (range)	n (%)	Mean age at diagnosis ±SD (range)
HPT	41 (95)	32±10 (11-61)	16 (53)	33±14 (14-65)	58 (78)	32±11 (11-65)
HPT first	31		13		44	
PET	28 (65)	38±15 (13-68)	6 (20)	30±13 (15-47)	34 (46)	37±15 (13-68)
Gastrin	14		3		17	
Insulin	4		0		4	
Glucagon	7		0		7	
VIP	2		0		2	
PP	9		3		12	
GHRH	1		0		1	
NF	6		2		8	
PET first	12		4		16	
PIT	21 (49)	35±14 (12-69)	7 (23)	31±13 (15-52)	28 (38)	34±14 (12-69)
Prolactin	10		4		14	
GH	4		0		4	
ACTH	1		0		1	
NF	8		4		13	
PIT first	6		4		10	
NET	6 (14)	40±9 (29-50)	0 (0)	NA	6 (8)	40±9 (29-50)
Stomach	1				1	
Bronchus	1				1	
Thymus	3				3	
Unknown	1				1	
NET first	2		0		2	
ADR	10 (23)	48±11 (30-62)	1 (3)	NA	12 (16)	48±11 (30-62)
NF	10		1		12	
ADR first	1		1		2	
Patients with no clinical expression	0	NA	13 (43)	NA	13 (18)	NA

*In one patient the diagnostic methods could not be determined.
ACTH corticotrophin; *ADR* adrenal adenoma; *GH* growth hormone; *GHRH* growth hormone releasing hormone; *HPT* hyperparathyroidism; *NA* not applicable; *NET* neuroendocrine tumour; *NF* non functioning; *PET* pancreatic endocrine tumour; *PIT* pituitary abnormality; P*P* pancreatic polypeptide; *VIP* vasoactive intestinal peptide.

nosed clinically (including 6 patients with a combined clinical and genetic diagnosis) and 30 (41%) patients were diagnosed by genetic screening. Of one patient the diagnostic method could not be determined.

Clinical manifestations

There were a median of 2 (IQR 1-3; range 0-5) prevalent manifestations in the study population. The prevalence of each individual manifestation is shown in

Table 3. Thirteen *MEN1* mutation carriers have not (yet) shown any clinical manifestation of the syndrome. The median duration of follow-up in this group was 2.5 years (IQR 0.5-4.75; range 0-5) and the mean age at the end of follow-up was 32 ± 13 years (range 16-66). In the 61 patients who have clinical expression of the syndrome, the first manifestation was primary hyperparathyroidism in 44 (72%) patients, a PET in 16 (26%) patients, a pituitary abnormality in 10 (16%) patients, a NET of the gastro-intestinal tract, thymus or bronchus in 2 (3%) patients and an adrenal adenoma in 2 (3%) patients. There are 11 patients in whom more than one manifestation occurred simultaneously at the time of the first manifestation. The mean age at first manifestation was 32 ± 13 years (range 11-68). In 16 (22%) patients the first manifestation occurred before MEN1 was diagnosed. The median time interval between the occurrence of the first manifestation and subsequent MEN1 diagnosis was 9.5 years (IQR 3.25-18.25; range 1-36).

Clinical versus genetic diagnosis

General characteristics of groups I and II are shown in Table 2. The mean age at MEN1 diagnosis was 34±14 years for clinically diagnosed patients and 30±14 for genetically diagnosed patients (*P*=0.151). Duration of follow-up was significantly (*P*=0.001) shorter in genetically diagnosed patients than in clinically diagnosed patients [median 11 years (IQR 4-17.0; range 0-31) *vs* 3 years (IQR 2.0-6.0; range 0-13) respectively]. The prevalence of manifestations at the end of follow-up in both groups is shown in Table 3.

The prevalence of manifestations at the time of MEN1 diagnosis in both groups is shown in Table 4. When comparing the number of manifestations at diagnosis between the two groups, significantly more manifestations were observed in patients diagnosed clinically ($P < 0.001$). One of the families in this study (previously reported by Dreijerink *et al.*) has shown a very low penetrance of the MEN1 syndrome[14]. Only two of the ten *MEN1* mutation carriers show clinical expression

Table 4. Prevalence of manifestations at the time of MEN1 diagnosis

No. of manifestations at MEN1 diagnosis	Group I 'clinical MEN1 diagnosis' (*n*=43) *n* (%)	Group II 'genetic MEN1 diagnosis (*n*=30) *n* (%)
None	0 (0)	19 (63)
1	17 (40)	6 (20)
2	11 (26)	5 (17)
3	3 (7)	0 (0)
4	2 (5)	0 (0)
5	1 (2)	0 (0)
unknown	9 (21)	0 (0)

Number (No.) of manifestations differs significantly between both groups (*P*<0.001)

Table 5. Prevalence of manifestations at the end of follow-up*

No. of manifestations at the end of FUP	Group I 'clinical MEN1 diagnosis' (*n*=18)† n (%)	Group II 'genetic MEN1 diagnosis' (*n*=30)‡ n (%)
None	0 (0)	13 (43)
1	2 (11)	8 (27)
2	5 (28)	5 (17)
3	6 (33)	4 (13)
4	3 (17)	0 (0)
5	1 (6)	0 (0)
unknown	1 (6)	0 (0)

Number (No.) of manifestations differs significantly between both groups (*P*=0.002)
*in patients with year of diagnosis ≥ 1994; †median follow-up: 5 years (range: 0-11); ‡median follow-up (FUP): 3 years (range 0-13); follow-up differed significantly between the two groups (*P*<0.0001)

of the syndrome and in all cases, except the index case, the diagnosis was made genetically. Because this family could bias the results, we also made the comparison without this particular family. In that case there were also significantly more prevalent manifestations at diagnosis in patients diagnosed clinically ($P < 0.001$). We also calculated the number of manifestations at the end of follow-up in each group (Table 5). To create more comparable groups only those patients diagnosed clinically in or after 1994 have been included in this analysis. Patients diagnosed genetically had fewer manifestations at the end of follow-up (*P*=0.002). When again excluding the aforementioned family, the difference between clinically and genetically diagnosed patients was still significant (*P*=0.036).

Malignancy and mortality

In the study population ten patients (14%) suffer(ed) from malignant NETs as defined by the presence of metastases. Six patients had a PET, three patients a NET of the thymus and one patient a NET of unknown origin. These patients were all diagnosed clinically.

The time of PET diagnosis ranged from 4 months before - 205 months after MEN1 diagnosis. Three patients already had metastases at the time of PET diagnosis, in the other three they developed after 20, 31 and 45 months, respectively. Three patients have died because of tumour progression (FU after diagnosis of metastases ranged from 39-65 months). The other patients are alive with disease 4, 32 and 61 months after the diagnosis of metastases.

The diagnosis of a NET of the thymus was made at initial MEN1 diagnosis in one patient and 12 and 371 months before MEN1 diagnosis in the other two patients. One patient had already metastases at NET diagnosis, in the other two metastases

developed after 51 and 239 months. All patients have died, one because of tumour progression. Of the other two the cause of death could not be determined. The follow-up after the diagnosis of metastases ranged from 28 to115 months. One patient had a metastasised NET of unknown origin at the time of MEN1 diagnosis. This patient died 2 months after the diagnosis from tumour progression. Three patients without malignant disease died from unknown causes. All were diagnosed clinically.

Clinical spectrum within mutations

The clinical spectrum within each mutation is shown in table 6. We only depict manifestations of which three or more representatives were found in our population. The results show that within each mutation there is a wide clinical spectrum. This goes for both the type of manifestations and for the number of manifestations in each individual patient. When comparing the different mutations, patients with the 357del4 mutation tend to have more PETs than patients with other mutations. In patients with the IVS3-6 (c>g) mutation, manifestations are generally few – eight patients have no clinical expression of the syndrome – and when manifestations occured no PETs or NETs were observed.

Table 6. Clinical spectrum for each mutation

	357del4	IVS3-6 (c>g)	Lys120del	Lys362Stop	IVS7+5 (g>a)	Ala390Val	1178ins8
Patients *(n)*	22	10	10	6	4	4	3
Manifestations							
HPT *(n)*	20	1	9	4	4	2	3
PET *(n)*	17	0	3	2	1	1	1
PIT *(n)*	9	1	5	0	2	1	1
ADR *(n)*	2	1	1	1	0	0	0
NET *(n)*	1	0	1	0	0	0	0
Manifestations per patient							
0 *(n)*	0	8	1	2	0	2	0
1 *(n)*	1	1	3	1	2	1	2
2 *(n)*	12	1	3	3	1	0	0
3 *(n)*	8	0	2	0	1	1	1
4 *(n)*	0	0	1	0	0	0	0
5 *(n)*	0	0	0	0	0	0	0
Unknown *(n)*	1	-	-	-	-	-	-

Only mutations with three or more representatives in our population are depicted.
ADR adrenal adenoma; *HPT* hyperparathyroidism; *NET* neuroendocrine tumour; *PIT* pituitary abnormality; *PET* pancreatic endocrine tumour.

Discussion

The prevalence of manifestations in our cohort using currently available diagnostic techniques is high. For hyperparathyroidism, PETs and pituitary adenomas, these prevalences are lower than those reported in recent literature[2, 12, 15-17]. This can be explained by the fact that in our population 18% of the patients have not (yet) developed any clinical manifestation of the MEN1 syndrome. When these patients are not included in the analysis we find the same prevalence as recent literature. The prevalences of other NETs and adrenal adenomas did not differ from recent literature[2, 12, 15-17].

We found that by presymptomatic screening for a *MEN1* mutation patients can be identified before manifestations occur. However, in our cohort, 11 of the 30 genetically diagnosed patients already harboured manifestations at the time of diagnosis. This leads us to the conclusion that screening for a *MEN1* mutation should be done at an early age. In our cohort the mean age at diagnosis did not differ (P=0.151) between clinically diagnosed patients (34 years) and genetically diagnosed patients (30 years). Recent guidelines recommend screening for a mutation at the age of five, because the earliest manifestation of MEN1 described in literature occurred at that age[13, 18]. In our population, which consisted of patients aged 16 years and older, the earliest clinical manifestation occurred at the age of 11 years.

Patients in our study population who were diagnosed genetically had a better outcome than those diagnosed clinically. Fewer manifestations were seen at the end of follow-up in the genetically diagnosed group. Malignancy and death only occurred in the clinically diagnosed group. These results should, however, be viewed with caution. The number of patients in each group is relatively small. In addition, although we compared patients in both groups diagnosed in the same time frame, temporal influences cannot be ruled out. The results can possibly be explained by the fact that patients diagnosed genetically are diagnosed earlier (lead-time bias). However, the age at diagnosis was comparable in both groups, and at the end of follow-up, comparisons were made only in patients diagnosed from 1994 onward. Longer term follow-up is needed to see if the effects we found are sustained. Still, we observed a better outcome and this corroborates the results of Lourenco-Jr *et al.* who also found a trend towards fewer and less aggressive manifestations in a genetically diagnosed subgroup compared with subgroups of index cases and clinically diagnosed cases[12]. Viewed together one might speculate that earlier intervention in patients with a genetic MEN1 diagnosis could play a role in the observed better outcome.

Asymptomatic *MEN1* mutation carriers should be periodically screened for tumour manifestations, possibly leading to diagnosis in an early stage, which would prevent complications. The same holds true for patients with a positive family history who refuse DNA analysis. In the older studies it was recommended that screening for hyperparathyroidism was enough because in virtually all cases this

was the first manifestation[19, 20]. We have now shown that all manifestations can occur as the first manifestation. Hyperparathyroidism as first manifestation is still most prevalent (72%), however a NET of the pancreas (26%) or pituitary adenoma (16%) were also frequently seen as the first manifestation. Even a NET of other origin (3%) and an adrenal adenoma (3%) occurred as first manifestation. This confirms the findings of a recent German MEN1 study, in which gastroenteropancreatic endocrine tumours and pituitary tumours were frequently seen as the first manifestation and adrenal adenomas and NETs of lung and thymus were also seen as first manifestation[17]. In genetically diagnosed patients all manifestations, with exception of NETs of thymus, bronchus or GI-tract, occurred as first manifestation. We have also shown that the clinical spectrum within a single mutation is wide. Though we observed fewer manifestations in patients with the IVS3-6(c>g) mutation and more PETs in patients with the 357del4 mutation, the number of patients is too small to draw conclusions from this observation. Reports regarding genotype-phenotype correlation in MEN1 vary. In contrast to specific genotype-phenotype correlations recent studies do show a relationship between type and location of mutations and clinical expression of the MEN1 syndrome[16, 17, 21]. How these results translate into clinical practice needs to be determined in future research.

In conclusion, Multiple Endocrine Neoplasia type 1 (MEN1) is a syndrome with a high morbidity. Early genetic diagnosis is recommended to identify patients before manifestations occur and to improve long-term outcome. In all asymptomatic MEN1 patients, screening for manifestations should include screening for all five MEN1-related tumours. For all MEN1 patients, regardless of the manner of diagnosis, we recommend periodical screening according to protocol. Once a manifestation is identified, we advise a tighter follow-up scheme with appropriate and early interventions (medical and/or surgical) according to current standards of care.

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Part II

Surgical strategy in MEN1 related hyperparathyroidism

Chapter 4

The optimal surgical treatment for primary hyperparathyroidism in MEN1 patients: a systematic review

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The optimal surgical treatment for primary hyperparathyroidism in MEN1 patients: a systematic review

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Abstract

Objective

The optimal surgical approach for patients with primary hyperparathyroidism (pHPT) and Multiple Endocrine Neoplasia type 1 (MEN1) is controversial. We sought to determine the optimal type of surgery for pHPT in MEN1.

Methods

We collected data on clinical presentation, surgery and follow-up of MEN1 patients with pHPT at the University Medical Center Utrecht and affiliated hospitals between 1967 and 2008. Furthermore, we performed a systematic review of the literature and meta-analysis. Surgical procedures were classified into less than subtotal (<SPTX) versus subtotal (SPTX) and total parathyroidectomy (TPTX).

Results

Fifty-two patients underwent primary surgery for pHPT, of which 29 had <SPTX, 17 SPTX, and 6 TPTX. Recurrent pHPT was most frequent after SPTX (65%) followed by <SPTX (59%). Persistent disease was most frequent after <SPTX (31%). Time to recurrence was 61 months longer after SPTX than after <SPTX. Although recurrent pHPT was not seen after TPTX, permanent hypoparathyroidism developed in 67% of these patients. The meta-analysis showed that after SPTX and TPTX, patients had the lowest risk of persistent and recurrent pHPT. TPTX had the highest risk of permanent hypoparathyroidism. Large non-comparative studies showed low recurrence rate after SPTX and TPTX.

Conclusion

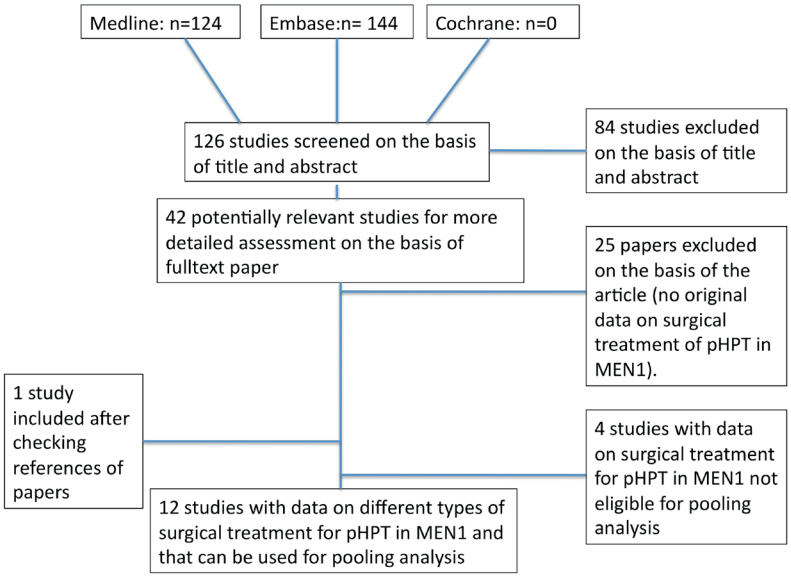
We believe that SPTX is the best surgical therapy for pHPT in MEN1. MEN1 patients with pHPT should not be treated with <SPTX because of the unacceptable high rate of recurrent and persistent pHPT. Additionally, a thymectomy should routinely be performed in these patients.

Introduction

The Multiple Endocrine Neoplasia syndrome type 1 (MEN1) is a rare autosomal dominant inherited disorder. MEN1 is caused by a germline mutation in the *MEN1* gene on chromosome 11. The prevalence is estimated to be 2-3/100,000[1]. Patients with MEN1 are prone to developing endocrinopathies. These endocrinopathies are primary hyperparathyroidism (pHPT), pancreatic endocrine tumours (PETs), and pituitary adenomas. Other less frequent manifestations are adrenocortical adenomas and neuroendocrine tumours of the stomach, thymus, and bronchus. Hyperparathyroidism is the most prevalent manifestation occuring in 78-90% of MEN1 patients[2, 3]. It is often the first presentation of the MEN1 syndrome[1].These patients tend to be younger than patients with sporadic pHPT. Most commonly, pHPT presents during the second and third decade[1, 4]. pHPT can be both asymptomatic and symptomatic. Signs and symptoms may consist of bone abnormalities, mental changes, weakness, nefrolithiasis, and marked hypercalcaemia[4]. MEN1 related pHPT tends to be more aggressive than sporadic pHPT and usually manifests as multiglandular disease[5].

The treatment for pHPT is primarily surgical. The goals for surgery are restoring calcium levels to normal permanently, while preventing hypoparathyroidism and minimizing the number of reoperations[4,6]. Parathyroidectomy reduces the risk of kidney stones, fractures (improved bone mineral density), and potential cardiovascular morbidity. It may even also improve quality of life. In MEN1 patients who also have a gastrinoma, parathyroidectomy may reduce the gastrin production[7]. Controversy exists on the optimal surgical strategy. Most authors have advocated

Figure 1. Flowchart of selection of articles screened and included in meta-analysis



subtotal (SPTX; resection of 3-3 ½ parathyroid glands) or total parathyroidectomy (TPTX, resection of 4 glands) with autotransplantation[4, 8, 9]. There is a high risk of recurrence after surgical intervention, even after extensive surgery. The downside of extensive surgery is the increased risk of permanent hypoparathyroidism (hypocalcaemia) and recurrent laryngeal nerve injury. Acute hypoparathyroidism may cause mild to severe neuromuscular symptoms ranging from neuromuscular irritability to seizures. Yet, less extensive surgery yields a higher risk of recurrent disease requiring reintervention, which increases the risk of complications even more.

To the best of our knowledge, there is no published systematic review with a meta-analysis of the results reported in the current available literature that compares different surgical therapies with respect to recurrent and persistent pHPT and permanent hypoparathyroidism.

The aim of this study was to determine the optimal surgical therapy for pHPT in patients with MEN1. To this end, we evaluated our experience with surgical treatment in these patients and carried out a meta-analysis.

Methods

From the MEN1 database at the University Medical Center Utrecht (UMCU), in the Netherlands, patients diagnosed with pHPT between 1967 and 2008 were selected. Patients were included in the MEN1 database if they had genetically proven MEN1, or three out of five manifestations of MEN1 or one out of five manifestations and a first-degree family member. The medical records of these patients were reviewed. Since the UMCU is a tertiary referral center, patients who were initially treated at other institutions and later referred to our institution were included.

The diagnosis of pHPT was confirmed when serum calcium or ionized calcium levels were elevated in combination with raised or inadequately suppressed PTH levels (normal reference value: 1.0-7.0 mmol/L). We have chosen to use ionized calcium levels (normal reference value: 1.15-1.32 mmol/L) in our analysis, as this became the standard parameter measured routinely since 1993 in our hospital. Before 1993 we used serum calcium levels to diagnose hyperparathyroidism.

Operative technique

Throughout the years, our operative strategy has evolved. In the past, only subtotal or total parathyroidectomy with autotransplantation were performed. In subtotal parathyroidectomy (SPTX), 3 – 3 ½ parathyroid glands were resected during a bilateral cervical exploration after identification of all parathyroid glands. In total parathyroidectomy (TPTX), 4 glands were resected. In TPTX one (partial) gland was used as a graft for autotransplantation into the brachioradial muscle of the nondominant forearm. The autotransplantation was performed during the

same operation, using fresh parathyroid tissue. The resected gland with the least abnormal macroscopic appearance (size, color, and vascularisation) was used for the transplant. The gland was cut into multiple small fragments a millimeter in size. Minimally invasive parathyroidectomy (MIP) has been performed in selected cases in order to reduce the morbidity of surgery and postponing more radical surgery. A MIP would be performed only if the affected parathyroid gland could be localized preoperatively with identical location of an enlarged gland on both ultrasound and sestamibi scan. During the procedure, intraoperative PTH measurement was carried out after the affected parathyroid gland was resected to determine if PTH levels had decreased ≥ 50% as a measure of success. If PTH levels did not decrease sufficiently, conversion to a bilateral cervical exploration would follow.

We did not routinely perform thymectomy. Our standard policy was to perform a thymectomy only if uncertainty remained whether the inferior parathyroid glands had been completely removed. We classified the surgical strategy based on operation reports and medical records.

Surgical outcome was defined as the initial success after surgery, persistence and recurrence rate, and complications particularly permanent hypoparathyroidism. Surgical cure was defined as a normalization of serum (ionized) calcium levels and PTH for a period of at least six months after the surgical procedure. If not, it was classified as persistent pHPT. Permanent hypoparathyroidism was defined as hypocalcaemia persisting beyond the first six months after surgery and requiring suplementation with calcium and an active form of vitamin D. Both duration of follow-up and time to recurrence were calculated in months. The duration of follow-up was defined as the time between the first surgical procedure and the date of final contact with the patient.

Meta-analysis

We searched Medline (1966-2008), Embase (1980-2008), the Cochrane database of systematic reviews, and the Cochrane Central Controlled Trials Register (2008) using predefined search terms (Appendix A). On the basis of title and abstract, we selected 126 studies. Only studies that compared different surgical treatments of pHPT were included (*n*=12; Fig. 1). We also included the results of the present study. Pooling was done for the following groups: patients who underwent resection of fewer than 3 parathyroid glands (<SPTX), 3-3 ½ parathyroid glands (SPTX), or all parathyroid glands (TPTX) including autotransplantation. We studied outcome of surgery for the different groups. Outcome was defined as the risk of persistent pHPT, recurrent pHPT, and permanent hypoparathyroidism. To determine the risk of recurrent pHPT, patients who had persistent pHPT were excluded. We performed a two-step meta-analysis. First, we studied if fewer than 3 parathyroid glands resected (<SPTX) or 3 or more glands resected (SPTX/TPTX) had a better outcome. Second, we compared SPTX to TPTX. The odds-ratio (OR), the 95% confi-

Table 1. Patient characteristics of pHPT in MEN 1 (*n*=54)

	Surgical treatment				
Patient characteristics	<SPTX	SPTX	TPTX	Overall	*P*-value
Age (years) [a]	35 (±12)	32 (±11)	29 (±8)	34 ± 11	ns
Preoperative Ca^{++} mmol/l	1.40 (±0.07)	1.39 (± 0.08)	1.45 (± 0.07)	1.40 ± 0.07	ns
Preoperative PTH mmol/l	8.60 (±4.18)	7.60 (±3.28)	9.16 (±4.14)	8.40 ± 3.90	ns

Data are given in mean ± standard deviation
[a] Mean age at the time of surgery
Ca^{++} mean ionized calcium levels, *PTH* Parathyroid Hormone levels
<SPTX less than 3 parathyroid glands resected , *SPTX* subtotal parathyroidectomy with 3 – 3 ½ parathyroid glands resected, *TPTX* total parathyroidectomy, *ns* not significant

Table 2. Overview of surgical treatment in patients with pHPT and MEN1 (*n*=52), postoperative laboratory results, and complications per surgical treatment.

	< SPTX	SPTX	TPTX	Overall	*P*
Number of procedures	29[a]	17[a]	6	52	
Postoperative Ca^{++} (mmol/l)	1.25 (± 0.12)	1.13 (±0.13)	1.07 (±0.76)	1.19 ±0.14[b]	0.011
Postoperative PTH (mmol/l)	5.70 (±4.26)	2.97 (± 1.72)	2.35 (±2.99)	4.42 ±3.70[c]	0.070
Complications					
Hypoparathyroidism					0.003
Transient	0	3 (18%)[e]	1 (17%)	4 (8%)	
Permanent[d]	2(7%)[e]	4 (24%)[e]	4 (67%)	10 (19%)	
Unknown duration	1 (3%)[e]	0	0	1 (2%)	
Recurrent laryngeal nerve injury					ns
Transient	1 (3%)	1 (6%)	-	2 (4%)	
Permanent	-	1 (6%)	-	1 (2%)	

[a]8 out of the 29 <SPTX underwent MIP (minimally invasive parathyroidectomy). Two patients underwent delayed conversion to bilateral neck exploration. One of the patients in the SPTX group underwent a MIP, but was immediately converted to a SPTX because of an insufficient drop in PTH (<50%).
[b]Overall preoperative Ca^{++} levels are significantly higher than postoperative Ca^{++} levels, *P*=0.0001.
[c]Overall preoperative PTH levels are significantly higher than postoperative PTH levels, *P*=0.002.
[d]Permanent hypoparathyroidism is defined as a duration of hypoparathyroidism of 6 months or longer.
[e]Of the patients with hypoparathyroidism, 3 of 4 after <SPTX developed recurrent pHPT and 4 of 6 after SPTX 3-3 ½ developed recurrent disease.

Ca^{++} ionized calcium levels, *PTH* parathyroid hormone levels, *<SPTX* less than 3 parathyroid glands resected, *SPTX* subtotal parathyroidectomy with 3 – 3 ½ parathyroid glands resected, *TPTX* total parathyroidectomy

dence interval (95% CI), and *P*-value were calculated. The I^2 test was used to check for quantative heterogeneity. This test measures the proportion of inconsistency between studies that cannot be explained by chance alone. Additionally, we included the results of the non-comparative studies, which could not be used in the meta-analysis, in the "Results" section.

Statistical analysis

SPSS 16.0 (SPSS Inc, Chicago, Il) was used for statistical analysis. Review manager 5.0 software (Cochrane Collaboration) was used for the meta-analysis. Depending on distribution, numerical data are depicted as mean ± standard deviation (SD) or median with its interquartile range (IQR). Presented percentages are calculated on the basis of the available data. When appropriate, the χ^2-test was used for statistical analysis of the data. To determine if the surgical procedure was associated with recurrent disease, univariate analysis was performed. For this purpose, patients were divided into 3 groups: <SPTX (less than 3 glands resected), SPTX (3 – 3 ½ glands resected, and TPTX (4 glands resected).

Results

From the MEN1 database at our institution, 54 patients with pHPT were identified. Twenty-one were men (39%). Ten patients were asymptomatic. Forty-one patients (76%) had one or more of the following symptoms: nefrolithiasis, mood disorders, fatigue and gastro-intestinal complaints. Baseline characteristics are given in Table 1.

Fifty-two patients underwent primary surgery either at our hospital *(n*=36) or another hospital *(n*=16). Two other patients did not undergo surgical treatment. Eight patients underwent a MIP, 21 underwent <SPTX, 17 SPTX and 6 TPTX. Three times, during an initial MIP, PTH levels remained elevated and the surgical procedure was converted to a conventional bilateral neck exploration with a TPTX either in the same session or a (few) day(s) later due to logistical reasons. An overview of the surgical procedures is given in Table 2. After the primary surgery, ten patients (19%) developed permanent hypoparathyroidism. In most cases, this occurred after TPTX. One patient had a transient recurrent laryngeal nerve injury and another patient had a laryngeal nerve injury of an unknown duration.

Eleven patients (21%) developed hypercalcaemia within 6 months, indicating persistent disease. Twenty-eight patients (54%) developed a recurrence after a median of 121 months (IQR 47-201). Time to recurrence was 61 months shorter in patients who underwent <SPTX (93 months) than in patients who underwent a SPTX (154 months), although this was not significant. None of the patients treated with TPTX had a recurrence. One patient did develop persistent disease after TPTX. Outcomes of surgical treatment for persistent and recurrent disease in follow-up are given in Table 3. Thirty-one percent of the patients with <SPTX had persistent disease. In addition, 59% of patients with <SPTX, developed recurrent

Table 3. Outcome of surgical treatment for pHPT in MEN1: cure, persistent and recurrent pHPT.

	< SPTX	SPTX	TPTX	Overall	*P*-value
Follow-up (months) [median (IQR)]	99 (44-162)	144 (71-207)	16 (4-236)	121 (47-201)	ns
Cure (%)	20 (69%)	13 (93%)	5 (83%)	38 (73%)	ns
Persistent pHPT[a]	9 (31%)	1 (7%)	1 (17%)	11 (22%)	ns
Recurrent pHPT	17 (59%)[b]	11 (65%)	-	28 (54%)	0.010
Time to recurrence (months; range)	93 (44-164)	154 (46-207)	-	127 (34-194)	0.088
No. pts who underwent reintervention (%)	16 (55%)	8 (47%)	1		
Reintervention number				25 (48%)	ns
1				16(31%)	
2				3 (6%)	
3				5 (9%)	
7				1 (2%)	
Second reoperation (No. glands resected)					
0	2	1	0		
1	7	6	1		
2	5	0	0		
3	1	0	0		
Autotransplantation	7	4	-		
Unknown	1	1			
Final TPTX status	6	6			
Outcome second reoperation					
Permanent hypoparathyrioidsm	2	2	0		
Recurrent pHPT	5	5			

IQR interquartile range, *<SPTX* fewer than 3 parathyroid glands resected, *SPTX* subtotal parathyroidectomy with 3 – 3 ½ parathyroid glands resected, *TPTX* total parathyroidectomy, *ns* not significant

[a] Hypercalciemia within six months after surgical intervention

[b] All 8 patients who underwent a MIP developed recurrent pHPT

disease. After SPTX, one patient (7%) had persistent pHPT, but 65% developed recurrent disease after a median of 13 years. Although none of the six patients with TPTX developed recurrent pHPT, one patient had persistent pHPT. Patients who had TPTX had the highest risk of permanent hypoparathyroidism (67%) compared to those who had <SPTX (7%) and SPTX (25%), (*P*=0.003; Table 2).

We performed 25 operations for persistent or recurrent pHPT. The number of reoperations per patient ranged from one to seven. During the second operation, one parathyroid gland was resected in 14 patients, 2 glands in 5 patients, and 3 glands in 1 patient. No parathyroid glands could be identified in 3 patients. After secondary surgery, calcium levels initially normalized in 19 patients (76%). Three patients developed hypoparathyroidism, two of whom had permanent hypoparathyroidism. None of the patients who developed hypoparathyroidism after the first operation or after reoperation has had a re-autotransplantation.

No reintervention was performed in 13 patients with persistent or recurrent disease. The patient who developed 8 episodes of recurrence underwent 7 re-interventions in both the cervical region and the arm where the autotransplant graft was located. She now suffers from permanent unilateral laryngeal nerve injury. She was the only patient in our series who had supernumery glands. Median follow-up was 121 months (IQR = 47-201). At the end of follow-up nine patients had died, six because of metastasized neuroendocrine tumors, one because of metastasized melanoma and two of an unknown cause.

Meta-analysis & Systematic Review

Twelve studies were included in our meta-analysis. First we compared patients who had undergone <SPTX with patients who had undergone SPTX or TPTX. After <SPTX, patients had a significantly higher risk of developing recurrent and persistent pHPT than did patients with SPTX or TPTX. The odds ratio for recurrent pHPT was 3.11 (95% CI = 2.00-4.84) for patients who underwent <SPTX (Fig. 2A). Patients with <SPTX had a significantly lower risk of developing permanent hypoparathyroidism (OR 0.24, 95% CI = 0.24-0.48; Fig. 2B) and a higher risk of persistent pHPT (Appendix C1). Second, we compared patients who had SPTX to patients who had TPTX. Patients with SPTX did not have a significantly higher risk of developing recurrent pHPT than patients with TPTX (OR = 2.15, 95% CI = 0.82-5.61, *P*=0.12; Fig. 3A). Neither did they have a higher risk of developing persistent pHPT (Appendix C2). After SPTX, patients had a significantly lower risk of permanent hypoparathyroidism than after TPTX (OR 0.25, 95%, CI = 0.11-0.54, *P*=0.0004; Fig. 3B). The I^2 test revealed moderate heterogeneity between the study outcomes on recurrent pHPT (percentage of total variation across the studies not due to chance alone was 38 and 46%). There was no heterogeneity for study outcomes with respect to permanent hypoparathyroidism. An overview of the surgical outcomes from studies that were included in the meta-analysis and those that could not be included is given in Appendix B.

Most noncomparative studies had large study populations[8-12]. The overall recurrence rates are difficult to compare because of different techniques used in the studies. One series showed an overall recurrence rate of 7.6% after SPTX (*n*=66) with bilateral thymectomy and <SPTX (<3 glands)[10]. Another series of 100 patients had a failure rate of 26% after <SPTX (*n*=37) and a combined failure rate of 11% after SPTX (*n*=43) and TPTX (*n*=11) after a follow-up period of 4.6 years[11]. A series of 51 patients who underwent 45 TPTX with thymectomy had only five recurrences; all in the autografts after 6.7 years of follow-up. The rate of permanent hypoparathyroidism in that series was 22%.

One large series including only patients who underwent reoperations for pHPT in MEN1 had a recurrence rate of 27% after 72 months; only 2 of 75 patients (3%) had permanent recurrent laryngeal nerve injury after their reoperation[12].

Figure 2A. Comparison of <SPTX versus SPTX/TPTX for recurrent pHPT

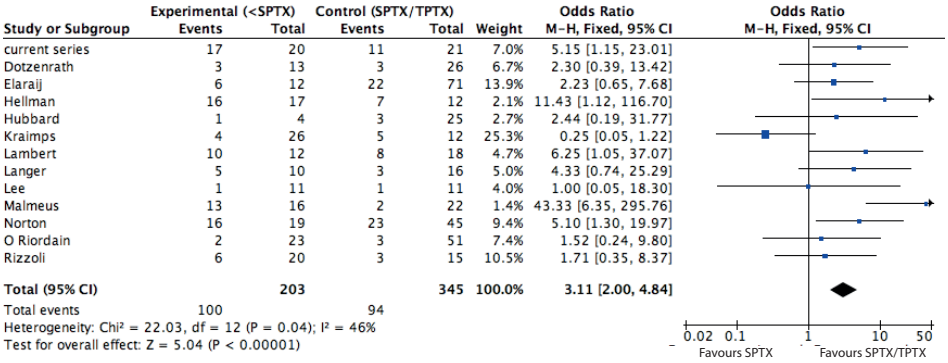


Figure 2B. Comparison of <SPTX versus SPTX/TPTX for permanent hypoparathyroidism

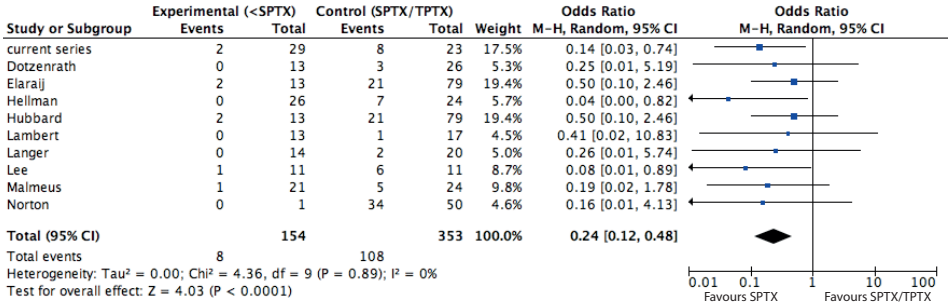


Fig. 2A. After <SPTX , patients are 3.11 times more likely to develop recurrent disease than after SPTX/TPTX (95% CI = 2.00-4.84, p<0.0001). Patients who had persistent pHPT were excluded from this analysis.
Fig. 2B. After <SPTX patients have a significantly lower risk of developing permanent hypoparathyroidism than after SPTX/TPTX (OR 0.24 95% CI 0.12-0.48, p<0.0001).

<SPTX less than 3 parathyroid glands resected; *SPTX* parathyroid glands resected; *TPTX* total parathyroidectomy with autotransplantation; SPTX and TPTX are analyzed together.

Discussion

This study confirms the difficulties of managing primary hyperparathyroidism in MEN1 patients. Contradictory findings on the surgical treatment have been reported. In our study, recurrent pHPT was most frequently seen after SPTX, yet the follow-up was long compared to other series (Appendix B). Even though none of the patients developed recurrent pHPT after TPTX, the majority developed permanent hypoparathyroidism (67%). Persistent disease was most frequently seen after <SPTX. These results and those of the meta-analysis and systematic review confirm that <SPTX should not be performed because it has the highest risk of persistent and recurrent disease.We propose that subtotal parathyroidectomy (SPTX) is the preferred treatment for pHPT in MEN1.

Some caution must be taken when interpreting the data presented here. There were no randomized controlled trials and most series were retrospective. Furthermore, the duration of follow-up was different in the studies. To evaluate permanent hypoparathyroidism only a few studies were available. The main limitation of our study is its retrospective nature. Some data were unavailable and there may have been confounding by indication for the type of surgery. Unfortunately, the results of the large non-comparative studies could not be included; they could have had a significant effect in the meta-analysis. An international randomized controlled trial with a long-term follow-up is necessary to answer some of the remaining questions. Nonetheless, such a study design would be very problematic to accomplish in view of the rarity of this disease.

Most authors agree that pHPT in MEN1 is caused by multiglandular disease[6, 9, 12-15]. The theory that MEN1-related pHPT develops asymmetrically and in time all parathyroid glands can become hyperplastic or develop adenomas is based on

Figure 3A. Comparison of SPTX versus TPTX on recurrent pHPT

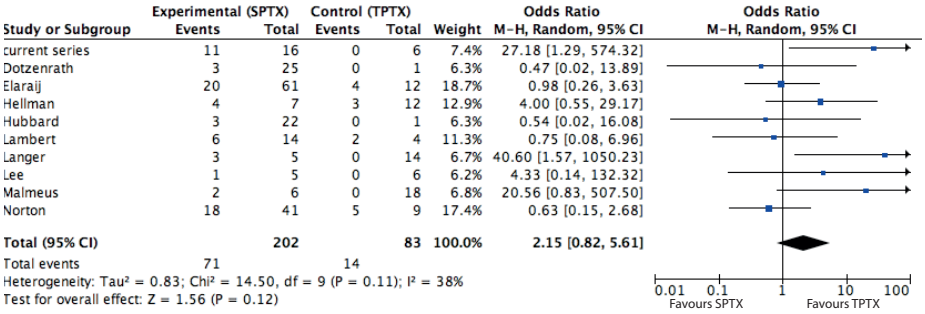


Figure 3B. Comparison of SPTX versus TPTX for permanent hypoparathyroidism

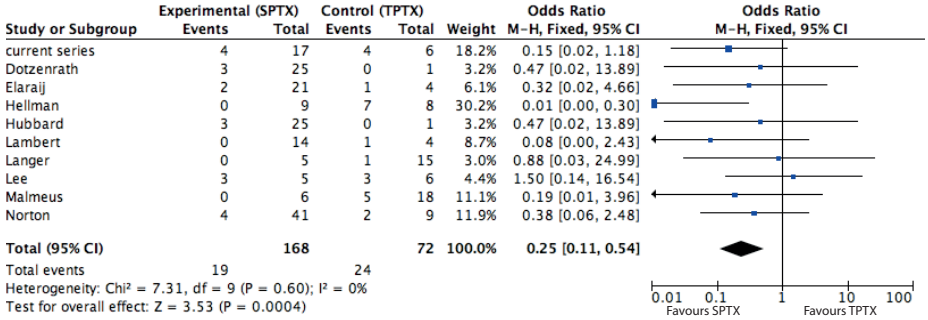


Fig. 3A. After SPTX, patients do not have a significantly higher risk of developing recurrent pHPT than after TPTX (OR 2.15 95% CI=0.82-5.61, p=0.12). Patients who had persistent pHPT have been excluded from this analysis.

Fig. 3B. After SPTX, patients have a significantly lower risk of developing permanent hypoparathyroidism than after TPTX (OR 0.25 95% CI=0.11-0.54, p=0.0004).

SPTX 3-3 ½ parathyroid glands resected; *TPTX* total parathyroidectomy with autotransplantation

the observation that there is a high risk of developing recurrent pHPT when limited resections are performed[5, 6]. Even parathyroid glands that appear normal can have a diffuse positive staining reaction for parathyroid hormone[16]. Most authors therefore recommend SPTX or TPTX [5, 8, 13, 15, 17, 18]. SPTX is generally considered to be superior to TPTX because the risk of permanent hypoparathyroidism is much lower. However, there might be an increased risk of recurrence compared to TPTX since some parathyroid tissue remains in site. The controlled-cohort studies and case series that we identified in our systematic review of literature showed that the recurrence rate of pHPT was high after SPTX (3-3 ½), ranging from 12 to 67% after eight years of follow-up (Appendix B). The noncontrolled series had large study populations and more favourable outcomes with respect to recurrence rates for SPTX and TPTX, but follow-up rates were shorter than in our series. In our series, the recurrence rate was 65% after a long median follow-up of 12 years. This risk of recurrence increases with time and does not seem to reach a plateau phase[8]. After total parathyroidectomy with autotransplantation, recurrence rates of 4-55% have been reported[8, 15, 18-20, 17]. The higher recurrence rate after SPTX in our series may be explained by the longer follow-up period than in other (non-comparative) series and the fact that in our series a cervical thymectomy was seldom performed.

The risk of severe and permanent hypoparathyroidism is highest after TPTX with autotransplantation. It ranges from 13 to 47% in the literature and was 67% in our series. After subtotal parathyroidectomy, this risk ranges from 0 to 22%[15, 18]. Patients with <SPTX are the least likely to develop permanent hypoparathyroidism[13]. These findings are confirmed by our meta-analysis. If patients with permanent hypocalcaemia require lifelong vitamin D and calcium supplements, this may well affect their quality of life. If left untreated, longstanding hypoparathyroidism may lead to extrapyramidal disorders (Parkinson's disease and dementia), skin disorders and cataract. These risks have to be balanced against the benefits of curing the patient by performing extensive parathyroid resections. Especially, since the time to develop recurrent disease after SPTX is long and a reoperation is often required only after several years.

Some authors have found uniglandular disease in MEN1[21, 22]. In a small number of our patients, we had performed minimally invasive parathyroidectomy (MIP) to reduce complications and mainly at the patient's request. This practice has been abandoned after poor results. In our experience and others', the recurrence rate after MIP was unacceptably high: 100%[6, 23]. Additionally, there is a higher risk of persistent disease after <SPTX, which was 31% in our series[13]. However, some authors have had better results with <SPTX, with cure rates of approximately 70% after 48 months[21] and 100% after 5 years[22].

The possibility of supernumerary parathyroid glands should also be considered in the surgical treatment of pHPT in patients with MEN1[24, 25]. Although some authors have not found supernumerary glands[9], these may occur in up to 30% of MEN1 patients (Appendix B). The superior parathyroid glands are frequently located wit-

hin the fascial sheeth of the thyroid. Therefore, this fascia should be removed to localise the parathyroid glands. If the fascial sheeth is not removed, subcapsular parathyroid glands may be overlooked [26]. Not only supernumerary glands, but ectopic ones as well can be found, in up to 33%[27]. These ectopic glands most often are located in the thymus[9]. Some authors advocate that during cervical exploration in these patients, a routine thymectomy should be performed to search for supernumerary and ectopic glands[3, 5, 6, 9, 10, 13, 17, 28]. After SPTX with thymectomy, calcium levels return to normal more often (84.1%) than after SPTX without transcervical thymectomy (57.6%, $P = 0.0001$)[29]. In our series thymectomy was rarely performed. Some authors even recommend removal of the fatty tissue in the central compartment to remove all (ectopic) parathyroid tissue[18].

Finally, one large case series studying reoperations for pHPT in MEN1 found a recurrent laryngeal nerve injury rate of 2% and a permanent hypoparathyroidism rate of 16%[12]. In our series only one patient, who underwent 7 reoperations, developed permanent recurrent laryngeal nerve injury.

Conclusion

Given the currently available evidence, limited resections of fewer than 3 glands should not be part of a primary operation for pHPT in MEN1 patients, since the persistence and recurrence rates are too high. SPTX might be the best surgical treatment for patients who have MEN1 and pHPT, despite the higher risk of recurrent pHPT. In addition, a thymectomy should routinely be performed in these patients.

Acknowledgements

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Appendix A. predefined search terms

MEN 1

"MEN 1" OR "MEN1" OR "MENI" OR "MEN I" OR "multiple endocrine neoplasia type 1" OR "multiple endocrine neoplasia syndrome type 1"

Primary hyperparathyroidism

"Hyperparathyroidism, Primary"[Mesh] OR "Parathyroid Neoplasms"[Mesh] OR "primary hyperparathyroidism" OR "hyperparathyroidism" OR "HPT" OR "parathyroid adenoma*" OR "parathyroid hyperplasia"

Surgical procedure

"Parathyroidectomy"[Mesh] OR "parathyroid surgery" OR "parathyroidectomy" OR "total parathyroidectomy" OR "subtotal parathyroidectomy" OR "conventional neck exploration" OR "unilateral neck exploration" OR "minimally invasive adenomectomy"

Appendix B. Summary of outcome of primary surgical treatment of pHPT in MEN1 patient in literature.

Study *Author year* *Study type* *No. MEN1-pHPT*	***Type of Surgery***		***Outcome of surgery***
		Persistent pHPT	*Recurrent pHPT*
Included in pooling analysis			
Dotzenrath 2001 Retrospective cohort study *n*=38	<SPTX *n*=13 SPTX *n*=25		*n*=3 *n*=3
Elaraj 2003 Retrospective cohort study *n*=92	<SPTX *n*=13 SPTX *n*=63 TPTX *n*=16		*n*=6 (46%) *n*=20 (61%) *n*=4 (16%)
Hellman 1998 Retrospective cohort study *n*=50	<SPTX *n*=26 SPTX *n*=9 TPTX *n*=15	n=9 (35%) *n*=2 (22%) *n*=0	*n*=16 (61%) *n*=4 (44%) *n*=3 (20%)
Hubbard 2006 Retrospective cohort study *n*=28	<SPTX *n*=4 SPTX *n*=21 TPTX *n*=4		*n*=1 *n*=1 *n*=2
Kraimps 1992 Retrospective case series *n*=38 MEN1 *n*=4 MEN2A	<SPTX *n*=26 SPTX *n*=14	*n*=0 n=2	*n*=4 *n*=5
Lambert 2005 Retrospective cohort study *n*=37	<SPTX *n*=16 SPTX *n*=16 TPTX 5		*n*=12 (92%) *n*=6 (43%) *n*=2 (50%)
Langer 2004 cohort study *n*=34	<SPTX *n*=14 SPTX *n*=5 TPTX *n*=15		
Lee 2005 Retrospective cohort study *n*=22	<SPTX *n*=11 SPTX *n*=5 TPTX n=6		*n*=0 *n*=1 *n*=0
Malmeus 1986 Retrospective cohort study *n*=39	<SPTX *n*=21 SPTX *n*=6 TPTX n=3	*n*=5 *n*=0 *n*=0	*n*=13 *n*=2 *n*=0
Norton 2008 Prospective cohort study *n*= 84	<SPTX *n*=35 SPTX n=40 TPTX *n*=9	*n*=15 (44%) *n*=5 (12%) *n*=0	*n*=16 (47%) *n*=18 (44%) *n*=5 (55%)
Riordain 1992 Retrospective cohort study *n*=84	<SPTX *n*=30 SPTX *n*=54	*n*=5 *n*=0	*n*=2 *n*=3
Rizzoli 1985 Retrospective cohort study *n*=61	<SPTX *n*=41 SPTX/TPTX *n*=20	*n*=21 (66%) *n*=2 (25%)	*n*=7 *n*=3

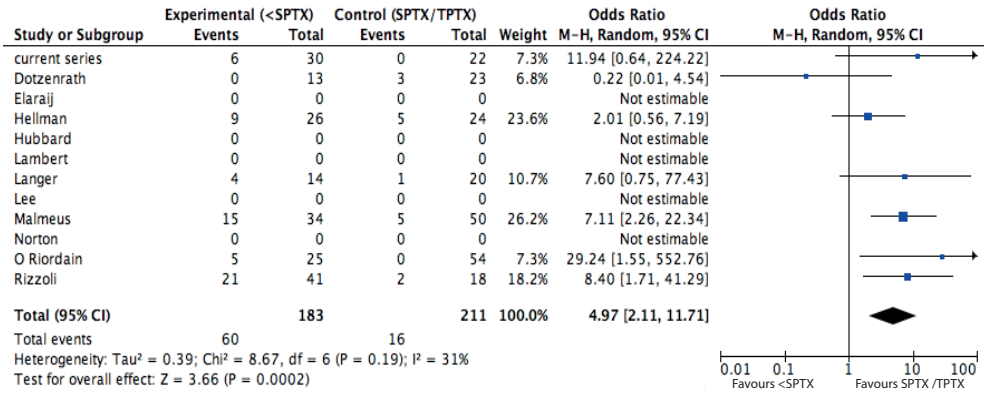
	FUP Median (range) or Mean ± SD	***Comments***
Permanent hypoparathyroidism		
n=2 *n*=3	54 (12-180) mo	Supernumerary glands *n*=2
15% 26% 46%	<SPTX 5.3 y SPTX/TPTX 6.1 y	Significant difference in time to recurrence between SPTX/TPTX and <SPTX Thymectomy *n*=79 (86%)
n=0 *n*=0 *n*=15 (100%)	8.2 ± 3.9 y 9.1 ± 3.9 y 5.2 ± 2.8 y	Recovery from hypoparathyroidism after 1-7 years.
n=0 *n*=2 *n*=1	152 (8-285) mo 62 (8-192) mo 167 (18-226) mo	
Overall *n*=4 (10%)	8 (1-46) y	Outcome MEN1 and MEN2 patients analysed together. Only 4 MEN2 pts included; none developed recurrent pHPT Supernumerary glands 13% Ectopic glands in 33%
n=1	4.0 y, FUP in *n*=13 4.6 y, FUP in *n*=14 4.6 y, FUP in *n*=4	Thymectomy 4/16 Thymectomy 5/16 Thymectomy 3/5 Supernumerary glands *n*=1 Ectopic glands n=8
	132 (6-240) mo 151 (84-264) mo 36 (6-192) mo	Supernumerary glands *n*=2 Ectopic glands *n*=2
n=1 *n*=3 *n*=3	7.0 (0.5-19.5) y 6.9 (1.5-15.5) y 7.7 (2-11.5) y	
n=1 *n*=0 *n*=3	Overall 6.5 (1-14) y	
n=1 (2%) *n*=4 (10%) *n*=2 (22%)	20.7 (± 1.9) y 14.5 (± 1.5 y 9.9 (± 1.5) y	Patients have pHPT and Zollinger-Ellison Syndrome
Overall *n*=7 (8%)	Overall 6.7 (2.5-11.1) y	Supernumerary glands *n*=6
	7.8 (1.3-12) y	

(Appendix B continued)

Not included in Pooling Analysis			
Arnalsteen 2002 Retrospective cohort study *n*=83	<SPTX *n*=13 SPTX *n*=66		Reoperation *n*=4 Reoperation *n*=5 Overall recurrence 7.6%
Burgess 1998 Retrospective case series *n*=37	SPTX *n*=37	*n*=3 (8%)	*n*=7 (19%)
Cougard 1994 Retrospective case series n=100	<SPTX *n*=37 SPTX *n*=43 TPTX *n*=11		Overall 'failure' 21.8% <SPTX 26% SPTX/TPTX 11%
Kivlen 2001 Retrospective case series *n*= 75 recurrent pHPT + reoperation	Median sternotomy 3 (%) Autograft resection *n*=12 (13%) Procedures for perstistent pHPT *n*=39 (42%) Procedures for recurrent pHPT *n*=55 (58%)	*n*=17 (27%)	
Tonelli 2007 Retrospective case series *n*=51	TPTX + thymectomy n=45	*n*=0	*n*=5 (autograft recurrences)

FUP follow-up mo months *SPTX* subtotal parathyroidectomy *TPTX* total parathyroidectomy y years

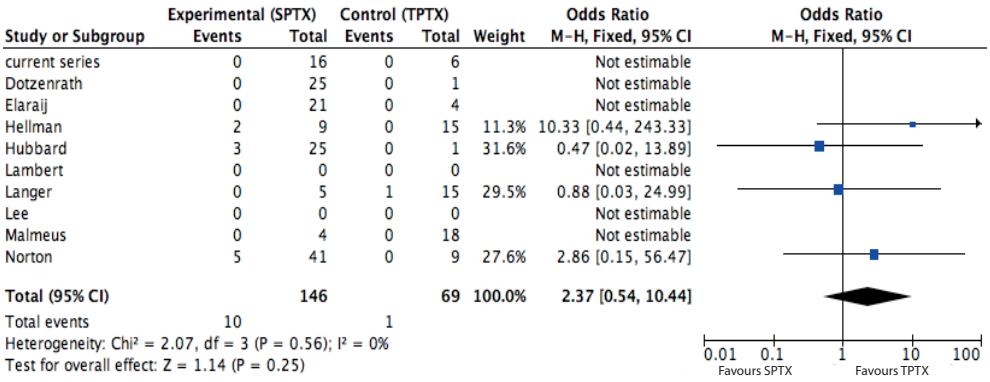
Appendix C1. Comparison of <SPTX versus SPTX/TPTX on persistent pHPT



App. C1. After <SPTX, patients are more likely to develop persistent pHPT than after SPTX/TPTX (OR 4.97 95% CI=2.11-11.71, *P*=0.0002).
<*SPTX* fewer than 3 parathyroid glands resected; *SPTX* 3-3 ½ parathyroid glands resected; *TPTX* total parathyroidectomy with autotransplantation.

	37 (± 34) mo 50 (± 54) mo no significant difference in FUP	SPTX: thymectomy *n*=55 Supernumerary glands *n*= 24 (30%) Reason exclusion pooling analysis: unclear recurrence rate.
13%		
n=9 (24%)	8 y	Overall recurrence rate after 8 y 67%
	4.6 y	Ectopic glands in 31% Thymectomy *n*=41
	Median 72 mo	Permanent RLN injury <1982 *n*=2 >1982 *n*=0 Mediastinal exploration *n*=1 Horner syndrome *n*=1 bleeding *n*=2 chylus fistula *n*=1 arrhythmia
n=10 (22%)	80 (± 62) mo	

Appendix C2. Comparison of SPTX versus TPTX for persistent pHPT



App. C2. After SPTX, patients do not have a significantly higher risk of developing persistent pHPT than after TPTX (OR 2.37 95% CI=0.54-10.44, *P*=0.25).
SPTX 3-3 ½ parathyroid glands resected; *TPTX* total parathyroidectomy with autotransplantation.

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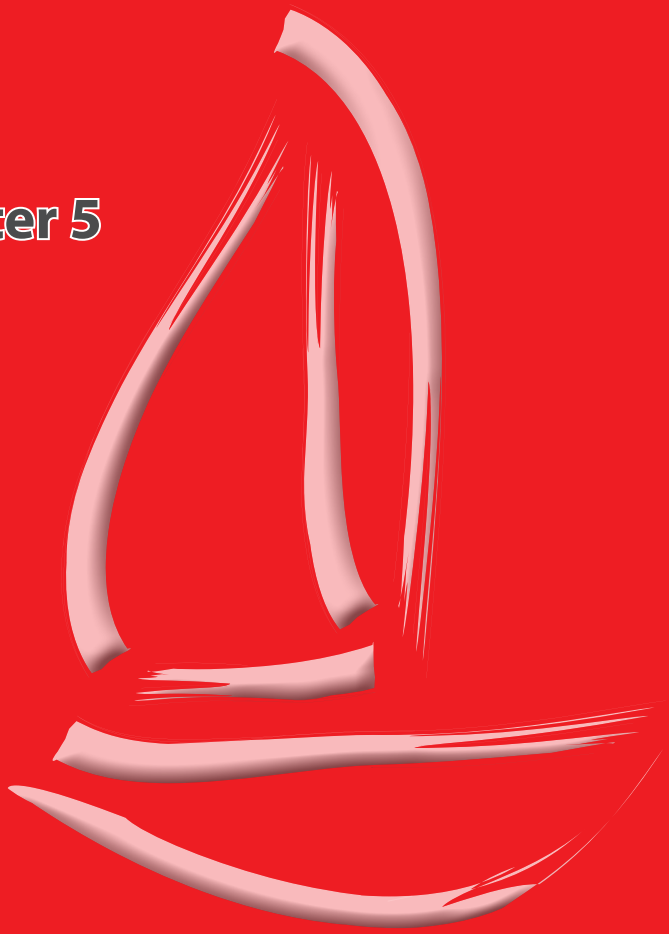
Chapter 5



Primary hyperparathyroidism in MEN1 patients: a cohort study with longterm follow-up on preferred surgical procedure and the relation with genotype

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Primary hyperparathyroidism in MEN1 patients: a cohort study with longterm follow-up on preferred surgical procedure and the relation with genotype

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On behalf of the DutchMEN1 Study Group

Abstract

Objective

To indentify the optimal surgical strategy for Multiple Endocrine Neoplasia Type 1 (MEN1) related primary hyperparathyroidism (pHPT). To describe the course of postoperative hypoparathyroidism and to assess whether genotype is associated with persistent/recurrent pHPT.

Summary background data

Surgery is the preferred treatment in MEN1-related pHPT, but the surgical procedure of choice is still uncertain.

Methods

This retrospective cohort study was performed at the Departments of Endocrinology of the University Medical Centers of Utrecht and Nijmegen, the Netherlands. Patients were selected from the Dutch MEN1 database, including all patients 16 years or older treated for MEN1 from 1990-2009. Data were collected by medical record review.

Results

Seventy-three patients underwent parathyroid surgery. Persistent/recurrent pHPT occurred in 53% after less than 3 parathyroids resected (<SPTX), 17% after subtotal resection (SPTX) and 19% after total resection with autotransplantation (TPTX). Persistent (≥6 months) postoperative hypoparathyroidism occurred in 24% after <SPTX, 39% after SPTX and 66% after TPTX. Median duration of hypoparathyroidism was 1.5 years, in 65% successful cessation of vitamin D/calcium was possible, even after more than ten years. After <SPTX, patients with nonsense or frameshift mutations in exons 2, 9 and 10 had a significantly lower risk of persistent/recurrent pHPT than patients with other mutations. After SPTX/TPTX persistence/recurrence did not differ with genotype. After SPTX/TPTX persistence/recurrence was more frequent (P=0.07) in patients without bilateral transcervical thymectomy (TCT).

Conclusions

SPTX with bilateral TCT is the procedure of choice for MEN1-related pHPT. Genotype seems to affect the chance of recurrence. Postoperative hypoparathyroidism lasting six or more months should not be considered permanent in MEN1.

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is a rare autosomal dominant endocrine tumor syndrome, caused by mutations in the *MEN1* gene on chromosome 11q13, coding for the menin protein[1]. Mutations in the *MEN1* gene predispose patients to primary hyperparathyroidism (pHPT); neuroendocrine tumors (NET) of the pancreas and duodenum; pituitary tumors, gastric, bronchial and thymic neuroendocrine tumors, and adrenal adenomas. pHPT is the most prevalent MEN1 manifestation, affecting 78-94% of the patients [2-7], and it is often the first manifestation of the disease [4, 8].

Surgery is the preferred treatment and pHPT accounts for most of the MEN1-related surgeries [9-11]. Because of multiglandular involvement[12], leaving behind parathyroid tissue has a high risk of recurrent pHPT [13-26]. Secondary surgery is more challenging because of postoperative fibrosis, leading to more difficulty in identifying the parathyroid glands and a higher risk of complications such as recurrent laryngeal nerve injury and hypoparathyroidism [27, 28].

However, after more extensive parathyroidectomies in MEN1, postoperative hypoparathyroidism frequently occurs [13-26]. It can be very severe, especially shortly after the operation and sometimes intravenous (IV) calcium and intensive monitoring is necessary. Less severe cases of hypoparathyroidism can also be challenging to treat.

Which surgical strategy best combines long-term cure while preventing postoperative hypoparathyroidism is still a matter of debate. Apart from the surgical technique there are no known predictors for persistent postoperative hypoparathyroidism in MEN1 [13-26]. Surgical strategy is also the most important determinant of recurrent pHPT [13-26].

Up until now no association has been found between *MEN1* genotype and the presence or severity of pHPT [29-33]. A relatively small study has assessed time to recurrence and has found no relation with *MEN1* genotype [34]. It would be relevant if a higher risk of recurrence in a subset of patients can be based on the *MEN1* genotype, because such an association might lead to more individualized decision making.

Therefore the aims of the present study were (1) to identify the optimal surgical strategy for MEN1-related pHPT, while taking into account both long-term recurrence rate and hypoparathyroidism, (2) to describe the course of postoperative hypoparathyroidism in MEN1 patients, and (3) to assess whether genotype is associated with persistent/recurrent pHPT.

Methods

This cohort study was performed at the Departments of Endocrinology of the University Medical Centers of Utrecht (UMCU) and Nijmegen (RUNMC), both tertiary referral centers in the Netherlands. Patients were selected from the Dutch national MEN1 database. All MEN1 patients aged 16 years or more and treated between 1990 and present were included in this database. Patients were identified

by a standard identification procedure using the hospital's diagnosis databases. Patients were excluded if they did not have parathyroid surgery, parathyroidectomy was performed for any other reason than pHPT, surgery was not performed at the UMCU or RUNMC, surgery was performed before 1990, cinacalcet was used before surgery, and follow-up after surgery was less than six months.

Clinical and demographic data were retrospectively collected by medical record review, including the original surgery and pathology reports. Data were collected regarding demographics, mutation (according to Human Genome Variation Society nomenclature), preoperative characteristics, surgical procedures, results of pathological examination and postoperative follow-up. If a patient underwent more than one surgical procedure, these procedures were combined to one overall procedure, which was used in the analyses. The total number of parathyroids removed was based on the results of pathological examination. Patients were considered to have a subtotal parathyroidectomy (SPTX) if 3 or more parathyroids were removed, leaving one or a partial gland behind. Any lesser resection was considered less than SPTX (<SPTX). Patients were considered to have a total parathyroidectomy (TPTX) if all parathyroids were removed (≥4), usually combined with autotransplantation of fresh parathyroid tissue into the nondominant forearm or the sternocleidomastoid muscle.

pHPT was defined as a raised serum total calcium (reference values 2.20-2.60 mmol/L, RUNMC from April 2008 2.20-2.65 mmol/L) or serum ionized calcium (reference values RUNMC 1.10-1.30 mmol/L UMCU 1.15-1.32 mmol/L) combined with a raised or inadequately normal parathyroid hormone (PTH; reference values RUNMC 1.0-6.5 pmol/L; UMCU before 2001 <15 pmol/L, 2001-2006 1-6 pmol/L, >2006 1-7 pmol/L).

The primary outcomes were persistent hypoparathyroidism and persistent or recurrent pHPT. Persistent hypoparathyroidism was defined as active vitamin D and/or calcium therapy for six months or more. Duration of this therapy was also taken into account. Persistent hyperparathyroidism was defined as hypercalcaemia with a raised or inadequately normal PTH within six months after surgery. Recurrent pHPT was defined as hypercalcaemia with a raised or inadequately normal PTH after six months or more after surgery. Persistent and recurrent pHPT were taken together as one group in the analyses. Time to recurrence was also taken into account.

The secondary outcome was the severity of postoperative hypoparathyroidism, measured by the need for IV calcium. Gender, age at surgery (<40 or ≥40), decade of surgery (1990-1999 or 2000-2009), duration of preoperative hypercalcaemia (≤24 months or >24 months), level of preoperative serum calcium (<2.70, 2.70-2.90, >2.90) and PTH (raised or normal), type of surgery (<SPTX, SPTX, TPTX), need for IV calcium, level of postoperative serum calcium (>2.20, 2.00-2.20, <2.00) and PTH (undetectable or detectable) and difference in pre- and postoperative serum calcium (delta <0.5 or ≥0.5) were taken into account as predictors for the presence and duration of hypoparathyroidism.

To assess a possible genotype-phenotype correlation the mutation and type of mutation were taken into account as a predictor for persistent/recurrent hyperparathyroidism on the basis of a previously reported genotype-phenotype correlation in pancreatic NETs in MEN1 patients [35].
Time periods are described in quarters (three-month time periods) unless otherwise specified. Duration of follow-up is counted from the moment of surgery. If a patient underwent more than one surgical procedure, follow-up is counted from the last surgery.
The Medical Ethics Review Committee of both the University Medical Center Utrecht and of the Radboud University Nijmegen Medical Center gave permission for conducting the study.

Surgical technique
In both the UMCU and the RUNMC parathyroid surgery is performed by two experienced surgeons, performing more than 50 parathyroid procedures per center per year. In both centers, SPTX or TPTX with autotransplantation are the preferred procedures in MEN1 patients. Standard UMCU procedure was to perform a transcervical thymectomy (TCT) only if uncertainty remained whether the inferior parathyroid glands had been completely removed. At the RUNMC TCT was routinely performed with SPTX/TPTX.
Minimally invasive parathyroidectomy (MIP) has been performed in selected cases and if the patient was reluctant to undergo SPTX, in order to reduce the morbidity of surgery and to postpone more radical surgery. An MIP was only performed if the affected parathyroid gland could be localized preoperatively with identical location of an enlarged gland on both an ultrasound and a sestamibi scan. In selected cases, based on imaging results, unilateral cervical exploration has also been performed. Because of high recurrence rates, <SPTX has now been abandoned as procedure of choice for MEN1 patients.

Statistical analysis
The mean with standard deviation (SD) or median with interquartile range (IQR) was used in describing the study population and outcomes, depending on the normal distribution. Differences in distribution of continuous variables were determined with the student's *t*-test or the Mann-Whitney test depending on the normal distribution. Differences in distribution of categorical variables were determined with the Pearson's χ^2 test. The relation between categorical variables and the primary outcomes regarding postoperative course (hypoparathyroidism and persistent/recurrent pHPT) were analyzed using Kaplan-Meier survival analysis. Comparisons were made using the log rank test. For the continuous variable 'age' Cox regression analysis was used. Differences were considered significant if $P < 0.05$. SPSS 17.0 (SPSS Inc., Chicago, IL) was used for the statistical analysis.

Results

Study population

A total of 138 patients were identified from the Dutch national MEN1 database and 73 patients were included (Fig. 1) with a median follow-up after surgery of 17 quarters (4.3 yrs; IQR 7-26 quarters). In 70 (96%) patients the causative mutation in the *MEN1* gene was known, either because of direct genetic testing or because the mutation was found in a first-degree relative. Among these 70 patients, 20 different mutations were found (Table 1).

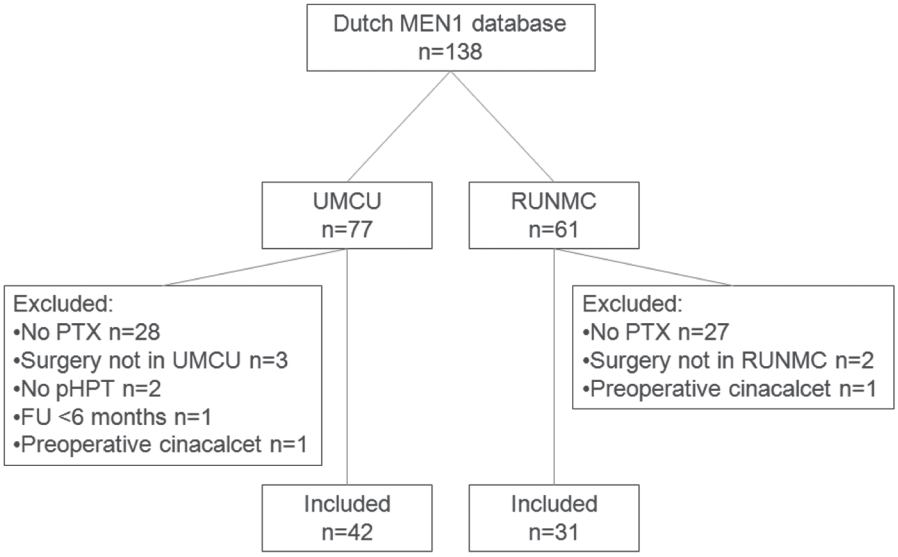
Surgery

For 42 (58%) patients the primary surgery was also the final surgery. Overall 119 surgical procedures were performed. The preoperative characteristics and the surgical procedures performed are described in Table 2. Patients with one surgical procedure had higher levels of preoperative calcium and PTH, compared to patients with multiple surgeries. Detailed information regarding the different surgical groups (<SPTX, SPTX, TPTX) is provided in Table 3.

Clinical-Pathological Correlation

We compared the number of parathyroids the surgeon expected to remove during the final surgery with the number of parathyroids confirmed at pathological examination. In 13% of the cases in which the surgeon expected to remove <3 parathyroids *(n*=40), pathological examination revealed less parathyroids than

Figure 1. The flowchart shows how the study population was determined.



FU follow-up; *MEN1* multiple endocrine neoplasia type 1; *pHPT* primary hyperparathyroidism; *PTX* parathyroidectomy; *RUNMC* Radboud University Medical Center Nijmegen; *UMCU* University Medical Center Utrecht.

Table 1. Overview Of Mutations In The Study Population*

Mutation	Location	Type	No. of patients
c.-110-?_669+?del (p.0?)	exon 1-3	deletion	1
c.249_252del (p.Ile85fs)	exon 2	frameshift	15
c.358_360del (p.Lys120del)	exon 2	in-frame deletion	9
c.122del (p.Leu41fs)	exon 2	frameshift	1
c.322C>T (p.Arg108X)	exon 2	nonsense	1
c.552G>T (p.Glu184Asp)	exon 3	missense	1
c.683T>C (p.Leu228Pro)	exon 4	missense	3
c.1024G>C (p.Ala342Pro)	exon 7	missense	1
c.1058T>A (p.Ile353Asn)	exon 7	missense	1
c.1099A>T (p.Lys367X)	exon 8	nonsense	5
c.1192C>T (p.Gln398X)	exon 8	nonsense	2
c.1100_1113dup (p.Val372fs)	exon 8	frameshift	1
c.1169C>T (p.Ala390Val)	exon 8	missense	1
c.1323G>A (p.Trp441X)	exon 9	nonsense	1
c.1339C>T (p.Gln447X)	exon 9	nonsense	1
c.1561dup (p.Arg521fs)	exon 10	frameshift	16
c.1430dup (p.Glu478fs)	exon 10	frameshift	5
c.1677_1684dup (p.Lys562fs)	exon 10	frameshift	2
c.1594C>T (p.Arg532X)	exon 10	nonsense	1
c.799-9G>A (p.?)	intron 4	splice site	2

* *n*=70; of three patients, the mutation was not known.
Mutation nomenclature according to HGVS guidelines (www.hgvs.org/mutnomen), with the A of the ATG initiation codon numbered as nucleotide number 1, and the exon containing the ATG initiation codon numbered as exon 2. The MEN1 reference sequence used is NM_000244.3.

expected. For cases in which the surgeon expected to remove 3, 3½, 3¾ *(n*=11) this was 18% and for ≥4 *(n*=22) this was 50%, taking autotransplantation into account.

Recurrent pHPT

Six months after surgery 34 (47%) patients were still on vitamin D and/or calcium therapy, 26 (36%) patients were normocalcaemic and 6 (8%) patients were hypercalcaemic. For 7 (10%) patients the status at six months was unknown. Overall, 19 (26%) patients had recurrent or persistent pHPT during follow-up: nine of 17 patients after <SPTX (53%), four of 23 after SPTX (17%) and six out of 32 after TPTX (19%; Table 3). Median time to recurrence after <SPTX was 3 quarters (IQR 0.5-12), and after SPTX/TPTX 12 quarters (IQR 3-27). The follow-up did not differ between patients who developed persistent/recurrent pHPT and those who did not [18 quarters (4.5 years) vs 16 quarters (4 years), respectively].

Compared to the patients who underwent SPTX or TPTX, patients after <SPTX were more likely to develop recurrent pHPT during follow-up (Fig. 2A). The results of SPTX and TPTX in terms of recurrence and time to recurrence were not different (Fig. 2B).

Patients who underwent SPTX or TPTX with bilateral TCT, had less recurrence than

patients without bilateral TCT, although this was not statistically different (one recurrence in 17 patients vs nine in 33 patients, *P*=0.07). For patients who underwent <SPTX recurrence did not differ between patients with or without bilateral TCT (*P*=0.807). The recurrence rate did not differ between patients with one or multiple surgeries (*P*=0.975). There was no association between recurrence and age at surgery, adjusted for type of surgery (Hazard Ratio 0.98; 95% CI 0.95-1.02).

Genotype-Phenotype Correlation

To assess if patients with a certain genotype were at greater risk for persistent/recurrent pHPT, we divided the patients into two groups based on their *MEN1* mutation. Group 1 consisted of patients with a nonsense or frameshift mutation

Table 2. Patient Characteristics

	Total (*n*=73)	One Surgery (*n*=42)	Multiple Surgeries (*n*=31)	*p**
Gender, No.				0.133
Male (%)	26 (36)	18 (43)	8 (26)	
Female (%)	47 (64)	24 (57)	23 (74)	
Median follow-up in quarters (IQR)	17 (7-26)	19 (9-31)	12 (7-21)	0.055
Mean age at surgery (SD)†	41 (14)	39 (13)	44 14)	0.146
Median duration of preoperative hypercalcaemia in months (IQR)†	21 (10-55)	14 (8-42)	35 (18-60)	0.06
Mean preoperative serum calcium, mmol/L (SD)†	2.72 (0.18)	2.76 (0.16)	2.66 (0.19)	0.023
Median preoperative serum PTH, pmol/L (IQR)†	8.9 (6.8-12)	9.8 (6.9-13.9)	8.3 (6.3-9.7)	0.027
Mean postoperative serum calcium, mmol/L (SD) †	2.32 (0.20)	2.32 (0.20)	2.33 (0.19)	0.933
Mean postoperative serum PTH, pmol/L (SD) †	4.0 (3.2)	3.8 (3.0)	4.4 (3.8)	0.596
After final surgery ‡ §				0.07
<3 Parathyroids removed (%)	17 (23)	14 (33)	3 (10)	
Subtotal resection (%)	23 (32)	16 (38)	7 (23)	
Total resection (%)	32 (44)	12 (29)	20 (66)	
Unknown (%)	1 (3)	0	1 (3)	
TCT				0.377
No. TCT (%)	35 (48)	23 (50)	12 (35)	
Bilateral (%)	19 (26)	12 (33)	7 (23)	
Unilateral (%)	12 (16)	6 (14)	6 (23)	
Nos (%)	4 (6)	1 (2)	3 (10)	
Unknown (%)	3 (4)	0	3 (10)	

* comparing patients with one surgery with patients with multiple surgeries, *P*-values are derived from the χ^2 test for categorical variables and from the student *t*-test or the Mann-Whitney test for continuous variables depending on the normal distribution.

† Characteristics at final surgery are reported

‡ All surgeries combined

§ Based on pathological examination

IQR interquartile range; *nos* not otherwise specified; *SD* standard deviation; *PTH* parathyroid hormone; *TCT* transcervical thymectomy.

Table 3. Surgical Characteristics*

	<SPTX† (*n*=17)	SPTX (*n*=23)	TPTX‡ (*n*=32)
No. of parathyroids removed			
1	8	0	0
2	9	0	0
3	0	18	0
3½, 3¾	0	4	0
4	0	0	27
$4^2/_3$	0	1	0
5	0	0	5
TCT			
No TCT	10	10	14
Bilateral	2	10	7
Unilateral	3	2	7
Nos	0	1	3
Unkown	2	0	1
Pathological examination thymus			
Thymus without parathyroid	1	9	13
Thymus with parathyroid	2	3	3
Unknown	2	1	1
Persistent/recurrent pHPT, *n*(%)	9 (53)	4 (17)	6 (19)
Persistent hypoparathyroidism, *n*(%)	4 (24)	9 (39)	21 (66)

*For one patient details regarding previous surgical procedures were missing, so the combined procedure could not be determined

† For 15 patients <SPTX was the intended procedure; in two of them, surgery was performed before MEN1 was diagnosed. For two patients the intended strategy was TPTX, but at pathological examination only one and two parathyroids were confirmed, respectively.

‡ In 28 patients, autotransplantation was performed.

pHPT primary hyperparathyroidism; *<SPTX* less than subtotal parathyroidectomy; *SPTX* subtotal parathyroidectomy; *TPTX* total parathyroidectomy; *TCT* transcervical thymectomy; *nos* not otherwise specified.

in exons 2, 9, and 10 (which are the N- and C-terminal regions of the gene). Group 2 consisted of the other patients. This subdivision was previously reported by Bartsch *et al.* [35].

There were no differences between groups 1 and 2 with regard to gender, age at surgery and preoperative levels of serum calcium and PTH. Patients in group 1 had SPTX/TPTX more often (84%) than patients in group 2 (58%). Therefore we performed stratified analyses. After <SPTX there was a higher risk of recurrence for patients in group 2 (Fig. 3A). After SPTX/TPTX the overall chance of persistence/recurrence was low (18%) and did not differ between group 1 and group 2 (Fig. 3B).

Hypoparathyroidism

Postoperative hypoparathyroidism occurred in 41 (56%) patients and was persistent (duration ≥6 months) in 34 (47%). The occurrence of persistent hypoparathyroidism did not differ between patients that underwent one or multiple surgeries

(*P*=0.32). Of the patients who had <SPTX, 4 (24%) developed hypoparathyroidism which lasted six months or longer. For SPTX and TPTX this was 9 (39%) and 21 (66%), respectively (Table 3).

There was no difference in occurrence and duration of postoperative hypoparathyroidism between patients with <SPTX and SPTX (*P*=0.43). Figure 4 shows that rate and duration of postoperative hypoparathyroidism are highest after TPTX.

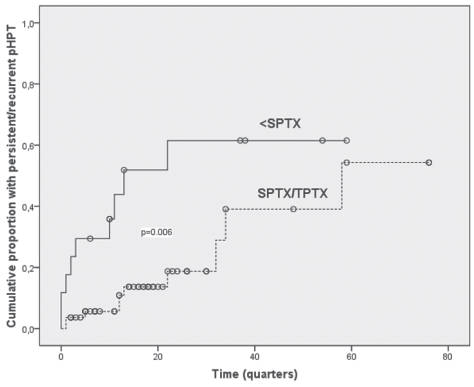
The patients with persistent hypoparathyroidism received vitamin D and/or calcium for a median duration of six quarters (1.5 years; IQR 4-16.5). Twenty-two (65%) patients had successful cessation of vitamin D/calcium during follow-up, resulting in only one case of recurrent pHPT. Successful cessation of vitamin D/calcium was noted up to 55 quarters (13.8 years) after surgery with subsequent normocalcaemia. In 12 (35%) patients vitamin D/calcium therapy was continued until the end of follow-up. However, in these patients follow-up was significantly shorter [median of 5.5 quarters (1.4 years) vs 23.5 quarters (5.9 years)].

Of the 34 patients with persistent hypoparathyroidism, ten (29.4%) patients needed IV calcium in the first quarter after surgery.

In patients who had SPTX, only a preoperative raised PTH was a weak predictor for postoperative hypoparathyroidism (*P*=0.047).

Figure 2 .

A



B

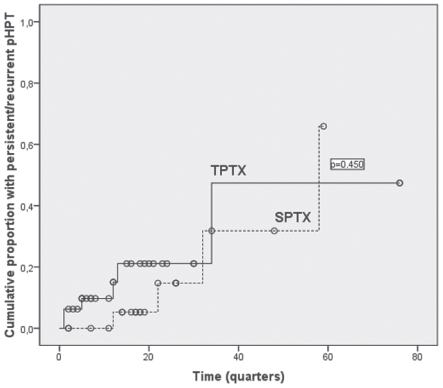


Fig. 2A, Kaplan-Meier survival curves showing persistent/recurrent pHPT and time to recurrence for patients who had <SPTX compared to patients who had SPTX or TPTX. Both curves differ significantly (log rank test *P*=0.006). (See Table 2a, Supplementary Digital Content 1, for the number of patients at risk for each group at each time point.)

Fig. 2B, Kaplan-Meier survival curve showing persistent/recurrent pHPT and time to recurrence for patients who had SPTX compared to patients who had TPTX. These curves are not different (log rank test *P*=0.450). (See Table 2b, Supplementary Digital Content 1, for the number of patients at risk for each group at each time point.)

Other surgical complications
Apart from the hypoparathyroidism described earlier, surgical complications occurred in two patients (3%). One patient had a transient vocal cord paresis for a duration of more than 12 months, after the fourth surgery (MIP). One patient had a hematoma after the first surgery, a conventional neck exploration with removal of one parathyroid.

Discussion
Our data show that recurrence is high (53%) if less than 3 parathyroids are removed and, for the first time, we can report a correlation between genotype and persistent/recurrent pHPT. We have also shown that after parathyroidectomy in MEN1 patients a substantial number (47%) developed persistent hypoparathyroidism, particularly after TPTX (66%).
Because of the retrospective nature of the study, and the fact that the patients were not randomized to undergo any surgical procedure, confounding by indication cannot be completely ruled out. Furthermore, statistically significant outcomes might be found by multiple testing in existing data sets, leading to overestimation of the value of coincidental findings. However, in our study, the study questions were formulated and the potential variables of interest were decided upon in a study protocol before the data were collected. In addition, we identified all eligible patients in the University Medical Centers (UMCs) by a standard identification procedure using all available hospital diagnosis databases. This minimizes the chance of selection bias. In addition, 85-90% of the Dutch patients with MEN1 are treated in a UMC, mostly in their own region, and therefore we expect that the patients from our cohort represent the general MEN1 population.
In our cohort, compared to literature, hypoparathyroidism occurred relatively frequently. After <SPTX hypoparathyroidism occurred in 23% of the cases compared with 0-15% in literature [13-15, 18-25]. After SPTX hypoparathyroidism occurred in 39% of the cases compared with 0-33% in literature [13-22, 24 ,25], with the exception of one series in which 60% hypoparathyroidism was reported [23]. After TPTX in 66% compared with 22-50% in the literature [14, 18, 20, 22-26], with one series reporting 87% hypoparathyroidism [19]. We defined persistent hypoparathyroidism as the use of active vitamin D and/or calcium for ≥ six months. A reason for this high rate of hypoparathyroidism might be unnecessary vitamin D and/or calcium therapy. However, the average PTH after six months for patients on vitamin and/or calcium was low and the average serum calcium was in the low normal range, indicating adequate therapy. Hypoparathyroidism rates did not differ between the UMCU and the RUNMC and the surgical procedure was performed by experienced surgeons. Therefore the surgeon cannot be seen as the explanatory factor for the higher rates of hypoparathyroidism. The difference might be explained by the various manners in which populations undergoing different types of surgery are defined. In our study, the operation strategy was based on the number of parathyroids identified by the pathologist and not on the intended strategy of the surgeon. Therefore, in our

Figure 3.

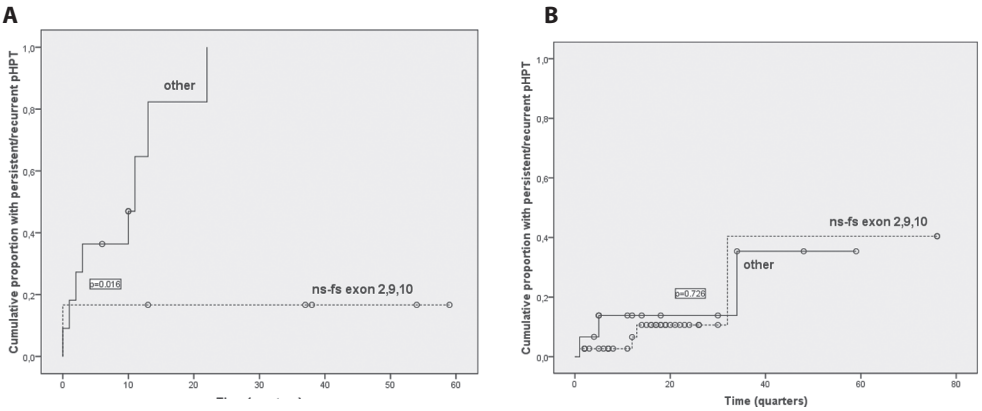
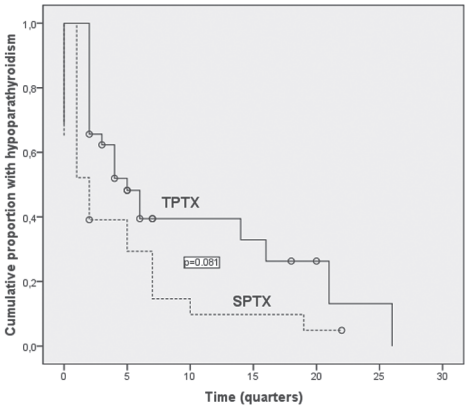


Fig. 3A, Kaplan-Meier survival curves of patients who had <SPTX. Patients with nonsense (ns) or frameshift (fs) mutations in exon 2, 9, 10 (group 1) are compared to patients with other mutations (group 2) with regard to persistent/recurrent pHPT and time to recurrence. Both curves differ significantly (log rank test *P*=0.016). (See Table 3a, Supplementary Digital Content 1, for the number of patients at risk for each group at each time point.)

Fig. 3B, Kaplan-Meier survival curves of patients who had SPTX or TPTX. Patients with nonsense (ns) or frameshift (fs) mutations in exon 2, 9, 10 (group 1) are compared to patients with other mutations (group 2) with regard to persistent/recurrent pHPT and time to recurrence. The curves are not different (log rank test *P*=0.726). (See Table 3b, Supplementary Digital Content 1, for the number of patients at risk for each group at each time point.)

Figure 4 .



Kaplan-Meier survival curves showing hypoparathyroidism and time to cure. Hypoparathyroidism is defined as the use of vitamin D and/or calcium supplements, and the moment of cure is defined as the moment of cessation of vitamin D and/or calcium therapy. A comparison is made between patients after SPTX and after TPTX. There is a trend towards less hypoparathyroidism after SPTX, although not reaching statistical significance (*P*=0.081) probably due to the number of patients. (See Table 4, Supplementary Digital Content 1, for the number of patients at risk for each group at each time point.)

cohort, SPTX and TPTX are true, pathology proven, subtotal or total procedures. Only in the most recent studies, populations were defined based on pathological examinations, whereas in older series this was not clearly described or groups were based on surgical reports only [13-26]. This is possibly reflected in our results: for <SPTX our rates of persistent/recurrent hyperparathyroidism are in line with literature (53% compared with 15-96%), but for SPTX (17% compared to 5-66%) and TPTX (19% compared to 0-56%) our recurrence rates are relatively low compared to other studies [14-21, 24-26]. One might argue that these high success rates are achieved at the cost of higher rates of postoperative hypoparathyroidism.

Another important finding of our study is the duration of postoperative hypoparathyroidism. In literature hypoparathyroidism is considered 'permanent' if this is still present at six months after surgery. We have shown that of the 34 patients with so-called 'permanent' hypoparathyroidism, vitamin D/calcium therapy was successfully stopped in 22 patients, with only one case of recurrent pHPT. The patients in our study were treated with vitamin D and/or calcium supplements for a median of 1.5 years and successful cessation of medication was possible even after 10 years. Hypoparathyroidism at six months after surgery should therefore not be considered permanent in MEN1 patients and we advise to try to taper off the medication even several years after surgery.

Hypoparathyroidism after SPTX cannot be predicted with standard laboratory or patient characteristics. The fact that MEN1 patients are screened for pHPT might be the reason for this lack of predictability. MEN1-related pHPT is usually detected in an early stage, as evidenced by the lower PTH levels found in MEN1 patients compared to sporadic patients [12].

In our series six patients had recurrent disease after TPTX. These recurrences can be caused by supernumerary glands or by autotransplant recurrences. In our series supernumerary glands were seen in 6 patients (8%), which is concurrent with reports in literature [13, 15, 21-22, 34], although occasionally rates as high as 30% are reported [36].

We confirm previous reports that, although not statistically significant, after SPTX or TPTX recurrence is more common in patients without a bilateral TCT [37-39]. In addition, in 8 of the 36 (22%) patients in whom some form of TCT was performed, a parathyroid gland was found in the thymus, reaffirming the significance of performing a TCT.

To our knowledge, we are the first to report a genotype-phenotype correlation in MEN1-related pHPT. In 2000 Bartsch *et al.* reported that patients with truncating nonsense or frameshift mutations in the C- or N-terminal regions of the *MEN1* gene (exon 2, 9, and 10) had a significant higher rate of malignant duodenopancreatic neuroendocrine tumors than patients with other mutations[35]. In two studies from this same group, this genotype-phenotype correlation could not be found in pHPT [30, 34]. However, this might be attributed to the small number of patients *(n*=38 and *n*=47). In other reports only the presence or absence of pHPT was compared between different genotypes and no correlation was found [29, 31-33].

The present data show that after SPTX/TPTX recurrence did not differ between the two genetic groups. After <SPTX persistence/recurrence was significantly lower in patients with nonsense or frameshift mutations in exon 2, 9 and 10.
This indicates that cure primarily depends on the amount of parathyroid tissue removed. If an extensive parathyroidectomy is performed, the overall chance of recurrence is relatively low and genotype had little effect on recurrence rates. Since TPTX frequently results in hypoparathyroidism, SPTX is the procedure of choice for all MEN1 patients. If an MIP is used as a staged procedure in individual cases (eg when the patient is reluctant to undergo SPTX), this might be more suitable for patients with a nonsense or frameshift mutation in exon 2, 9 and 10, because most recurrences after <SPTX are seen in patients with other mutations. However, these outcomes need to be confirmed in future studies.
The molecular pathophysiology of many mutations in human disease-causing genes is not exactly known, even if such mutations have been proven to have a pathogenic effect. For instance, a nonsense or frameshift mutation may either lead to nonsense mediated mRNA decay, or, alternatively, to the synthesis of a truncated protein. Therefore, the mutated allele may either be completely silenced (a so called 'null allele') or may drive the synthesis of an abnormal protein.
Furthermore, the specific consequences of a mutation in the *MEN1* gene are difficult to predict. This is because menin, the protein product of the *MEN1* gene, has many interacting proteins, including nuclear receptors such as the vitamin D, estrogen and PPAR (peroxisome proliferator-activated receptor) gamma receptors in different endocrine tissues [40-42]. Truncated proteins, and proteins harboring amino acid substitutions due to missense mutations could interfere with normal cellular processes. Compared with null-alleles, where the mutant allele produces no protein, such mutations could have a more deleterious effect. On the contrary, some mutant proteins may still retain part of their normal function, for instance, because important functional domains are still included in the mutant protein. This might cause a less severe phenotype than mutations that lead to a complete loss of normal menin function. Evidently, such models need to be supported by results from in vitro functional studies on the mutations observed in MEN1 patients.
Based in part on the results of the present study, we have changed our surgical strategy. SPTX combined with bilateral TCT is the preferred procedure now. If <SPTX is used as a staged procedure in individual cases, this might be more suitable for patients with nonsense or frameshift mutations in exon 2, 9 or 10.

Conclusion and recommendations
For patients with MEN1-related pHPT subtotal parathyroidectomy combined with bilateral TCT is the best surgical approach, providing the best balance between cure and postoperative hypoparathyroidism. If less than subtotal parathyroidectomy is used as a staged procedure in individual cases, this might be more suitable for patients with nonsense or frameshift mutations in exon 2, 9 or 10.
In postoperative hypoparathyroidism lasting six months or longer, it is important to realize that it might take months or even years to successfully taper off the medication, but it will be successful in nearly two thirds of the patients.

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Supplementary Digital Content 1

Table 2A.
No. at risk

Time (quarters)	0	10	20	30	40	50	60	70
<SPTX	17	11	5	4	2	2	0	0
SPTX/TPTX	55	38	19	10	5	4	2	2

Table 2B.
No. at risk

Time (quarters)	0	10	20	30	40	50	60	70
SPTX	23	20	10	5	3	2	0	0
TPTX	32	18	9	5	2	2	2	2

Table 3A.
No. at risk

Time (quarters)	0	10	20	30	40	50	60
ns-fs exon 2, 9, 10	6	5	4	4	2	2	0
other	11	8	2	1	0	0	0

Table 3B.
No. at risk

Time (quarters)	0	10	20	30	40	50	60	70	80
ns-fs exon 2, 9, 10	37	27	14	4	3	2	2	2	0
other	15	9	5	5	2	1	0	0	0

Table 4.
No. at risk

Time (quarters)	0	5	10	15	20	25
STPX	15	6	2	2	1	0
TPTX	22	11	6	5	2	1

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Part III

Natural course of thoracic and pancreatic neuroendocrine tumours in MEN1

Chapter 6

Thoracic and duodenopancreatic neuroendocrine tumors in multiple endocrine neoplasia type 1: natural history and function of menin in tumorigenesis

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Thoracic and duodenopancreatic neuroendocrine tumors in multiple endocrine neoplasia type 1: natural history and function of menin in tumorigenesis

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Abstract

Mutations of the multiple endocrine neoplasia type 1 (MEN1) gene lead to loss of function of its protein product menin. In keeping with its tumor suppressor function in endocrine tissues, the majority of the MEN1-related neuroendocrine tumors (NETs) show loss of heterozygosity (LOH) on chromosome 11q13. In sporadic NETs, *MEN1* mutations and LOH are also reported, indicating common pathways in tumor development.

Prevalence of thymic NETs (thNETs) and pulmonary carcinoids in MEN1 patients is 2-8%. Pulmonary carcinoids may be underreported and research on natural history is limited, but disease-related mortality is low. thNETs have a high mortality rate. Duodenopancreatic NETs (dpNETs) are multiple, almost universally found at pathology and associated with precursor lesions. Gastrinomas are usually located in the duodenal submucosa while other dpNETs are predominantly pancreatic. Duodenopancreatic NETs are an important determinant of MEN1-related survival, with an estimated 10-year survival of 75%. Survival differs between subtypes and apart from tumor size there are no known prognostic factors. Natural history of non-functioning pancreatic NETs needs to be redefined because of increased detection of small tumors. MEN1-related gastrinomas seem to behave similar to their sporadic counterparts, while insulinomas seem to be more aggressive. Investigations into the molecular functions of menin have led to new insights into MEN1-related tumorigenesis. Menin is involved in gene transcription, both as an activator and repressor. It is part of chromatin-modifying protein complexes indicating involvement of epigenetic pathways in MEN1-related NET development. Future basic and translational research aimed at NETs in large unbiased cohorts will clarify the role of menin in NET tumorigenesis and might lead to new therapeutic options.

Introduction

Thoracic and duodenopancreatic neuroendocrine tumors (dpNETs) can occur either sporadically or as a manifestation of an inherited syndrome, most importantly the Multiple Endocrine Neoplasia type 1 (MEN1) syndrome. This is an autosomal dominantly inherited disease that is caused by germline mutations in the *MEN1* gene. NETs associated with MEN1 are lung NETs, thymic NETs (thNET), gastric NETs and dpNETs. MEN1-related NETs are an important cause of morbidity and presently malignant dpNETs and thNETs are the main cause of MEN1-related death (Schaaf, *et al.* 2007; Goudet, *et al.* 2010).

In the past decade understanding of the genetic and molecular aspects of NETs has increased and important steps have been made in the therapy of advanced disease. New tumor classification and staging systems have improved patient care and uniformity in patient selection for clinical trials. It is important to recognize similarities in the tumorigenesis of MEN1-related and sporadic NETs, because MEN1-related NETs may be regarded as a model for sporadic disease. On the other hand, it is also essential to be aware of potential differences in tumor behavior between these two entities, as this influences diagnostic and therapeutic strategies.

In this review, we provide a comprehensive overview of the literature concerning tumor development of MEN1-related dpNETs and thoracic NETs (Box 1). The complete spectrum, from epidemiologic characteristics and natural history to important molecular findings associated with loss of the *MEN1* gene, is discussed. Differences between and similarities with their sporadic counterparts are highlighted. Table 1 provides a list of some of the abbreviations used in the text.

Table 1. Abbreviations used in the text

AC	Atypical carcinoid
DIPNECH	Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia
dpNET	Duodenopancreatic neuroendocrine tumor
H3K4me3	Trimethylation of lysine 4 on histone 3
HDAC	Histone deacetylase
LOH	Loss of heterozygosity
MEN1	Multiple endocrine neoplasia type 1
NET	Neuroendocrine tumor
NF	Nonfunctioning
NF-pNET	Nonfunctioning pancreatic neuroendocrine tumor
NR	Nuclear receptor
pNET	Pancreatic neuroendocrine tumor
thNET	Thymic neuroendocrine tumor
TC	Typical carcinoid
TF	Transcription factor
VIPoma	Vaso-active intestinal peptide producing neurendocrine tumors

MEN1 gene

The *MEN1* gene was initially localized to chromosome 11q13 by linkage analysis and tumor deletion mapping studies (Larsson, *et al.* 1988; Friedman, *et al.* 1989; Byström, *et al.* 1990; Lubensky, *et al.* 1996), which led to the identification of the gene in 1997 (Chandrasekharappa, *et al.* 1997; Lemmens, *et al.* 1997). More than 450 different germline *MEN1* mutations have been identified in MEN1 patients (Lemos&Thakker 2008). *MEN1* consists of ten exons and mutations are found scattered throughout the gene. The protein product is the 610 amino-acid protein, called menin. Most *MEN1* gene mutations are predicted to lead to truncation of the protein (Lemos&Thakker 2008). Missense mutations have been reported in about 20% of the cases. Both truncated and missense mutations result in reduced levels of protein due to proteolytic degradation via the ubiquitin-proteasome pathway (Yaguchi, *et al.* 2004). A small percentage of patients who are considered to have the MEN1 syndrome (based on the clinical definition) may not harbor a germline mutation within the coding region of the *MEN1* gene (Agarwal, *et al.* 1997). Possibly, these patients have mutations in the promoter region or large deletions on chromosome 11q13 (Cavaco, *et al.* 2002). Currently in clinical practice, inconclusive DNA sequencing is followed by multiplex ligation-dependent probe amplification analysis for detection of large deletions. An alternative explanation for the MEN1 syndrome of these patients may include epigenetic silencing of *MEN1* (eg by DNA methylation) or mutations in other genes, which cause MEN1-like manifestations.

The *MEN1* gene is a tumor suppressor gene for endocrine tissues. According to Knudson's "two hit hypothesis", biallelic inactivation of *MEN1* is required for tumor development (Knudson 1971). This second hit typically involves large chromosomal deletions in chromosome 11q13. Loss of heterozygosity (LOH) of MEN1 is demonstrated in most reported MEN1-related pancreatic NETs (pNETs; Lubensky *et al.* 1996; Debelenko, *et al.* 1997*b*; Hessman, *et al.* 2001; Perren, *et al.* 2007).

However, the frequency of LOH of chromosome 11q13 in MEN1-related primary duodenal gastrinomas is only 21-45% (Lubensky *et al.* 1996; Debelenko *et al.* 1997*b*). LOH of chromosome 11q13 has also been shown in MEN1-related pulmonary carcinoids (Debelenko *et al.* 1997b; Dong, *et al.* 1997). Intriguingly, no

Box 1. Search strategy

The contents of this review are based on the experience of the authors and an extensive search in PubMed. The following terms were used in the search string: "MEN1" and all relevant synonyms OR "menin" and all relevant synonyms. For lung NET this search string was combined with "bronchial" OR "pulmonary" OR "lung" (and relevant synonyms) AND "carcinoid" OR "neuroendocrine". For thNET this search string was combined with "thymic" OR "thymus" OR "mediastinal" AND "carcinoid" OR "neuroendocrine". For dpNET this search string was combined with all relevant synonyms for "pancreas" and "duodenum" AND all relevant synonyms for "neuroendocrine tumor" OR "gastrinoma" OR "insulinoma" OR "glucagonoma" OR "VIPoma".

LOH was found in thNETs (Teh, *et al.* 1994; Teh, *et al.* 1998; Hessman *et al.* 2001; Gibril, *et al.* 2003). In these cases other events might be involved in silencing the second *MEN1* allele. Somatic mutations in MEN1-related tumors have been reported as an alternative mechanism leading to inactivation of this second *MEN1* allele (Pannett&Thakker 2001). Post-transcriptional reduction in menin levels by specific microRNAs may mimic the second hit (Luzi, *et al.* 2012). In sporadic NETs somatic mutations of the *MEN1* gene have been found. The reported frequency for *MEN1* mutations in sporadic pNETs is up to 44% in well-differentiated tumors (Jiao, *et al.* 2011). In accordance with Knudson's hypothesis, LOH of chromosome 11q13 is also observed in sporadic pNETs (Debelenko *et al.* 1997*b*; Hessman, *et al.* 1998; Gortz, *et al.* 1999). Also, in sporadic pulmonary carcinoids mutations in the *MEN1* gene and LOH of chromosome 11q13 are reported with a frequency of 18-45% (Debelenko *et al.* 1997*b*; Walch, *et al.* 1998; Gortz *et al.* 1999; Petzmann, *et al.* 2001; Vageli, *et al.* 2006; Veschi, *et al.* 2012) and up to 73% in a single report (Finkelstein, *et al.* 1999). Apparently, MEN1-related tumors and their sporadic counterparts share common pathways in tumor development.

Thoracic NETs in MEN1

*Pathology and pathogenes*is

According to the World Health Organization lung and thymus NETs are classified into typical carcinoids (TC), atypical carcinoids (AC) and high-grade neuroendocrine carcinomas based on mitotic count and the presence of necrosis (Travis, *et al.* 2004). High-grade tumors are divided into large-cell neuroendocrine carcinomas and small-cell carcinomas. Small (< 0.5 cm) pulmonary tumors with carcinoid morphology are called tumorlets (Travis *et al.* 2004). For thNETs alternative grading systems exist, which is important to realize when comparing and interpreting results from different studies (Fukai, *et al.* 1999; Moran&Suster 2000*a*; Gal, *et al.* 2001; Gaur, *et al.* 2010).

Pulmonary carcinoids have a different clinical presentation and genetic profile compared to high-grade tumors and must be regarded as a separate entity (Swarts, *et al.* 2012). High-grade lung NETs are not seen in association with MEN1. Moreover, in contrast to sporadic pulmonary carcinoids, mutations in the *MEN1* gene and LOH at chromosome 11q13 are rare in high-grade lung NETs (Swarts *et al.* 2012). This sharp distinction is absent in thNETs (Moran&Suster 2000*b*) and MEN1-related thNETs include both well- and poorly-differentiated neuroendocrine carcinomas.

The cell of origin for pulmonary carcinoids is thought to be the pulmonary neuroendocrine cell (Swarts *et al.* 2012), although, some suggest an uncommitted progenitor cell (Warren&Hammar 2006). Pulmonary neuroendocrine cells are evenly distributed throughout the airways, but absent from the alveoli, and comprise 0.4% of all lung epithelial cells (Boers, *et al.* 1996). In response to various triggers reactive neuroendocrine cell hyperplasia can occur, which is not associated with the development of pulmonary carcinoids. Diffuse Idiopathic Pulmonary Neu-

roendocrine Cell Hyperplasia (DIPNECH) on the other hand, is a rare disorder that is considered preneoplastic to pulmonary carcinoids (Aguayo, *et al.* 1992; Travis *et al.* 2004). Only one case of a MEN1-patient with DIPNECH has been published to date (Davies, *et al.* 2007). However, in published cases of MEN1-related pulmonary carcinoids, pathology of surrounding lung tissue is not reported, so the true prevalence of DIPNECH among MEN1-patients is unknown.
The cell of origin for thNETs is not known and there are no known precursor lesions. ThNETs were first described in 1972 and in the same year the association with MEN1 was reported (Rosai&Higa 1972; Rosai, *et al.* 1972). It was then hypothesized that these tumors arise from neuroendocrine cells residing within the normal thymus. In different series family clustering of MEN1-related thNETs is demonstrated (Teh, *et al.* 1997; Teh *et al.* 1998; Ferolla, *et al.* 2005; Goudet, *et al.* 2009). In those series no apparent *MEN1* genotype-phenotype correlation was seen, suggesting the involvement of other genetic factors. (Teh *et al.* 1998).

Epidemiology
The prevalence of thNETs among MEN1 patients is 2-8% (Teh *et al.* 1997; Burgess, *et al.* 1998*b*; Gibril *et al.* 2003; Goudet, *et al.* 2011; Sakurai, *et al.* 2013). Approximately one fifth of all thNETs are MEN1-related (Teh *et al.* 1997), therefore the diagnosis of a thNET should always prompt further evaluation of a possible underlying MEN1 syndrome (de Laat, *et al.* 2012). Mean age at diagnosis of thNETs in MEN1 is 39-47 years (Teh *et al.* 1997; Teh *et al.* 1998; Gibril *et al.* 2003; Ferolla *et al.* 2005; Goudet *et al.* 2009; Sakurai *et al.* 2013). In series from USA, Europe and Australia 95-100% of the patients are male (Teh *et al.* 1997; Teh *et al.* 1998; Gibril *et al.* 2003; Ferolla *et al.* 2005; Goudet *et al.* 2009) whereas in a recent Japanese series 64% of thNETs occurred in males (Sakurai *et al.* 2013). Age at diagnosis is 43-58 years in sporadic thNET and male predominance, although less pronounced (67-86%), is also seen (de Montpreville, *et al.* 1996; Soga, *et al.* 1999; Moran&Suster 2000*a*; Gaur *et al.* 2010; Hamaji, *et al.* 2012). It is important to note that most MEN1-patients with thNETs are heavy smokers (Teh *et al.* 1997; Teh *et al.* 1998; Gibril *et al.* 2003; Ferolla *et al.* 2005).
The exact prevalence of pulmonary carcinoids in MEN1 is unknown. Commonly reported figures are 3-8% (Marx, *et al.* 1998; Karges, *et al.* 2000; Goudet *et al.* 2011), However, in a large Tasmanian family *(n*=129) prevalence among patients screened with thoracic computed tomography ranged from 11% if only pathology proven cases were included to 31% based on radiological findings (Sachithanandan, *et al.* 2005). Reported age at diagnosis of MEN1-related pulmonary carcinoids is mid-forties (Sachithanandan *et al.* 2005). Although initially a female predominance was reported (Duh, *et al.* 1987; Farhangi, *et al.* 1987; Shepherd 1991; Sachithanandan *et al.* 2005), the prevalence appears to be equal between genders in a large recent study (Goudet *et al.* 2011).

Natural history and prognostic factors – pulmonary carcinoids

Very little is known of the natural course and prognosis of pulmonary carcinoids in MEN1. Evidence is limited to one small series and several case reports or descriptions, either separately published or mentioned within larger MEN1 patient series. Among sporadic pulmonary carcinoids, TCs are much more frequent than ACs (10-27% in series also including non-surgical patients; Fink, *et al.* 2001; Pusceddu, *et al.* 2010; Naalsund, *et al.* 2011; Okoye, *et al.* 2013). This seems to be similar in MEN1, but classifications are rarely reported (Murat, *et al.* 1997; Snabboon, *et al.* 2005; Lourenco-Jr, *et al.* 2007; Abe, *et al.* 2008; Divisi, *et al.* 2008; Matsuda, *et al.* 2010; Montero, *et al.* 2010). As in other MEN1 manifestations multiplicity seems to be common in pulmonary carcinoids (Marx *et al.* 1998; Sachithanandan *et al.* 2005). In its sporadic counterpart, multiplicity is seen in <1-9% (Daddi, *et al.* 2004; Garcia-Yuste, *et al.* 2007; Ferolla, *et al.* 2009; Okoye *et al.* 2013). Ectopic hormone production is not reported in MEN1-related pulmonary carcinoids, in contrast to sporadic disease (Boddaert, *et al.* 2012; Garby, *et al.* 2012; Simonds, *et al.* 2012).

The overall survival of MEN1-related pulmonary carcinoids is unknown. In series focusing on MEN1-related mortality, 5-9% of the MEN1-related deaths occurring before 1990 were attributed to pulmonary carcinoids (Wilkinson, *et al.* 1993; Goudet *et al.* 2010), with no deaths due to pulmonary carcinoids reported after 1990 (Geerdink, *et al.* 2003; Wilson, *et al.* 2008; Goudet *et al.* 2010). In line with these findings, pulmonary carcinoids do not give an increased risk of death in MEN1 patients (Goudet *et al.* 2010).

The prevalence of lymph node or distant metastases is difficult to establish in MEN1-related pulmonary carcinoids. In a total of only 33 cases reported in literature information on metastases is available, with 24% lymph node metastases and 12% distant metastases (Underdahl, *et al.* 1953; Williams&Celestin 1962; Dry, *et al.* 1976; Farhangi *et al.* 1987; Shepherd 1991; Murat *et al.* 1997; Sachithanandan *et al.* 2005; Snabboon *et al.* 2005; Lourenco-Jr *et al.* 2007; Abe *et al.* 2008; Divisi *et al.* 2008; Waldmann, *et al.* 2009; Fabbri, *et al.* 2010; Matsuda *et al.* 2010; Montero *et al.* 2010).

Given the paucity of data on pulmonary carcinoids in MEN1 and the absence of head-to head comparisons, it is unclear whether the natural history differs between MEN1-related and sporadic pulmonary carcinoids. There are a few studies in sporadic tumors that show somatic *MEN1* mutations, LOH at 11q13 or reduced *MEN1* gene expression to be an adverse prognostic factor (Debelenko, *et al.* 1997*a*; Petzmann *et al.* 2001; Swarts, *et al.* 2011).

Factors predicting development of metastases or survival are not known in MEN1-related pulmonary carcinoids. In their sporadic counterparts ACs, lymph node metastases, distant metastases, and higher proliferation rate (Ki67 labeling index or mitotic index) have been repeatedly identified as adverse prognostic factors (Cao, *et al.* 2011; Daddi, *et al.* 2013). Results on the prognostic values of gender, age and tumor size are contradictory.

Natural history and prognostic factors – thNET

Six case-series including more than five patients have been published on thNETs in MEN1 (Teh *et al.* 1997; Teh *et al.* 1998; Gibril *et al.* 2003; Ferolla *et al.* 2005; Goudet *et al.* 2009; Sakurai *et al.* 2013). In these series, classifications are often not reported (Teh *et al.* 1997; Teh *et al.* 1998; Gibril *et al.* 2003; Sakurai *et al.* 2013). When mentioned, 100% are AC in one series and 38% poorly-differentiated neuroendocrine carcinomas in another (with the distinction between TC and AC for the other 62% not reported; Ferolla *et al.* 2005; Goudet *et al.* 2009). In sporadic thNETs the reported frequencies vary greatly, TCs are reported in 0-67% in different series and 81% in a literature review from 1999 (de Montpreville *et al.* 1996; Fukai *et al.* 1999; Soga *et al.* 1999; Moran&Suster 2000*a*; Gaur *et al.* 2010; Cardillo, *et al.* 2012). Patient selection and differences in the use of grading systems may explain this variation. Cushing syndrome due to ectopic adrenocorticotropic hormone production is rare in MEN1-related thNETs (0-5%; Teh *et al.* 1997; Teh *et al.* 1998; Gibril *et al.* 2003; Ferolla *et al.* 2005; Goudet *et al.* 2009; Sakurai *et al.* 2013), but it has been observed in 5-31% of the sporadic cases (Moran&Suster 2000*a*; Kondo&Monden 2003; Cardillo *et al.* 2012).

Among the manifestations of the MEN1 syndrome, thNETs carry the highest risk of death (Goudet *et al.* 2010) with an estimated 10-year survival of 30-36% (Goudet *et al.* 2009; Sakurai *et al.* 2013). Although mortality is high, the course of MEN1-related thNETs may be protruded, with one series reporting a median survival of 9.6 years (Goudet *et al.* 2009). When comparing six MEN1 patients with thNETs to 22 patients with sporadic thNETs, Crona *et al.* found no survival difference between these groups (2013).

In MEN1-related thNETs 90% disease-related mortality was reported among patients with advanced stage disease in a series with mean 3.6 years follow-up (Teh *et al.* 1997). In the other series, patients were followed for a mean 5-7 years and metastases occurred in 32-71% of the patients. Disease-related mortality in these series ranged from 0-43% (Teh *et al.* 1998; Gibril *et al.* 2003; Ferolla *et al.* 2005; Goudet *et al.* 2009; Sakurai *et al.* 2013). In one of the two series reporting no mortality, the majority was discovered incidentally at prophylactic thymectomy or by screening (Gibril *et al.* 2003).

No data are available on prognostic factors with regard to overall survival, recurrence or metastases in MEN1 patients. In series reporting on sporadic thNETs prognostic factors related to decreased survival are higher tumor grade, more advanced disease, higher Ki67 labeling index (cut-off 10%), and larger tumor size (Moran&Suster 2000*a*; Gal *et al.* 2001; Gaur *et al.* 2010; Cardillo *et al.* 2012; Crona *et al.* 2013).

dpNETs in MEN1

Pathology and pathogenesis

dpNETs are classified according to the European Neuroendocrine Tumor Society/

World Health Organization grading system into three grades based on proliferation rate (Rindi, *et al.* 2006; Bosman, *et al.* 2010).
The hallmark of dpNETs in MEN1 is multiplicity, which is in contrast to the mostly solitary sporadic dpNETs (Thompson, *et al.* 1984; Pipeleers-Marichal, *et al.* 1993; Crippa, *et al.* 2012). All histologic subtypes can occur in MEN1. At pathology all MEN1 patients have multiple micro-adenomas (pNETs <5 mm without a clinical syndrome) dispersed throughout the pancreas associated with one or more NETs ≥ 5 mm (Thompson *et al.* 1984; Kloppel, *et al.* 1986; Le Bodic, *et al.* 1996; Anlauf, *et al.* 2006*b*). These multiple dpNETs in MEN1 arise from independent clonal events, as demonstrated by different allelic deletion and retention patterns in synchronous tumors (Debelenko *et al.* 1997*b*; Hessman, *et al.* 1999; Perren *et al.* 2007). Apart from these tumors other lesions such as islet cell hyperplasia/ enlargement, nesidioblastosis and atypical or monohormonal endocrine cell clusters are frequently observed in the MEN1 pancreas, leading to different theories as to the cell of origin for pNETs (Thompson *et al.* 1984; Le Bodic *et al.* 1996; Vortmeyer, *et al.* 2004; Perren *et al.* 2007). Normal pancreatic islets and alternatively ductal/acinar cells are proposed to be the precursor cells for pNETs (Vortmeyer *et al.* 2004; Perren *et al.* 2007).
Gastrinomas take a special place among the MEN1-related dpNETs, as the vast majority is not pancreatic but located submucosal in the duodenum (Pipeleers-Marichal, *et al.* 1990). Sporadic gastrinomas are located in the duodenum less frequently than in MEN1 (Pipeleers-Marichal *et al.* 1990; Donow, *et al.* 1991; Pipeleers-Marichal *et al.* 1993; Anlauf, *et al.* 2006*a*). Duodenal NETs in MEN1 are almost always multiple, while sporadic duodenal NETs are usually solitary (Pipeleers-Marichal *et al.* 1990; Donow *et al.* 1991; Anlauf *et al.* 2006a). In MEN1, they are associated with multifocal hyperplasia of gastrin and somatostatin producing cells, which are proposed to be precursor lesions (Anlauf, *et al.* 2005).

Epidemiology
The clinical prevalence of dpNETs in MEN1 is over 50% in recent large series (Goudet *et al.* 2011; Sakurai, *et al.* 2012*a*) and the penetrance of clinically manifest dpNETs at the age of 80 is 84% (Triponez, *et al.* 2006a).
dpNETs are classified as hormonally active or non-functioning (NF) based on combinations of clinical features, laboratory results, and findings at immunohistochemistry. In the MEN1 syndrome, synchronous dpNETs may secrete different hormones based on immunohistochemistry (Le Bodic *et al.* 1996; Anlauf *et al.* 2006*b*). As these findings do not always correlate with clinical symptoms, classifications should not be based on immunochistochemistry alone.
When sought for, additional NF-pNETs are found in all patients undergoing surgery for functional tumors, so the prevalence of NF-pNETs is probably equal to dpNETs in general (Tonelli, *et al.* 2006; Lopez, *et al.* 2011; Giudici, *et al.* 2012). Gastrinoma is the most prevalent hormonally active dpNET (29-55% of all dpNETs

in studies published in the last decade), followed by insulinoma (2-24%) and rare functioning tumors seen in <10% such as glucagonoma, Vaso-active Intestinal Peptide producing NET (VIPoma), and somatostatinoma (Lourenco-Jr *et al.* 2007; Vierimaa, *et al.* 2007; Pieterman, *et al.* 2009; Waldmann *et al.* 2009; Goudet *et al.* 2011; Sakurai *et al.* 2012*a*). It is important to realize that 76% of all cases of growth hormone (GH)-releasing hormone-producing pNETs reported in literature are MEN1-related (Garby *et al.* 2012). This diagnosis should therefore always raise suspicion of an underlying MEN1 syndrome. Separating different types of dpNETs in MEN1 is somewhat artificial, because most patients with hormonally active tumors will harbor additional NF-pNETs (Tonelli *et al.* 2006; Lopez *et al.* 2011; Giudici *et al.* 2012), patients with NF-pNETs can develop hormonally active tumors (Thomas-Marques, *et al.* 2006; Davi, *et al.* 2011) and co-occurrence of different hormonally active tumors has also been described (Tonelli *et al.* 2006; Giudici *et al.* 2012). MEN1-related dpNETs are seen one to two decades earlier than their sporadic counterparts (Jensen 1998; Nikfarjam, *et al.* 2008; Anlauf, *et al.* 2009; Crippa *et al.* 2012; Singh, *et al.* 2012). Insulinomas have the lowest age of onset (patients are usually in their twenties or thirties at diagnosis), patients with gastrinomas and NF-pNETs are usually diagnosed in their thirties (Jensen 1998; Cougard, *et al.* 2000; Triponez *et al.* 2006*a*; Sakurai, *et al.* 2012*b*). At the age of 60 the penetrance of gastrinoma is significantly higher in men (55%) compared to women (33%), while the other dpNET types do not show gender differences (Goudet *et al.* 2011).

Natural history

dpNETs are the most important determinant of MEN1-related survival. In historical series, ulcer disease due to gastrinoma was the most important cause of MEN1-related death (Ballard, *et al.* 1964) while this presently is malignant dpNETs (Schaaf *et al.* 2007; Goudet *et al.* 2010). In patients with MEN1-related dpNETs, estimated 10-year survival is 75% (Carty, *et al.* 1998; Kouvaraki, *et al.* 2006). Risk of death seems to differ between the various subtypes, with the rare functioning tumors presenting the highest risk, followed by NF-pNETs and gastrinoma, while insulinomas do not seem to increase the risk of death (Goudet *et al.* 2010).

However, the natural history of NF-pNETs is not well-established yet. Estimated ten-year survival rates of 23-62% have been reported (Levy-Bohbot, *et al.* 2004; Kouvaraki *et al.* 2006), whereas this was 100% in a recent series (Lopez *et al.* 2011). One has to keep in mind that with endoscopic ultrasound more small NF-pNETs are currently diagnosed. They are usually indolent and demonstrate slow growth, with a doubling time of 5-10 years (Kann, *et al.* 2006; Sakurai, *et al.* 2007). When 46 patients with NF-pNETs <2 cm without surgical treatment were followed over ten years, 17% showed increase in size, 11% developed a functional syndrome, 65% displayed stable disease, 2% died due to metastastic NF-pNETs, 2% due to other causes and 2% was lost to follow-up (Triponez F, Goudet P, AFCE, & GTE, unpublished observation presented at ENETS 2013, Barcelona, Spain). In the largest reported series on NF-pNETs *(n*=108), metastases, mostly distant, are seen in 19% and

disease specific survival is 91% after a mean follow-up of 4 years (Triponez *et al.* 2006*a*). In smaller series from the last decade, distant metastases are reported in 6-22% (Bartsch, *et al.* 2005; Davi *et al.* 2011; Lopez *et al.* 2011), whereas in a report from 1992 distant metastases were observed in 57% (Grama, *et al.* 1992). Mean tumor size in this latter series was 6.7 cm (Grama *et al.* 1992).
In MEN1-related insulinomas, reported survival rates in series with more than ten patients are 93-100% after 9-10 years of follow-up (Van Box Som, *et al.* 1995; Cougard *et al.* 2000; Proye, *et al.* 2004). Multiple insulinomas are seen in 25-83% (Van Box Som *et al.* 1995; Thompson 1998; Giudici *et al.* 2012), whereas in sporadic insulinomas multiplicity is seen in ~4% (Nikfarjam *et al.* 2008; Anlauf *et al.* 2009; Crippa *et al.* 2012).
Malignancy in MEN1-related insulinomas has been reported in 5-27% in series including more than ten patients (Cougard *et al.* 2000; Proye *et al.* 2004; Crippa *et al.* 2012). In these malignant insulinomas, liver metastases were only seen once (Proye *et al.* 2004).

The natural history of gastrinomas in MEN1 is difficult to establish for several reasons. Firstly gastrinomas in MEN1 are predominantly located in the duodenum (Pipeleers-Marichal *et al.* 1990). In series on MEN1-related gastrinomas high rates of pancreatic tumors might be reported, but most of these will not be the gastrinomas. Rates of pancreatic gastrinomas are only 0-18% in series that include immunohistochemistry in the classification of pNETs as gastrinoma (Tonelli *et al.* 2006; Dickson, *et al.* 2011; Imamura, *et al.* 2011; Lopez, *et al.* 2013). Second, MEN1-related gastrinomas are almost invariably accompanied by NF-pNETs (Thompson 1998; Dickson *et al.* 2011). If distant metastases arise, these can not only be caused by the gastrinoma, but also by the accompanying NF-pNETs, and even by NETs of other locations, which cannot be separated if no pathology or immunohistochemistry results are available. Third, when interpreting the results of clinical series it is important to realize that in surgical series synchronous metastases will most likely be underrepresented, because diffuse liver metastases are seen as a contraindication for surgery, whereas in series with low surgical rates nodal status will most likely be underrepresented, since this is difficult to establish on imaging (Skogseid, *et al.* 1998). Finally, since the publication of guidelines for periodic evaluation, MEN1 patients must be viewed as a screened population, making comparison with sporadic cases more difficult (Thakker, *et al.* 2012).
The reported 10-year survival of gastrinomas in MEN1 is 86-94% (Thompson 1998; Norton, *et al.* 2001; Ito, *et al.* 2013), with two series reporting 63% and 75% (Melvin, *et al.* 1993; Ruszniewski, *et al.* 1993). In MEN1-related gastrinoma, synchronous lymph node metastases are reported in 45-69% (Ruszniewski *et al.* 1993; Thompson 1995; Weber, *et al.* 1995; Jensen 1998; Norton, *et al.* 1999; Norton *et al.* 2001; Imamura *et al.* 2011; Singh *et al.* 2012; Ito *et al.* 2013), with two series reporting 23-35% (Thompson 1998; Cadiot, *et al.* 1999) and two series reporting 80%. (Dickson *et al.* 2011; Lopez *et al.* 2013). Synchronous distant metastases are

reported in 4-29% in series also including nonsurgical patients (Jensen 1998; Ito *et al.* 2013).
Due to its rarity, very few data are available on functioning pNETs other than gastrinomas and insulinomas. The largest combined experience comes from the French Endocrine Tumor Study Group, reporting on five glucaconomas, three VIPoma and two somatostatinomas in MEN1, comprising 3.3% of the MEN1-related dpNETs (Levy-Bohbot *et al.* 2004). Four of these ten patients had liver metastases (40%). Ten-year survival was 54% (Levy-Bohbot *et al.* 2004).

Natural history : comparison with sporadic dpNET
Some series including MEN1 and sporadic dpNETs of all subtypes report MEN1 to be associated with better survival. However, no separate baseline characteristics are provided for MEN1 patients so selection bias cannot be excluded (Tomassetti, *et al.* 2005; Fendrich, *et al.* 2007; Rindi, *et al.* 2012). In one study including only patients operated upon for advanced dpNETs (all subtypes), patients with MEN1 had a trend towards better survival and developed no new distant metastases while 46% of the patients with sporadic dpNETs did develop new distant metastases (Fendrich, *et al.* 2006). The meaning of these findings is difficult to discern giving the highly selected source population.
With regard to different subtypes of dpNETs, no studies are available comparing MEN1-related and sporadic NF-pNETs. In series comparing MEN1-related with sporadic insulinomas a higher rate of malignancy was seen in MEN1 (Service, *et al.* 1991; Anlauf *et al.* 2009; Goretzki, *et al.* 2010; Crippa *et al.* 2012). On gastrinoma more data are available, mostly from different reports of the prospective study on Zollinger-Ellison Syndrome by the National Institutes of Health (Weber *et al.* 1995; Jensen 1998; Norton *et al.* 1999; Yu, *et al.* 1999). In two of these reports MEN1 patients had a better overall survival than patients with sporadic gastrinomas, but also less advanced disease at baseline, indicating potential lead time bias (Weber *et al.* 1995; Jensen 1998). When comparing patients in the same stage of disease, no survival difference was observed between MEN1-related and sporadic gastrinomas (Weber *et al.* 1995; Norton *et al.* 1999). Several other studies also point to a similar natural course for MEN1-related and sporadic gastrinomas (Stabile&Passaro 1985; Ruszniewski *et al.* 1993; Yu *et al.* 1999; Ellison, *et al.* 2006). In contrast, in one series a survival benefit was found for MEN1 patients, but separate baseline characteristics were not provided (Melvin *et al.* 1993). Another series also found survival benefit in MEN1, with no significant baseline differences in liver metastases (MEN1 6% vs sporadic 24% P=0.24), but this might be due to small number of patients and selection or referral bias cannot be excluded (Singh *et al.* 2012). Overall the available data seem to point to a similar natural course for MEN1-related and sporadic gastrinoma.

Prognostic factors

The most important adverse prognostic factor related to overall survival in MEN1-related dpNETs is the presence of liver or distant metastases (Stabile&Passaro 1985; Cadiot *et al.* 1999; Kouvaraki *et al.* 2006; Triponez *et al.* 2006*a*; Ito *et al.* 2013). In a series including all subtypes of dpNETs, the estimated 10-year survival for patients with distant metastases was 34% (Kouvaraki *et al.* 2006). In patients with diffuse liver metastases from gastrinoma, 10- and 15-year survival of 88% and 52% is reported, while in patients with metastases from NF-pNETs eight-year survival was 34% (Norton *et al.* 2001; Triponez *et al.* 2006a). Lymph node metastases are not related to survival (Gibril, *et al.* 2001; Kouvaraki *et al.* 2006; Ito *et al.* 2013). Contradictory evidence exists with regard to the prognostic value of age. An older age (Burgess, *et al.* 1998*a*; Cadiot *et al.* 1999; Kouvaraki *et al.* 2006; Vierimaa *et al.* 2007) as well as younger age are reported as adverse prognostic factors (Gibril *et al.* 2001; Ito *et al.* 2013). Reports regarding the prognostic significance of pancreatic tumor size vary. No relation between tumor size and metastases, malignancy or overall survival is found in several series reporting on MEN1-related dpNETs (hormonally active and NF; Grama *et al.* 1992; Lowney, *et al.* 1998; Lairmore, *et al.* 2000; Bartsch *et al.* 2005; Kouvaraki *et al.* 2006; Lopez *et al.* 2011). In the subset of NF-pNETs larger tumor size was related to a higher rate of metastases and a decreased overall survival (Triponez *et al.* 2006*a*; Triponez, *et al.* 2006*b*). In gastrinoma series pancreatic tumors size >3 cm was found to be associated with an adverse outcome (Cadiot *et al.* 1999; Gibril *et al.* 2001; Ito *et al.* 2013). However, it is unclear if these pNETs used as prognostic indicator were all gastrinomas and not (in part) coexisting NF-pNETs. Moreover, results from liver biopsy immunohistochemistry are often not reported, so the origin of the metastases cannot be verified. In the natural course of gastrinoma, an aggressive and non-aggressive variant based on tumor growth can be distinguished, with high prognostic relevance (Weber *et al.* 1995; Sutliff, *et al.* 1997; Yu *et al.* 1999; Gibril *et al.* 2001). In MEN1-related gastrinomas aggressive disease is reported in 15% and in sporadic gastrinomas in ~25% (Yu *et al.* 1999; Gibril *et al.* 2001; Ito *et al.* 2013). In MEN1 patients, 5-year survival was 100% for patients with non-aggressive disease and 88% for patients with aggressive disease (Gibril *et al.* 2001). Factors found to be associated with aggressive disease were pancreatic tumor size, liver and bone metastases, markedly increased fasting gastrin level, and the presence of a gastric NET (Gibril *et al.* 2001). Mitotic count or Ki67 labeling index has proved to be a very important prognostic factor in sporadic dpNETs (Ekeblad, *et al.* 2008; Scarpa, *et al.* 2010; Rindi *et al.* 2012), but no information is available for MEN1-related dpNETs.

With regard to possible prognostic value of genotype, results are contradictory. Nonsense and frameshift mutations in exon 2, 9, and 10 were found to be associated with a more malignant dpNET phenotype (Bartsch, *et al.* 2000; Bartsch *et al.* 2005) and inactivating and frameshift mutations showed a trend towards more frequent occurrence in deceased patients (Ito *et al.* 2013). Others did not find any

relation between genotype and the course of dpNETs (Lairmore *et al.* 2000; Kouvaraki, *et al.* 2002).

Molecular background of MEN1

Menin

The *MEN1* gene product, menin, is highly conserved from nematodes and fruit flies to humans. Interestingly, the gene is absent in organisms like yeast and *Caenorhabditis elegans*. It is predominantly a nuclear protein, which is ubiquitously expressed in both endocrine and non-endocrine organs (Guru, *et al.* 1998; Stewart, *et al.* 1998; Ikeo, *et al.* 2000). It has been challenging to elucidate its biological function, as menin lacks enzymatic activity and initially no homologous domains to other proteins were found. Abolition of menin during mouse embryogenesis is lethal at mid gestation and results in defects of neural tube, liver, and heart (Bertolino, *et al.* 2003*a*). Its function is tissue-specific, sometimes showing opposite effects between different organs. Many interacting proteins involved in gene transcription and various signaling pathways have been identified (Matkar, *et al.* 2013). Recently, the crystal structure of menin has been elucidated (Murai, *et al.* 2011; Huang, *et al.* 2012). Menin contains a deep pocket that can bind Mixed-Lineage Leukemia 1 (MLL1 or KMT2A) protein or the transcription factor (TF) JUND, with opposite effects on gene transcription (Huang *et al.* 2012). Further, evidence

Box 2. Gene transcription regulation

In the nucleus of eukaryotic cells, DNA is wrapped around octamers of histone proteins to form nucleosomes. Chromatin is formed by repeating nucleosomes to form beads on a string structures that are converted to the higher order chromatin structures. Chromatin architecture is dynamic and changes in chromatin status influence gene transcription activity (Fig. 1).

Gene transcription in eukaryotic cells depends on formation of the so-called pre-initiation complex, which consists of RNA polymerase II and general TFs in relation to the chromatin context. The formation and recruitment of the pre-initiation complex to DNA of gene promoters is modulated by cofactors. These processes are initiated when DNA sequence specific TFs (eg JUND) bind to their corresponding response element on the DNA upstream of the promoter region. Several mechanisms are required for tight control of transcription regulation in a gene specific and tissue-specific manner. Chromatin status is one important mechanism, as DNA accessibility is a prerequisite for gene transcription.

Post-translational covalent modifications of histone tails are involved in regulation of gene transcription, either directly by changing chromatin packing or through recruitment of other effector proteins to chromatin (chromatin 'readers'; Fig. 1). Histone modifications are 'written' or 'erased' by histone-modifying enzymes (Kouzarides 2007). Menin is involved in trimethylation of lysine 4 on histone 3 (H3K4me3). This methylation mark is associated with activation of gene transcription (Kouzarides 2007). Histone acetylation is correlated with activation of gene transcription and deacetylation of histone tails with transcription repression. Epigenetic alterations, including deregulation of histone modifications contribute to cancer development (Chi, *et al.* 2010). Deregulation of chromatin-modifying complexes by loss of menin is involved in MEN1 tumorigenesis.

supports a role for menin in DNA repair, through association with replication protein A2 (RPA2; Sukhodolets, *et al.* 2003) and Fanconi anemia complementation group D2 protein (FANCD2; Jin, *et al.* 2003). Subsequent functional experiments characterized menin both as an activator and a repressor of gene transcription. Growing evidence indicates that menin is involved in epigenetic regulation of gene transcription as menin has been shown to be part of chromatin-modifying protein complexes (Box 2). However, it is important to note that most studies focusing on menin interaction partners and its function were conducted in non-endocrine cell lines (Table 2).

Menin as an epigenetic repressor of gene transcription

Menin associates with proteins in removing acetylation marks from histones (Gobl, *et al.* 1999; Kim, *et al.* 2003). These histone-deacetylases (HDACs) form complexes with menin through the general co-repressor mSin3A (Kim *et al.* 2003; Fig. 2A). Deacetylation of histones at promoters of target genes is associated with downregulation of gene transcription. *GAST* (gastrin) was identified as a potential target of menin/mSin3A/HDAC complexes (Mensah-Osman, *et al.* 2011).

Recently, menin was shown to interact directly with protein arginine methyltransferase 5 (PRMT5), resulting in repression of the Hedgehog signaling pathway through increasing PRMT5-mediated dimethylation of arginine 3 on histone 4 (H4R3me2) at the *Gas1* and *Gli1* promoter (Gurung, *et al.* 2013*a*; Gurung, *et al.* 2013*b*; Fig. 2B). The Hedgehog signaling pathway is involved in various biological processes including (neuroendocrine) tumorigenesis (McMillan&Matsui 2012). Menin can be recruited to the promotor of the homeobox gene *GBX2* through interaction with the histone lysine methyltransferase SUV39H1. This interaction induced H3K9 trimethylation at the gene promoter, providing the repressive chromatin environment for downregulation of *GBX2* transcription (Yang, *et al.* 2013; Fig. 2C).

Menin as an epigenetic activator of gene transcription

Menin stably associates with MLL1 and MLL2 (KMT2B) containing protein complexes (Hughes, *et al.* 2004; Yokoyama, *et al.* 2004). The functional domain in MLL protein family members is the so-called SET domain that harbors histone methyltransferase activity for trimethylation towards lysine 4 of histone 3 (H3K4me3; Ruthenburg, *et al.* 2007). H3K4me3 is associated with activation of gene transcription (Santos-Rosa, *et al.* 2002; Guenther, *et al.* 2007). MLL translocations leading to MLL1-fusion proteins are frequently seen in mixed lineage leukemia (Krivtsov&Armstrong 2007). In contrast to other menin interactors, the menin-MLL1/2 interactions are rather stable and have been detected in several cellular systems (Hughes *et al.* 2004; Yokoyama *et al.* 2004). The menin-MLL1/2 complexes induce trimethylation on H3K4, and menin disease-derived mutants fail to recruit histone methyltransferase activity (Hughes *et al.* 2004). Genome-wide analysis

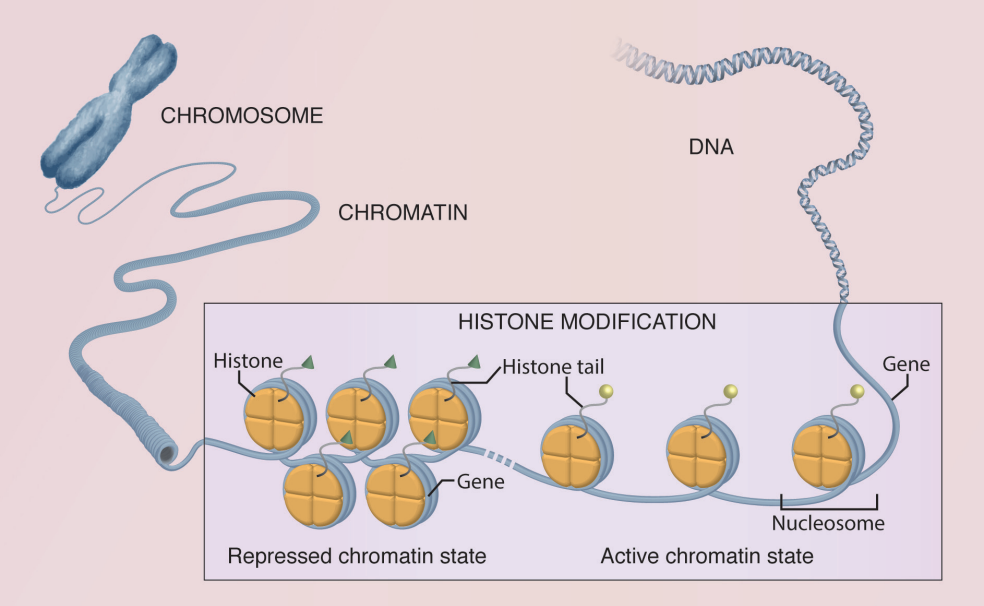
showed menin occupancy on promoters of many active genes, which is often accompanied with MLL1 or MLL2 and H3K4me3 (Scacheri, *et al.* 2006; Agarwal&Jothi 2012). Menin-MLL1/2 complexes are positive regulators of several target genes, including genes of the *HOX* cluster (Hughes *et al.* 2004; Yokoyama *et al.* 2004) and cyclin-dependent kinase (CDK) inhibitor genes (Milne, *et al.* 2005; Fig. 3A). *HOX* genes are characterized by a conserved DNA sequence, the homeobox. They encode homeodomain-containing TFs, which are essential in cell differentiation and the body plan during embryogenesis. Several *HOX* genes are identified as direct menin-MLL1/2 targets, such as *HOXA9*, Hoxc6, and Hoxc8 (Hughes *et al.* 2004; Yokoyama *et al.* 2004; Huang *et al.* 2012). It was shown in pancreatic islet-like endocrine cells that *HOX* gene expression is regulated by menin through H3K4 methylation (Agarwal&Jothi 2012). Menin-MLL1 complexes stimulate the expres-

Table 2. Cell systems used to study menin interaction partners

Reference	Protein complex	Cell type (origin)	Menin level
Hughes *et al.* (2004)	Menin-MLL2	293T (human embryonic kidney)	Endogenous
Yokoyama *et al.* (2004)	Menin-MLL1/2	K562 (myelogenous leukemia)	Endogenous
Milne *et al.* (2005)	Menin-MLL1/2	HeLa (human cervical cancer)	Endogenous
Yokoyama&Cleary (2008)	Menin-MLL1/2 -PSIP1	REH cells (human leukemia)	Endogenous
Huang *et al.* (2012)	Menin-MLL1 -PSIP1	Recombinant protein	*In vitro*
		293T (human embryonic kidney)	Overexpressed
van Nuland, *et al.* (2013)	Menin-MLL1/2	HeLa (human cercival cancer)	Overexpressed
Agarwal *et al.* (1999)	Menin-JUND	293T (human embryonic kidney)	Endogenous, overexpressed
Gobl *et al.* 1999)	Menin-JUND	Recombinant protein	*In vitro*
Mensah-Osman *et al.* (2011)	Menin-JUND	AGS (human gastric adenocarcinoma)	Overexpressed
Huang *et al.* (2012)	Menin-JUND	Recombinant protein	Bacterial expressed
		293T (human embryonic kidney)	Overexpressed
Kim *et al.* (2003)	Menin-HDAC -mSin3A	293T (human embryonic kidney)	Overexpressed
Kaji *et al.* (2001)	Menin-Smad3	Cos7 (monkey kidney)	Overexpressed
Heppner *et al.* (2001)	Menin-NFκB	293 (human embryonic kidney)	Endogenous, overexpressed
Sierra *et al.* (2006)	Menin-β-catenin	CRC (human colorectal cancer)	Endogenous
Dreijerink *et al.* (2006)	Menin-ERα	Recombinant protein	*In vitro*
Dreijerink *et al.* (2009)	Menin-PPARγ	Recombinant protein	*In vitro*
Gurung *et al.* (2013*b*)	Menin/PRMT5	293 (human embryonic kidney)	Endogenous, overexpressed
Yang *et al.* (2013)	Menin-SUV39H1	293T (human embryonic kidney)	Endogenous, overexpressed
Shi *et al.* (2013)	Menin-Hlbx9	MIN6 (mouse insulinoma)	Endogenous, overexpressed

This table summarizes studies referred to in this review.

Figure 1. Chromatin structure and histone modifications



DNA is wrapped around octamers of histones into nucleosomes. Chromatin state is influenced by post-translational modifications of histone tails. These modifications are associated with chromatin accessibility for effector proteins such as transcription factors and lead to an active or a repressed chromatin state. For simplicity, not all histone tails are represented in this figure. Adapted from the National Institutes of Health Common Fund Website, source: http://commonfund.nih.gov/epigenomics/figure.aspx, with permission.

sion of *CDKN1B* and *CDKN2C* genes encoding $p27^{Kip1}$ and $p18^{Ink4c}$ proteins respectively. Loss of function of menin or MLL1 resulted in downregulation of $p27^{Kip1}$ and $p18^{Ink4c}$ and displayed effects on cell growth (Milne *et al.* 2005). $p27^{Kip1}$ and $p18^{Ink4c}$ belong to two distinct families of CDK inhibitors which regulate cell-cycle progression (Besson, *et al.* 2008). Reduced expression of these proteins contributes to tumor development in various tissues (Malumbres&Barbacid 2001).

Recruitment of menin-chromatin modifying protein complexes to target genes

Proteins can be recruited to gene promoters through specific interactions with DNA sequence-specific TFs. Menin stably interacts with the DNA sequence-specific TF JUND (Agarwal, *et al.* 1999; Huang *et al.* 2012; Fig. 2A). Several MEN1-derived missense mutants failed to bind JUND efficiently *in vitro* and their repressive effect on transcription was lost (Agarwal *et al.* 1999). Interactions between menin and other TFs are less stable than menin-JUND interactions. Menin can be tethered to DNA through the nuclear receptor (NR) for estrogen ERα, the NR peroxisome-proliferator-activated receptor gamma (PPARγ), and the vitamin D3 receptor (Dreije-

Figure 2 . Menin in epigenetic repression of gene transcription

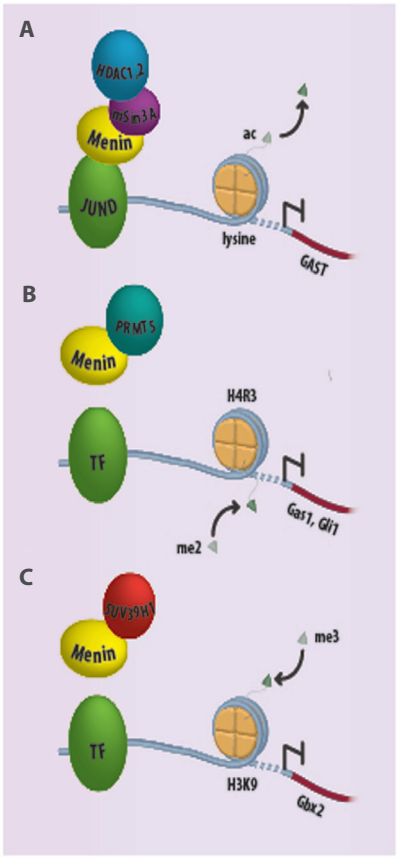


Figure 3. Menin in epigenetic activation of gene transcription

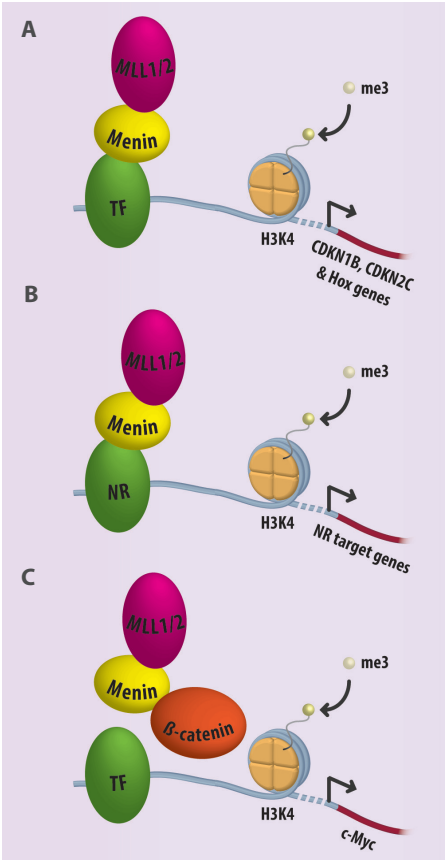


Fig. 2A Menin transiently interacts with mSin3A and represses *GAST* transcription via recruitment of HDAC1,2 and acetylation (ac) of histone tails. The protein complexes bind to the transcription factor (TF) JUND.
Fig. 2B Menin transiently interacts with PRMT5 and represses gene transcription of *Gas1* and *Gli1* through dimethylation (me2) of histone H4R3. In this case it is not known to which TF the protein complexes bind.
Fig. 2C Menin transiently interacts with SUV39H1 and represses gene transcription of *Gbx2* through trimethylation of histone H3K9. For this cases it is not known to which TF the protein complexes bind.

Fig. 3A Menin interacts with MLL1/2, which results in trimethylation (me3) of histone H3K4 (H3K4me3) and activation of transcription of *CDKN1B*, *CDKN2C* and *HOX* genes. The interacting transcription factor (TF) is not known in this case.
Fig. 3B Menin-MLL1/2 complexes bind to nuclear receptors (NRs), induce H3K4me3, and activate transcription of NR-target genes.
Fig. 3C Interaction of menin-MLL1/2 protein complexes with β-catenin activates *c-Myc* transcription through H3K4me3. The interacting TF is not known in this case.

rink, *et al.* 2006; Dreijerink, *et al.* 2009; Fig. 3B). NRs have the ability to bind DNA directly and translate changes in hormone levels into alterations in gene transcription. Transcriptional activation through menin-NR interactions is associated with H3K4me3 upregulation (Dreijerink *et al.* 2006; Dreijerink *et al.* 2009). Furthermore, menin-MLL1/2 complexes are transcriptional co-activators of the Wnt-signaling pathway. Together with the TF CTNNB1 (β-catenin), the menin-MLL2 complex was shown to be recruited to the enhancer of the oncogene *c-Myc* (Sierra, *et al.* 2006; Fig. 3C). Menin interacts and regulates NFKB1 (NF-κB) TFs (Heppner, *et al.* 2001). Transforming growth factor beta (TGFβ) signaling causes inhibition of proliferation in various cell types. Menin interacts with the TGFβ-regulated TF SMAD3. Inactivation of menin in pituitary cells disrupted SMAD3 binding to DNA, thereby blocking TGFβ signaling (Kaji, *et al.* 2001). Recently, transcription factor Hlxb9 was shown to be a menin interaction partner specifically in mouse β-cells and to be involved in regulating β-cell proliferation rate and expression of insulin-modulating genes (Shi, *et al.* 2013).
Besides DNA sequence-specific TF mediated recruitment of menin-MLL1/2 complexes to target genes, interactions with chromatin binding protein PC4 and SFRS1 interacting protein 1 (PSIP1; also known as LEDGF/ p75), are important for tethering these complexes to target genes. The transcription co-activator PSIP1 co-localizes with menin-MLL1 complexes at specific menin target genes, including *HOXA9*, *CDKN1B* and *CDKN2C* (Yokoyama&Cleary 2008; Huang *et al.* 2012). The association of several menin mutants with PSIP1 was disrupted, resulting in reduced transcription of *HOXA9* (Yokoyama&Cleary 2008).

Contribution of menin loss to NET development

Several studies have addressed the role of *MEN1* in endocrine pancreatic cell function and proliferation. Absence of *MEN1* does not seem to affect the initial pancreatic differentiation process from embryonic stem cells *in vitro* (Agarwal&Jothi 2012). Beta-cell specific disruption of *MEN1* leads to the formation of insulinomas (Bertolino, *et al.* 2003*b*; Crabtree, *et al.* 2003; Biondi, *et al.* 2004). Alpha-cell specific knockout of *MEN1* was found to lead to transdifferentiation into insulin-producing cells and subsequent insulinoma development (Lu, *et al.* 2010). Disturbance in epigenetic regulation of gene transcription is thought to contribute to MEN1-associated tumorigenesis. The most convincing evidence supporting this mechanism was reported recently (Lin, *et al.* 2011). Mice with β-cell specific knockout of *MEN1* showed reduced tumor formation and increased survival in combination with gene knock-out of the retinoblastoma-binding protein 2 (RBP2 also known as JARID1A, KDM5A), which is a histone demethylase for H3K4me2/3. This indicates that compensation of the loss of H3K4 trimethylation mark on certain target genes may restore the function of menin in pancreatic tumors. Identification of relevant menin target genes could provide further insight into the development of MEN1 related tumors. Currently, it is not clear how the tumor-suppressing roles of menin in cultured cells are related to suppression of MEN1-associated tumor

development. *HOX* genes are important for the development of endocrine organs (Manley&Capecchi 1998). Comparison of *HOX* gene expression profiles in MEN1 associated parathyroid tumors and non-familial parathyroid tumors, revealed differently expressed genes between these groups. This indicates a role for *HOX* genes in MEN1-associated parathyroid tumor development (Shen, *et al.* 2008). This has not been shown for other NETs. Several animal studies support that menin target genes *CDKN1B* and *CDKN2C* are involved in endocrine tumorigenesis. $p27^{kip1}$ or $p18^{ink4c}$ deficient mice develop pituitary tumors and hyperplasia in multiple organs, including the thymus, without elevation in GH levels (Fero, *et al.* 1996; Kiyokawa, *et al.* 1996; Nakayama, *et al.* 1996; Franklin, *et al.* 1998). Strikingly, mice lacking both $p27^{kip1}$ and $p18^{ink4c}$ developed hyperplasia and/ or tumors predominantly in endocrine organs including the pancreas and duodenum. The tumor spectrum seen in these mice showed remarkable overlap with the tumor spectrum seen in MEN1 patients (Franklin, *et al.* 2000). Inactivating germline mutations in *CDKN1B* have been identified in patients with a MEN-like phenotype. Although hyperparathyroidism and pituitary tumors are the most commonly described manifestations (Pellegata, *et al.* 2006; Georgitsi, *et al.* 2007), pNETs have also been described in association with *CDKN1B* mutations (Agarwal, *et al.* 2009; Occhi, *et al.* 2013). Based on these studies, a role for $p27^{kip1}$ and $p18^{ink4c}$ in MEN1-related tumor development seems reasonable. Menin-MLL1/2 complexes inhibit proliferation of pancreatic islet cells in mice by promoting H3K4me3 and transcription of $p27^{kip1}$ and $p18^{ink4c}$ (Karnik, *et al.* 2005). Interestingly, $p18^{ink4c}$ and menin collaborate in repressing development and growth rate of mouse pNETs. This synergetic effect was not observed with $p27^{kip1}$ (Bai, *et al.* 2007). Studies focusing on $p27^{kip1}$ protein and mRNA expression in pNETs from MEN1 patients show conflicting results (Milne *et al.* 2005; Lindberg, *et al.* 2008; Occhi *et al.* 2013).

Tissue selectivity in MEN1-related tumorigenesis

Regarding the ubiquitous expression of menin, it is difficult to explain the tissue selectivity of tumorigenesis in MEN1 patients. Unfortunately, most studies focusing on menin interaction partners and its target genes are performed in nonendocrine cell lines (Table 2 and Supplementary Table 1). Menin acts as a tumorsuppressor in endocrine organs, but it is an essential oncogenic cofactor in leukemogenesis (Yokoyama, *et al.* 2005; Yokoyama&Cleary 2008). Understanding the predominance for endocrine tumor development resulting from *MEN1* loss, might help to develop targeted therapies for MEN1 patients. Several factors have been suggested as potential important players in the tissue selectivity of this endocrine tumor syndrome (Gracanin, *et al.* 2009). Tissues may differ in their ability and requirement to compensate for the loss of one *MEN1* allele (Gracanin *et al.* 2009). Physiological regulation of menin levels in response to increased insulin was shown to be important in adaptive β-cell proliferation during pregnancy in mice (Karnik, *et al.* 2007). Intriguingly, mice with liver specific loss of menin did not develop tumors (Scacheri, *et al.* 2004). The expression levels of menin in lym-

phoblastic cell lines derived from MEN1 patients did not differ from healthy controls (Wautot, *et al.* 2000) and downregulation of menin could activate the *MEN1* promoter in a compensatory manner in non-endocrine cell lines (Zablewska, *et al.* 2003). However, it has been suggested that menin haploinsufficiency through loss of one *Men1* allele contributes to pNET development in mice (Crabtree *et al.* 2003; Lejonklou, *et al.* 2012). In regard to tissue-specific regulation of menin expression, microRNAs are interesting candidates for further evaluation (Gracanin *et al.* 2009; Luzi&Brandi 2011). Menin interaction partners might be involved in the tissue-specific tumor formation in MEN1. For example, the TF HLXB9 was shown to be a β-cell specific menin interaction partner (Shi *et al.* 2013). NRs are also potential candidates as they have tissue specific functions.

Implications for further research

Although in the past decade significant progress has been made in understanding menin function, many questions remain. Its tumor suppressive role in endocrine organs is not well understood and elucidating underlying biology should be an important focus for future studies. Regarding the observed tissue selectivity in MEN1-related tumorigenesis, it is important to study menin-protein interactions and target genes in endocrine cell lines specifically. To date, most studies addressing menin interactions and target genes were performed in non-endocrine cell lines. Not only basic research projects but also translational studies in unbiased MEN1 patient cohorts are needed. These studies should clarify which molecular pathways involving menin actually contribute to MEN1 NET tumorigenesis and are clinically relevant. With regard to novel therapeutic strategies, the involvement of altered epigenetic regulation of gene expression resulting in MEN1 tumorigenesis is an interesting candidate for further evaluation. The development of compounds that interfere with epigenetic regulation of gene transcription has gained a lot of attention recently and such drugs have shown to have therapeutic potential in cancer treatment (Dawson&Kouzarides 2012). These findings highlight the importance of better insight into MEN1 tumorigenesis for the improvement of MEN1 patient care.

From a clinical point of view, identifying natural course and prognostic factors has been hampered by the rarity of the disease and generally low number of events regarding distant metastases and disease-related mortality. Therefore it is important to follow large unselected cohorts over a long period of time, by national or even international collaboration.

When comparing the natural history of MEN1-related NETs to their sporadic counterparts, insulinomas in MEN1 seem to be more aggressive, while natural history in MEN1-related gastrinomas seems to be similar to sporadic gastrinomas. Data on NF-pNETs and thoracic NETs are insufficient to permit comparisons. However, currently available evidence does not support MEN1-related NETs to be more indolent than sporadic NETs.

Among MEN1-related NETs, thNETs occur with low frequency and show a remarkable gender difference. Compared to other NETs their prognosis is poor. These different epidemiologic and natural history characteristics cannot be explained with the currently available evidence and warrants further research.
Pulmonary carcinoids and NF-pNETs in MEN1 share the fact that they are more common than previously thought. Identification of these NETs will further increase in the coming decade due to increased sensitivity of imaging techniques and standardized screening. As little is known about the natural history of small NETs in MEN1, clinical significance of these findings remains to be determined. To assist clinical decision-making in this respect, studies with a long-term follow-up in unselected patient cohorts are needed.
All dpNETs are potentially malignant and dpNETs are the most important determinant of long-term survival in MEN1 patients. Although the estimated 10-year survival rate is 75%, it is important to remember that MEN1-patients are usually in their thirties when these tumors develop. Moreover, unless a total duodenopancreatectomy is performed, MEN1 patients are always at risk for developing new dpNETs and subsequent malignant transformation. This means that a satisfactory 10-year survival rate not automatically equals normal life expectancy. Although, the percentage of MEN1 patients with dpNETs that develop distant metastases is small, prognosis is poor in this group. At present, apart from tumor size, there are no known clinical or tumor characteristics that reliably predict the development of distant metastases. This means that the impact of therapeutic interventions has to be weighed against the overall change of distant metastases and disease-related mortality. Identification of additional clinical and molecular prognostic factors in MEN1-related dpNETs, should therefore be an important research focus. Factors known to be of prognostic value in sporadic dpNETs should be validated in MEN1 and new prognostic indicators sought for. These efforts should lead to early identification of tumors with an aggressive phenotype and subsequent individualized patient care based on risk stratification.

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Supplementary table 1 Cell types used to study target genes of menin-protein complexes

Reference	Protein complex	Cell type (origin)	Menin level	Technology
Hughes *et al.* (2004)	Menin-MLL2	MEFs, wt or menin null (mouse embryonic fibroblast)	Endogenous, re-expression	ChIP with anti-menin Ab at Hoxc8 locus
Milne *et al.* (2005)	Menin-MLL1/2	MEFs, wt or menin null (mouse embryonic fibroblast)	Endogenous, re-expression	ChIP with anti-menin Ab and anti-MLL Ab at Cdkn1b and Cdkn2c loci
Yokoyama&Cleary (2008)	Menin-MLL1/2	U937 (human lymphoma)	Endogenous	ChIP with anti-menin Ab, anti-MLL Ab and anti-PSIP1 Ab at HOXA, MEIS1, CDKN1B and CDKN2C loci
Huang *et al.* (2012)	Menin-MLL1-PSIP1	MEFs, menin null (mouse embryonic fibroblast)	Re-expression	ChIP with anti-menin Ab and anti-MLL Ab at Hoxc8 locus
Mensah-Osman *et al.* (2011)	Menin-JUND	AGS (human gastric carcinoma)	Overexpression, knockdown	ChIP with anti-menin Ab and anti-JUND Ab at gastrin gene locus
Scacheri *et al.* (2006)	Menin	HeLa (human cervical cancer)	Endogenous	ChIP-chip with anti-menin Ab
		HepG2 (human hepatocellular carcinoma)	Endogenous	ChIP-chip with anti-menin Ab
		Human pancreatic islets	Endogenous	ChIP-chip with anti-menin Ab
		Mouse pancreatic islets, wt or menin null	Endogenous	ChIP-chip with anti-menin Ab
Sierra *et al.* (2006)	Menin-β catenin	C2C12 (mouse myoblast)	Endogenous	ChIP with anti-menin Ab at c-Myc locus
Dreijerink *et al.* (2006)	Menin-ERα	MCF7 (human breast cancer)	Overexpression, knockdown	ChIP with anti-menin Ab and anti-ERα Ab at TFF1 locus
Dreijerink *et al.* (2009)	Menin-PPARγ	MEFs, wt or menin null (mouse embryonic fibroblast)	Endogenous, re-expression	ChIP with anti-menin Ab and anti-PPARγ Ab at Fabp4 and Aqp7 loci
Gurung *et al.* (2013b)	Menin-PRMT5	MEFs, menin null (mouse embryonic fibroblast)	Endogenous, re-expression	ChIP with anti-menin Ab and anti-PRMT5 Ab at gastrin gene locus
Gurung *et al.* (2013a)	Menin-PRMT5	MEFs, menin null or floxed (mouse embryonic fibroblast)	Endogenous, re-expression	ChIP with anti-menin Ab and anti-PRMT5 Ab at Gli1 locus
		PIME1, floxed (mouse pancreatic islet)	Endogenous	ChIP with anti-menin Ab and anti-PRMT5 Ab at Gli1 locus
(Yang *et al.* (2013)	Menin-SUV39H1	MEFs, wt or menin null (mouse embryonic fibroblast)	Endogenous, knockout, re-expression	ChIP with anti-menin Ab and anti-SUV39H1 Ab at Gbx2 locus

This table summarizes studies referred to in this review Ab, antibody; ChIP, chromatin immunoprecipitation; wt, wildtype

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Chapter 7

Natural course and survival of neuroendocrine tumors of thymus and lung in MEN1 patients.

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Natural course and survival of neuroendocrine tumors of thymus and lung in MEN1 patients.

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Abstract

Context

The natural course and survival of neuroendocrine tumors (NETs) of thymus (Th) and lung in multiple endocrine neoplasia type 1 (MEN1) patients are still unknown.

Objective

Our objective was to assess prevalence, tumor growth, and survival of Th and lung NETs in an unselected MEN1 population with long-term follow-up.

Design

This was an observational study.

Patients and Methods

A longitudinal study was performed using the Dutch national MEN1 database, including >90% of the Dutch MEN1 population >16 years of age. Patients under care of the Dutch University Medical Centers (1990–2011; *n*=323) were included.

Main Outcome Measures

The prevalence and survival of Th and lung NETs were assessed. Linear mixed-models analysis was applied to assess tumor growth with age as a possible confounder and gender, genotype and baseline tumor size as possible effect modifiers.

Results

Th NETs occurred in 3.4% of patients, almost exclusively in males with a 10-year survival of 25% (95% confidence interval = 8%–80%). A thoracic computed tomography scan was available in 188 patients (58.2%). A lung NET was identified in 42 patients (13.0%) with a 10-year survival of 71.1% (95% confidence interval = 51%–100%). Tumor volume of lung NETs increased 17% per year $P<.001$; tumor doubling time 4.5 years). Tumor doubling time in males was 2.5 vs 5.5 years in females ($P = .05$). Lung NET growth was not associated with genotype or with baseline tumor size (< 1 vs ≥ 1 cm).

Conclusion

In MEN1 patients, Th NETs almost exclusively occurred in males and had a very low prevalence and a high mortality. Lung NETs occurred more often than previously thought, had an indolent course, and occurred equally in both sexes. Tumor growth in males was double compared with female patients.

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant inherited disease, which is caused by the inactivation of the *MEN1* gene located on chromosome 11q13 encoding the tumor suppressor protein menin[1]. The classic clinical manifestations are parathyroid hyperplasia or adenomas leading to primary hyperparathyroidism, neuroendocrine tumors (NETs) of pancreas and duodenum, and pituitary adenomas[2]. However, the clinical spectrum of MEN1 includes more than 20 different tumors, including adrenal hyperplasia and adenomas, gastric NETs, thymic (Th) NETs, and lung NETs. Patients with MEN1 have a shorter life expectancy than the general population, mainly caused by the malignant potential of the MEN1-related NETs[3–5]. The 20-year survival of MEN1 patients is estimated to be 64%[3].

Even though Th and lung NETs are relatively rare in MEN1, with an estimated prevalence of 2.8% to 8% and 1.4% to 9.5%, respectively, these tumors are of major concern[6–12]. Th NET is an important cause of death among MEN1 patients[4, 13]. The discovery of lung NET often leads to extensive diagnostic imaging and sometimes to (major) thoracic surgery; however, the significance of lung NET for morbidity and survival are yet unclear[5].

Periodic screening for tumor manifestations and subsequent treatment of *MEN1* mutation carriers seems to lead to a more favorable survival[6, 14]. According to the recently published clinical practice guidelines for MEN1, radiological screening for Th and lung NETs is recommended every 1 to 2 years with a thoracic computed tomography (CT) scan or magnetic resonance imaging scan[15].

However, at present, very little is known about the natural course and prognosis of MEN1-related Th and lung NETs[5, 7, 8, 10, 16–19]. Consequently, the optimal screening, follow-up, and treatment of these tumors in MEN1 are unclear up to now[19]. Therefore, in the present study, we assessed prevalence, tumor growth, and survival of patients with Th and lung NETs in a large and unselected cohort of MEN1 patients.

Abbreviations:
CI, confidence interval; CT, computed tomography; DMSG, DutchMEN1 Study Group; MEN1, multiple endocrine neoplasia type 1; NET, neuroendocrine tumor; TNM, Classification of Malignant Tumours; UMC, university medical center.

Patients and Methods

Study design

For the present analysis, patients were selected from the Dutch national MEN1 database of the DutchMEN1 Study Group (DMSG). All MEN1 patients diagnosed according to the recently updated clinical practice guidelines, aged 16 years and older, treated at one of the Dutch university medical centers (UMCs), were inclu-

ded in the database[15]. In each UMC, MEN1 patients were identified by a standard identification procedure using the hospital diagnosis databases. This longitudinal database with 21 years of follow-up includes >90% of the total Dutch MEN1 population[20]. Clinical and demographic data were collected retrospectively by medical record review in a standardized manner using predefined definitions. Data of all identified patients were collected from every quarter of every available year of follow-up during the period 1990 to 2011. The study protocol was approved by the medical ethical committees of all UMCs in the Netherlands. Detailed information on the DMSG database methods have been described previously[21].

Patient selection

Patients with Th NET were diagnosed based upon the results of a pathology examination. Patients were considered to have a lung NET if (1) pathology examination showed lung NET or (2) radiological examination was positive for lung NET. Positive radiology was defined as a lesion on CT scan suspicious for lung NET at the discretion of a senior radiologist. MEN1 patients with histological proven lung metastases from other NETs (NET with pancreatic, gastric, or duodenal origin) were excluded from the analysis. Patients with other NETs in their history and lung lesions on imaging without histology were reviewed by an expert panel and excluded if the lesions were more suspect for metastases than a primary lung NET. All pathology results from thymic and lung NETs were reviewed by an expert panel, and revision of pathology was performed when tumor classification was in doubt. Follow-up of patients with Th NET and lung NET was assessed from the moment of diagnosis according to the predefined inclusion criteria.

Outcome measures

Primary outcomes were 10-year survival and the growth rate of Th and lung NETs. Furthermore, we assessed the influence of gender, age at MEN1 diagnosis, prophylactic thymectomy, age at Th and lung NET diagnosis, type of mutation (according to Human Genome Variation Society nomenclature), possible clustering within families, immunohistochemical outcomes of pathological examinations, Classification of Malignant Tumours (TNM)/World Health Organization (WHO) stage on tumor growth and survival[22–25].

Statistical analysis

Survival was estimated using the Kaplan-Meier method. Survival curves were compared with the log-rank test for gender, tumor size (<10 or ≥10 mm), mitotic rate (<10 or ≥10 high-power fields), tumor extension in adjacent organs, and genotype. Genotype was dichotomized to nonsense and frameshift mutations in exon 2, 9, or 10 vs other mutations, according to previous literature[26, 27].

Linear mixed-models analysis was applied to assess changes over time in volume of preoperative lung NETs. Time was used as a continuous variable and defined in

years. Because of non-normal distribution, logarithmic transformation of the size of the lung nodules was performed. Because of the repeated observations, multilevel analysis was used accounting for clustering within patients. Possible effect modification was assessed for gender, genotype, and the size of the lung nodules ≥10 or <10 mm at the first radiograph.

Clinical characteristics were reported as means and SDs or medians with interquartile ranges when appropriate. Continuous variables were analyzed by using independent-sample *t* test or Mann-Whitney U test. Dichotomous variables were compared with Fisher's exact test or χ^2 test.

In general, statistical significance was set at $P < .05$; however, for the analysis of the interactions in the mixed-model analysis, statistical significance was set at $P < .10$. All analyses were conducted using SPSS version 17.0 and R version 2.9.2.

Results

Study population

A total of 323 MEN1 patients were included in the database. Median age of diagnosis of MEN1 was 38 (range 8–80) years. The patients were part of 121 different MEN1 families. There was a female predominance *(n* = 188, 58.2%).

Thymic NETs

A Th NET was diagnosed in 11 MEN1 patients during follow-up (3.4%). Median age at diagnosis of Th NET was 45.0 (range 36.0–60.0) years. Clinical characteristics of the patients are summarized in Table 1. In 1 case (patient 11), the Th NET was hormonally active showing ectopic production of ACTH. Th NET predominantly occurred in male patients (91%). A total of 97 patients (29.9%) underwent prophylactic transcervical thymectomy during parathyroidectomy (males 26.7% vs females 32.3%). None of these patients developed Th NET during follow-up ($P = .038$) after a median exposed time of 8 (range 0–40) years. The median age at which prophylactic thymectomy was performed was 35.5 (range 17.0–66.0) years, and median age of these patients at end of follow-up was 47.0 (range 20.0–78.0) years. No genotype-phenotype association with the occurrence of Th NET was found; the patients with Th NET were all from different MEN1 families, and all had a different mutation. Additionally, of 8 patients with a Th NET, several family members (median 3, range 1–20) were included in the DMSG database and none developed a Th NET in the course of follow-up.

Median survival of the 7 deceased patients after the diagnosis of Th NET was 4.4 (range 0.75–5.25) years. The Kaplan-Meier curve is shown in Figure 1A. The 10-year survival of patients with Th NET was 25% [95% confidence interval (CI) = 8%–80%] No association was found between survival and genotype, tumor size,

Figure 1.

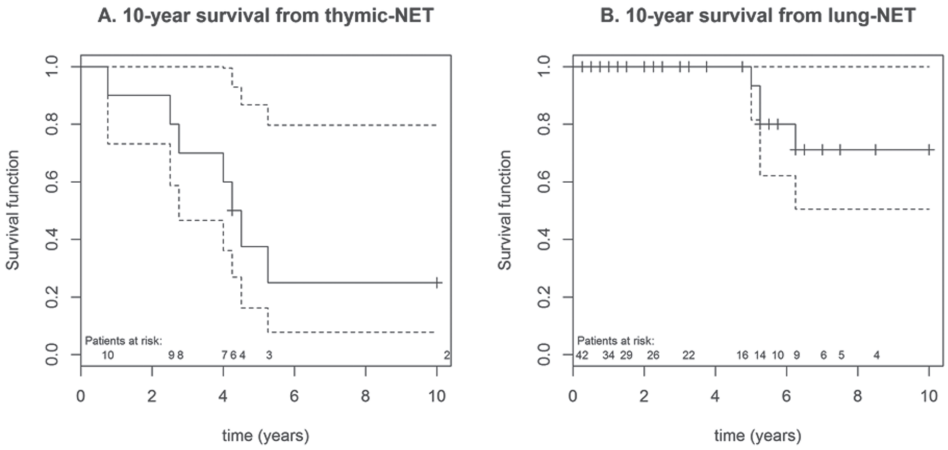

Fig. 1A, Survival curve with 95% CI for MEN1 patients with Th NET. Fig. 2B, Survival curve with 95% CI for MEN1 patients with lung NET. Solid and dashed lines represent the survival curve and 95% CI, respectively; vertical lines represent censored patients.

mitotic rate, or tumor extension into adjacent organs. The growth rate of Th NET could not be assessed because all patients were operated when the diagnosis Th NET was made. Pathological results can be found in Table 1.

Lung NETs

Lung NET was diagnosed in a total of 42 patients (13.3%): in 15 patients by a combination of imaging and pathology, 26 patients by radiological imaging, 1 patient who was operated in 1971 by pathology (original radiological reports were not available). Pathology was obtained by surgery in all histologically proven cases. In 1 patient with a small suspect lesion on imaging, fine-needle aspiration biopsy was performed that showed no tumor cells. Routine diagnostic screening of the thorax for lung NET was performed in 188 of the total 323 MEN1 patients. There were no differences in gender and type of mutation between the patients who were screened and those who were not screened ($P = .78$ and $P = .14$, respectively), whereas the age at MEN1 diagnosis was higher in the unscreened population ($P = .04$). In 51 of the screened patients, pulmonary nodules were identified. Ten patients with pulmonary nodules were excluded from the analysis because the nodules were histologically proven metastases of other NETs ($n = 2$) or were probably metastases from other NETs ($n = 8$).

Forty-eight percent of patients with lung NETs were male. The median age of diagnosis was 42 (interquartile range, 38–48) years. None of the lung NETs were hormonally active. In 26 patients, the tumor size at diagnosis was <10 mm, and in 16, the tumor size was ≥10 mm. Fourteen patients had lung NETs of both lungs (2

Tabel 1. Clinical, genetic and pathological characteristics of patients with thymic NET

Patient	Sex	Genetic mutation	Smoking	Age at diagnosis (y)	Length of Follow-up (y)	Status at end of follow-up	Tumor size on PA (mm)	Lymphadenectomy (positive/ total number of lymph nodes)	Mitotic index (per 10 hpf)	WHO Classification	TNM Classification[a]
1	F	frameshift exon 10: c.1430dupG(p.Glu478fs)	Former smoker	36	10.3	alive	40	Y (2/2)	3	atypical carcinoid	pT2N1cMo
2	M	frameshift exon 2: c.249_252del4(p.Ile85fs)[b]	Current smoker	46	4.3	Died from metastastic Th-NET	155	N	ND	atypical carcinoid	pT2cN0M0
3	M	frameshift exon 8: c.1100_1113dup14(p.Val372fs)	Current smoker	42	4.5	Died from locally advanced Th-NET (tracheal compression)	70	Y (2/4)	9	atypical carcinoid	pT3N1cM0
4	M	frameshift exon 10: c.1561dup(p.Arg521fs)	Former smoker	39	5.3	Died from metastatic Th-NET	100	Y (4/4)	ND	atypical carcinoid	pT3N1cM0
5	M	Missense exon 3: c.482G>A(p.Gly161Asp)	Current smoker	42	4.3	alive	140	Y (2/19)	20	LCNEC	pT1N3cM0
6	M	Nonsense exon 9: c.1258C>T(p.Arg420X)	Non-smoker	57	4.0	Died from metastatic Th-NET	65	N	ND	atypical carcinoid	pT3cNxMx
7	M	Nonsense exon 5: c.810G>A(p.Trp270X)	Non-smoker	60	3.8	Died from metastatic pancreatic-NET	ND	Y (1/1)	ND	atypical carcinoid	pTxN3cM0
8	M	Missense exon 2: c.116T>G(p.Leu39Trp)	Non-smoker	45	15.8	alive	ND	Y (0/2)	7	atypical carcinoid	pT2cN0M0
9	M	frameshift exon 10: c.1680_1683delTGAG (p.Ser560fs)	Current smoker	46	0.8	Died from hypercalcemia and Th-NET	140	N	0	typical carcinoid	pT3cN0M0
10	M	Nonsense exon 9: c.1258C>T(p.Arg420X)	Current smoker	45	2.5	Died from metastatic Th-NET	130	N	13	LCNEC	pT1cN0M0
11*	M	Missense exon 2: c.3G>C(p.Met1?)	unknown	43[c]	0	Died from locally advanced Th-NET (epicardial ingrowth)	ND	N	ND	ND	ND

[a]at diagnosis

[b]based on family mutation (no genetic analysis was performed for this patient)

[c]PA at obduction (age 43)

NET, neuroendocrine tumor; *Th-NET*, thymic neuroendocrine tumor *F,* Female; *M,* Male; *LCNEC,* Large cell neuroendocrine carcinoma; *NA* , Not Applicable; *ND,* Not determined; *y,* years; *PA,* pathology

patients had nodules ≥10 mm in both lungs, 6 patients had bilateral nodules <10 mm, and 6 patients had nodules ≥10 mm at one side and nodules <10 mm at the other side). The median follow-up from the moment of diagnosing lung NET to the end of follow-up was 3.25 years [13 (interquartile range 5–22)quarters]. Table 2 shows the clinical characteristics of patients of whom a histological diagnosis was available. There was no genotype-phenotype association with regard to occurrence of lung NET.

Five patients died during follow-up. Mortality was not related to lung NET, and the overall 10-year survival was 71.1% (95% CI = 51%–100%; Fig. 1B).

In 16 patients a lung NET was surgically removed. The indications for surgery were the intention to cure in 13, diagnostic in 2, and unknown in 1 patient. Most patients undergoing surgery for lung NET were asymptomatic. Only 3 patients had complaints of flushes and diarrhea in the absence of other NETs. These symptoms did not improve after lung NET surgery. In the subgroup of operated patients, 4 patients died and median follow-up was 5.25 years [21 (range 2–145) quarters; Table 2]. Patients not operated for lung NET had a median follow-up of 2.25 (range 1–34) years, and 1 patient died during follow-up. The survival of patients with lung NETs was not different for operated and nonoperated patients ($P = .68$).
Genotype, gender, and tumor size at diagnosis of lung NET did not significantly influence survival (log-rank test $P = .56$, $P = .21$, and $P = .77$, respectively).

Increase of tumor volume of lung NETs was 17% per year (4% per quarter-year; $P < .001$) with a subsequent tumor doubling time of approximately 4.5 years. Lung NET growth rate was higher in males compared with females ($P = .05$) with an increase of tumor volume of 36% for males ($P < .001$) and 13% for females ($P = .01$). This results in a tumor doubling time of approximately 2.5 years in male and 5.5 years in female patients. In stratified analysis, the association between gender and tumor growth was not influenced by age of lung NET diagnosis, indicating that the tumor growth rate was the same in pre- and postmenopausal women. Lung NET growth was not significantly associated with type of mutation ($P = .53$) or with baseline tumor size (≥10 or <10 mm; $P = .60$).

Discussion

This nationwide longitudinal study shows that Th NETs almost exclusively occurred in male MEN1 patients, with a prevalence of 3.4% and a 10-year survival of 25%, indicating that Th NET has a poor prognosis. Lung NET was a more common MEN1 manifestation with a prevalence of 13% in patients who underwent thoracic imaging. Overall 10-year survival of lung NET was 71%, and the cause of death of the deceased patients was not related to lung NET. Increase of tumor volume of lung NET was approximately 17% per year, resulting in an overall tumor doubling

Tabel 2. Clinical, genetic and pathological characteristics of patients with pathological diangnostics of lung NET

Patient	Sex	Genetic mutation	Smoking	Age at diagnosis (yrs)	Length of Follow-up (yrs)	Status at end of follow-up	Type of surgery	Lymphadenectomy (positive/ total number of lymph nodes)	Tumor size on PA (mm)	Mitotic index (per 10 hpf)	WHO Classification	TNM Classification	Relapse
1	F	Frameshift exon 2: c.249_252del(p.Ile85fs)	NS	44	1.3	alive	wedge resection upper left lobe	N	7	1	typical carcinoid	pT1cN0M0	N
2	M	Frameshift exon 10: c.1561dup(p.Arg521fs)	NS	62	6	alive	wedge resection lower left lobe	Y (0/ND)	23	0	typical carcinoid	pT1N0cM0	N
3	F	Frameshift exon 10: c.1430dupG(p.Glu478fs)	NS	42	6.8	alive	wedge resection upper left lobe	N	5	ND	typical carcinoid	pT1(m)cN0M0	Y: nodule <10 mm ipsilateral lung
4	F	Frameshift exon 2: [a] c.249_252del(p.Ile85fs)	NS	43	1.5	alive	unknown	ND	ND	0	typical carcinoid	ND	N
5	M	Deletion exon 1 to 3: c.-110-?_669+?del(p.?)	S	46	5.3	died[b]	wedge resection upper right lobe	N	14	0	typical carcinoid	pT1cN0M0	Y: nodule >10mm ipsilateral lung
6	M	Frameshift exon 10: c.1561dup(p.Arg512fs)	FS	62	5	died[c]	wedge resection	Y (0/11)	18	1	typical carcinoid	pT1N0cN0M0	ND
7	M	Frameshift exon 10: c.1561dup(p.Arg521fs)	S	39	5.3	died[d]	wedge resection middle right lobe	Y (0/11)	30	<1	typical carcinoid	pT1N0cM0	N
8	F	Missense exon 4: c.683T>C(p.Leu228Pro)	NS	54	16	alive	lobectomy upper right lobe	N	7	<2	typical carcinoid	pT1cN0M0	Y: Nodule unknown size ipsilateral lung
9	M	Nonsense exon 6: c.819T>G(p.Y273X) or c.819T>A(p.Tyr273X)	NS	39	7.5	alive	wedge resection in upper and lower left lobe	Y (1/>6)	15	5	atypical carcinoid	pT1N1cM0	Y: Nodule unknown size ipsilateral lung
10	F	Frameshift exon 3: c.653_660del(p.Ala218fs)	NS	25	29	alive	segmentectomy middle right lobe	N	ND	>2	atypical carcinoid	pT1cN0M0	N
11	F	Nonsense exon 8: c.1074C>G(p.Tyr358X)	NS	22	5.3	alive	bilobectomy of middle and lower right lobes	Y (0/7)	14	<1	typical carcinoid	pT1N0pM1	ND
12	F	Splice mutation intron 4: c.799-9G>A(p.?)	NS	38	1	alive	lobectomy upper left lobe	Y (0/6)	25	2	atypical carcinoid	pT1N0cM0	Y: nodule <10 mm ipsilateral lung
13	F	Nonsense exon 2: c.377G>A(p.Trp126X)	FS	52	2.3	alive	lobectomy lower right lobe	Y (1/3)	20	ND	typical carcinoid	pT1N1cM0	N
14	F	Nonsense exon 10: c.1594C>T(p.Arg532X)	NS	41	2.5	alive	lobectomy middle right lobe	Y (0/ND)	12	<2	typical carcinoid	pT1N0cM0	Y: nodule <10 mm ipsilateral lung
15	F	In frame deletion exon 2: c.358_360del(p.Lys120del)	FS	44	0.3	alive	segmentectomy lower left lobe	N	10	0	typical carcinoid	pT1cN0M0	ND
16	M	In frame deletion exon 2: c.358_360del(p.Lys120del)	ND	37	36.3	died[e]	lobectomy middle right lobe	Y (0/5)	35	2	atypical carcinoid	pT2N0cM0	Y: nodule >10mm contralateral lung

[a]based on family mutation (no genetic analysis was perfomed for this patient)

[b-e]Cause of death: [b]adenocarcinoma of unknown origin [c]prostate carcinoma [d]metastatic Thymic NET [e]complicated surgery (not MEN1-related) *NET,* neuroendocrine tumor; *yrs,* years; *CS,* current smoker; *F,* Female; FS, former smoker; *m,* multiple tumors; *hpf,* high-power field; *M,* Male; *N,* no; *ND*, not determined; *NS,* nonsmoker; *PA,* pathology; *VATS,* video assited thoracoscopy; *Y,* yes.

time of approximately 4.5 years. Intriguingly, the lung NET growth rate was much higher in male patients compared with female patients.

Strengths and limitations

In this study, we analyzed the prevalence, long-term course, and survival of Th NET and lung NET in a national cohort of MEN1 patients. To our knowledge, this is the first study assessing the growth rate of lung NET in MEN1 patients. For the DutchMEN1 study group database data of >90% of the total Dutch MEN1 patient population were collected, and this database is therefore a true population-based database, hereby reducing the chance of selection bias. In addition, data were collected according to a predefined protocol for each quartile that was based on the predefined study questions, enabling a reliable modeling of tumor volume.

Some limitations should, however, be discussed. First is the retrospective nature of the study. The decision to perform surgery for lung NET was made at the discretion of the treating physicians. In addition, for the analysis of tumor growth rate, we had to rely on data from imaging studies performed in the course of patient care that were not blinded or standardized. Interpretation of imaging was made by different radiologists who were aware of previous results from the same patient. However, in the analysis, individual repeated observations of the size of lung nodules were taken into account in the multilevel analysis to prevent overestimation of the precision of the outcomes. Furthermore, by excluding patients with radiological suspected and/or histologically proven metastases from another NET, we intended to include only lung nodules of patients highly suspected for lung NET.

Second, biannual radiological screening by CT or magnetic resonance imaging scan for lung and Th NET as recommended by current guidelines was not performed in roughly 40% of our cohort. This concerned patients who were generally followed up during the earlier years of the study. This might have led to an underestimation of the true lung NET prevalence. Still, we found a higher prevalence of lung NET than was expected based on available literature, indicating that the prevalence of lung NET is often underestimated[6, 11–13]. This discrepancy in the prevalence of lung NET is probably caused by the use of plain chest radiographs and only sporadic use of CT scans in the screening for lung NETs by older studies and the improved quality of current CT scans, which enabled us to include more small lesions suspect for lung NET[11, 12, 19]. Pathology was not available for these smaller lesions, but the longitudinal analysis showed that small nodules (<10 mm) had a growth rate similar to larger lesions (≥10 mm), indicating that the small lesions were indeed lung NETs.

Finally, we could not demonstrate a significant correlation between clinical characteristics and survival in Th NET. This should be interpreted with some caution.

Even though we studied a relatively large cohort of MEN1 patients, the absolute number of patients with Th NETs was low.

Comparison with other literature

The prevalence, male predominance, and mean age at diagnosis of Th NET found in our study is in line with previous results from other studies[7, 8, 10, 13, 28, 29]. Survival analysis showed a 10-year survival of only 25%, which is comparable with the 10-year survival of 36% in a previous study[9]. Although 2 previous studies found small clusters of Th NET within MEN1 families, our results do not confirm this[7, 16].

The protective value of prophylactic thymectomy has been subject to debate. In our study, no Th NET was found in patients who underwent preventive cervical thymectomy. Development of Th NET after thymectomy is possible, because usually only a cervical thymectomy is performed during hyperparathyroidism surgery, presumably leaving an intrathoracic part of the thymus behind. Although previous case reports show that prophylactic cervical thymectomy does not completely prevent the development of a Th NET, our data show a significant reduction in risk for Th NET[7, 9, 29, 30]. Also, most patients in our group who underwent prophylactic cervical thymectomy had reached the age at which a Th NET can be expected by the end of follow-up. These findings are supported by another study[16].

The increase in tumor volume over time of lung NETs appeared to be low with an overall tumor doubling time of approximately 4.5 years. Intriguingly, we also found that the growth rate of lung NETs was significantly higher in male patients compared with female patients. Gender-related differences in MEN1 phenotype are increasingly recognized. A recent large MEN1 cohort showed gender-related differences in the prevalence of gastrinoma and insulinoma[31]. To our knowledge, we are the first to report gender-related differences in the natural course of a MEN1-related NET. At present, these gender-related differences have not been explained. The recently discovered interactions between menin and estrogen receptor-α-mediated transcription might play a role in gender-related differences in the MEN1 phenotype[32].

Lung NETs turned out to be a far more common MEN1 manifestation than is currently presumed. In contrast to most previous studies, we also analyzed small lesions identified on imaging classified as lung NET[12, 13, 19, 33–35]. The longitudinal analysis showed that small nodules (<10 mm) had a growth rate that was similar to larger lesions (≥10 mm), indicating that the small lesions were indeed lung NETs. The prevalence of a histological diagnosis of lung NET in our study was 4.9%, which is in line with estimations of lung NET prevalence in previous studies[9, 19]. In contrast to sporadic cases of lung NETs, the lung NETs in our cohort were not clinically functional, which is consistent with previous reports in MEN1[5].

The survival analysis showed that lung NETs have a good prognosis with 10-year

survival of 71.1%, which is consistent with other studies in MEN1 patients[13, 17–19]. Of the deceased lung NET patients, none died because of the lung NET.
Interestingly, the 4 patients with atypical carcinoids also had a relatively good prognosis in our cohort. These patients had mitotic rates of 2% to 5%, which is in the low range for atypical carcinoid. This might explain the good prognosis. Additionally, in recent years, higher 10-year survival rates have been reported in sporadic atypical carcinoids[36]. Data on MEN1-related atypical carcinoids in literature are too scarce to draw conclusions about potential differences in tumor behavior between sporadic and MEN1-related atypical carcinoids.

Clinical implications
Because of the aggressive course of Th NET, frequent imaging is currently advised [15]. However, due to the low incidence of Th NET, especially after cervical thymectomy, the number needed to be repeatedly screened to timely identify a patient with a Th NET is very high. More research on the impact of prophylactic thymectomy on Th NET incidence is necessary to establish a definitive recommendation on screening the total MEN1 population for Th NET. In the current guidelines, surgery is still advised for lung NET in MEN1 patients[15]. However, given the relatively indolent behavior of lung NET in combination with the lack of a beneficial effect on survival and symptoms, the clinical indications and intended benefit of (early) surgery for MEN1-related lung NET, especially in females, can be debated. Specifically, little is still known about the prevalence and course of atypical carcinoids in MEN1.

Conclusion
In conclusion, Th NETs almost exclusively occur in males and have a very low prevalence. However, mortality from Th NET is high. Cervical thymectomy significantly reduces the incidence of Th NET. Lung NETs are much more common than was reported in previous literature but have a good prognosis. Lung NETs occur equally in both sexes, but the increase in tumor volume in males is much higher compared with females, with tumor doubling times of approximately 2.5 years and 5.5 years, respectively.

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Chapter 8

Long-term natural course of small non-functional pancreatic neuroendocrine tumors in MEN1: results from the DMSG

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Long-term natural course of small non-functional pancreatic neuroendocrine tumors in MEN1: results from the DMSG

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Abstract

Background

Pancreatic neuroendocrine tumors (pNETs) are highly prevalent in patients with Multiple Endocrine Neoplasia type 1 (MEN1), and metastatic disease is an important cause of MEN1-related mortality. Especially small non-functional (NF) pNETs pose a challenge to the treating physician and more information is needed regarding their natural course. We assessed long-term natural history of small NF-pNETs and its modifiers in the Dutch MEN1 population.

Patients and Methods

Retrospective longitudinal observational cohort study of patients with small (<2 cm) NF-pNETs from the Dutch national MEN1 database which includes >90% of the Dutch MEN1 population. Modifiers of long-term natural course were analyzed using linear mixed-models analysis.

Results

Growth rate of the 115 included small NF-pNETs from 99 patients was slow (0.4 mm/y; 95% Confidence Interval, 0.15-0.59). Seventy percent of the tumors was stable and a subgroup of 30% of the tumors was growing (1.6 mm/y; 95%, Confidence Interval, 1.1-2.0). No differences in clinical characteristics were identified between growing and stable tumors. Within the subgroup of growing tumors, germline missense mutations were significantly associated with accelerated growth compared to nonsense and frameshift mutations.

Conclusion

The majority of small NF-pNETs are stable at long-term follow-up, irrespective of the underlying *MEN1* genotype. A subgroup of tumors are slowly growing but cannot be identified on clinical grounds. In this subgroup, tumors with missense mutations exhibited faster growth. Additional events appear necessary for pNETs to progress. Future studies should be aimed at identifying these molecular driving events, which could be used as potential biomarkers.

Introduction

Pancreatic neuroendocrine tumors (pNETs) occur sporadically but also in the context of familial tumor syndromes, such as Multiple Endocrine Neoplasia type I (MEN1). MEN1 is caused by inherited mutations in the *MEN1* tumor suppressor gene on chromosome 11, encoding the MENIN protein[1].

In MEN1 pNETs are highly prevalent[2, 3]. Non-functional pNETs (NF-pNETs) are the most frequent type and the pancreas usually harbors multiple tumors[3, 4]. At the age of 80 years the penetrance of pNETs is over 80% and metastatic disease is the most important cause of MEN1-related mortality[2, 3, 5, 6].

A major challenge in determining the optimal management strategy for individual MEN1 patients with pNETs, is the inability to predict natural behavior and metastatic potential[7, 8]. Especially small (<2 cm) NF-pNETs detected through periodical screening pose a challenge to the treating physician. They seem to have an indolent course, but are not devoid of malignant potential[3]. Up until today, based on what is customary in oncology, treatment decisions are based on tumor size and growth. Current expert-based MEN1 guidelines suggest follow-up for NF-pNETs smaller than 1 cm, unless tumors exhibit substantial growth; other groups advocate a conservative approach for tumors up to 2 cm[9, 10]. More information on the natural course of these tumors and factors associated with tumor growth and behavior is necessary to come to an evidence-based personalized treatment strategy.

We aimed to clarify the long-term natural course of small MEN1-associated NF-pNETs and its modifiers in a retrospective national longitudinal cohort study. This knowledge will aid management decisions in MEN1-related small pNETs, but might also prove useful in the subset of sporadic NF-pNETs harboring somatic *MEN1* mutations, because exome sequencing of sporadically occurring pNETs has identified inactivating mutations in the *MEN1* gene in 44% of tumors[11].

Patients and methods

Data were retrieved from the MEN1 database of the DutchMEN1 study group (DMSG), which includes more than 90% of the Dutch MEN1 population. MEN1 was diagnosed according to current guidelines[9]. See previous reports for details regarding this database[12-14]. The study protocol was approved by the ethical boards of all university medical centers in the Netherlands.

Study design and study population

We studied the growth of small (<2 cm) NF-pNETs and the development of new pNETs (incidence) in a retrospective longitudinal observational cohort study.

All patients included in the DMSG database with a pNET were identified based on histopathological analysis or, if unavailable, by consecutive imaging (Supplemental Materials and Methods).

Criteria for inclusion and exclusion

From each patient, the largest tumor in the pancreatic head as well as the largest tumor in the pancreatic body-tail was eligible for inclusion in the growth analysis. Exclusion criteria for tumors for the growth analysis were as follows: (1) baseline size ≥ 2 cm, (2) functional tumor, (3) < 2 scans, (4) unclear tumor location or size.

Functional tumors were defined as follows. An insulinoma was defined as a positive 72-hour fast and curation after surgical resection. Because most gastrinomas in MEN1 have a duodenal origin[15], a pancreatic tumor was only deemed a gastrinoma if immunohistochemistry was positive for gastrin and biochemical cure was obtained for at least six months after its removal. When in doubt regarding functionality, tumors were not included in the growth analysis.

Exclusion criteria for patients for incidence analysis were: (1) <2 scans, (2) unclear tumor numbers, (3) previous pancreatic surgery.

Tumor size and the total number of pNETs were analyzed from the time a tumor was consecutively identified until the termination of follow-up, surgical removal or systemic anti-tumor therapy [with exception of somatostatin analogues (SSA)] for any neuroendocrine tumor (NET). Follow-up time for this study was defined as the time between the first and last scan.

Outcome measures

Primary outcome was the growth rate of NF-pNETs <2 cm and secondary outcome was the number of incident tumors per patient as assessed on computed tomography (CT) or magnetic resonance imaging (MRI). For the analysis the tumor size and number of pNETs on CT/MRI as reported by the senior radiologist was used.

Genotype was dichotomized to nonsense and frameshift mutations vs missense mutations in the *MEN1* gene. Other types of mutations were excluded from this analysis because at present only in case of nonsense, frameshift and missense mutations, the effect on the MENIN protein can be predicted. In combination with loss of the wild type allele, nonsense and frameshift mutations are expected to be more severe and lead to complete absence of MENIN while in case of *MEN1* missense mutations some functional MENIN may still be present, probably leading to a milder phenotype[16, 17]. We also assessed previously reported genotype-phenotype associations[18-21].

Statistical analysis

The development of tumor size over time for each individual tumor was graphically depicted using spaghetti curves, with tumor size in mm on the y-axis and time in quarters on the x-axis.

Linear mixed-models analysis, accounting for clustering of observations within patients, was performed to assess changes of tumor size over time (growth). The assumptions of the model were met. A two-level model with tumor size as the lowest level and tumor as the second level was used. Adjustments for tumor loca-

tion and patient level did not influence outcomes (-2 log likelihood test). The model was constructed with tumor size in millimeters (residuals followed a normal distribution) as dependent variable and time in quarters as independent variable with a random intercept as well as random slopes of time at the tumor level.
In addition, we assessed the best fit for the association between tumor size and time (e.g. linear, second to fifth order polynomials (-2 log likelihood test) and exponential (R^2).
Determinants of growth [gender, age (dichotomized on the median), genotype, concomitant biochemical gastrinoma, newly diagnosed tumor versus tumor visible at first screening, use of SSA] were separately tested as effect modifiers.
Progressive tumors were compared to stable tumors in a subgroup analysis, and determinants of growth were separately tested as effect modifiers in stable and progressive tumors.
Tumor growth exceeding the mean tumor growth in the study population by 1.28SE [corresponding with 80% confidence interval (CI)] was defined as progressive. Sensitivity analysis showed that the identified subgroups did not change if the 70% or the 95% CI was used to identify progressive tumors.

For the assessment of incident pNETs a Poisson mixed-models analysis was used. A two-level model with repeated measurements of tumor number as the lowest level and patient as the second level was used. Subgroups with stable and progressive tumor numbers were defined in the same fashion as the tumor size subgroups.

Clinical characteristics were reported as mean [± standard deviation (SD)] or median [interquartile range (IQR)] as dictated by the distribution. Continuous variables were compared by the independent-sample *t*-test or Mann-Whitney *U* test. Dichotomous variables were compared with Fisher's exact test.

Statistical significance was set at $P < 0.05$. For the analysis of effect modification in the mixed-models analysis, statistical significance was set at $P < 0.10$. All analyses were conducted using IBM SPSS Statistics for Windows, Version 23.0. (IBM Corp. Armonk, NY). Spaghetti plots were constructed in RStudio Version 0.98.501 (RStudio, Inc., Boston, MA).

Results

A pNET was diagnosed in 205 (52%) patients (*n*=94 pathologically confirmed, *n*=107 imaging only, *n*=4 imaging or pathology before 1990 only). Prevalence of a pNET was higher in patients with nonsense/frameshift mutations, compared to patients with missense mutations (Kaplan-Meier survival analysis, Supplemental Fig. 1, log-rank $P<0.05$). Loss to follow-up in the entire cohort was 8% (31/392).

Study population
Flow charts of included patients are shown in Fig.1A (growth analysis) and 1B (analysis of incidence), and patient characteristics are shown in Table 1. More information including genotype, reason for exclusion and tumor size, can be found in Supplemental Table 1.

Growth of NF-pNETs <2 cm
The baseline size of the 115 included tumors was 10 ± 4 mm and the tumors were followed up to a maximum of 16 years (median follow-up 3 years with a median number of 4 scans). Tumor characteristics are shown in Table 2.

Overall a linear relation between time and tumor size was the best fit and most representative for the outcomes of tumor growth over time. From 13 years follow-up onwards, a third order polynomial (growth at the start, followed by a stable period and a second period of growth) seemed to be a better fit. However, this relation was based only on the three tumors with the longest follow up (see Fig. 2A).

The growth curves of the individual pNETs are shown in Fig. 2A, and modeled growth is shown in Fig. 2B. In the total group, the estimated growth was very slow; less than 1 mm per year (0.4 mm/y; 95% CI=0.15 to 0.59; *P*=0.002; (Fig. 2B). None of the assessed effect modifiers was associated with growth rate (Table 3).
Of the 115 included tumors, 35 tumors of 34 patients were progressive vs 80 stable tumors in 65 patients. From the 16 patients of whom two tumors were analyzed, eight patients had one progressive and one stable tumor, seven patients two stable tumors and one patient two progressive tumors. Neither tumor characteristics (Table 4) nor patient characteristics (data not shown) differed between stable and progressive tumors.
Growth curves of the individual progressive pNETs are shown in Fig. 2C and modeled growth is shown in Fig. 2D. Even in progressive tumors, growth was only 1.6 mm/y (95% CI 1.1 to 2.0; $P < 0.001$). Stable tumors did not show any growth during follow-up (estimated tumor increase –0.01 95% C= –0.12-0.11; *P*=0.9).
In the subgroup of progressive tumors, genotype was a significant effect modifier for growth (Table 3). Unexpectedly, growth rate was faster in tumors with germline missense mutations (Table 3; Fig. 2E) compared to nonsense/frameshift mutations.
Associations with previously reported genotype-phenotype associations are shown in Table 3. No effect modification was seen for the other factors assessed in the progressive tumors (Table 3).

Adverse events in NF-pNETs <2 cm
Three patients developed pNET-related liver metastases (Table 1). In one case, apart from the small NF-pNET (stable in size and not surgically removed), no other NETs were present, making this the most likely source of the liver metastases.

In the other two cases, there was a concomitant tumor >2 cm in the pancreatic body-tail and a previously removed tumor >2 cm at baseline, respectively, which could also have been the source of the liver metastases. There was no disease-related mortality after a median follow-up of five years after the first scan (IQR 3-8 years).

Incidence of new pancreatic tumors

Incidence of new tumors was low, with a rate ratio of 1.04 per year. In most patients, the number of pancreatic tumors remained stable, and progression was seen in 36 patients (30%). There was no association between growth of individual tumors and an increasing number of tumors (Fisher's exact test; *P*=1).

Discussion

In this longitudinal cohort study we have shown that most MEN1-related small pNETs (<2 cm) were stable during long-term follow-up. There was a subgroup of tumors that were progressive in size, substantiating the current notion that there may be distinct subtypes of NF-pNETs[9]. In this subgroup, we found that genotype was associated with growth rate.

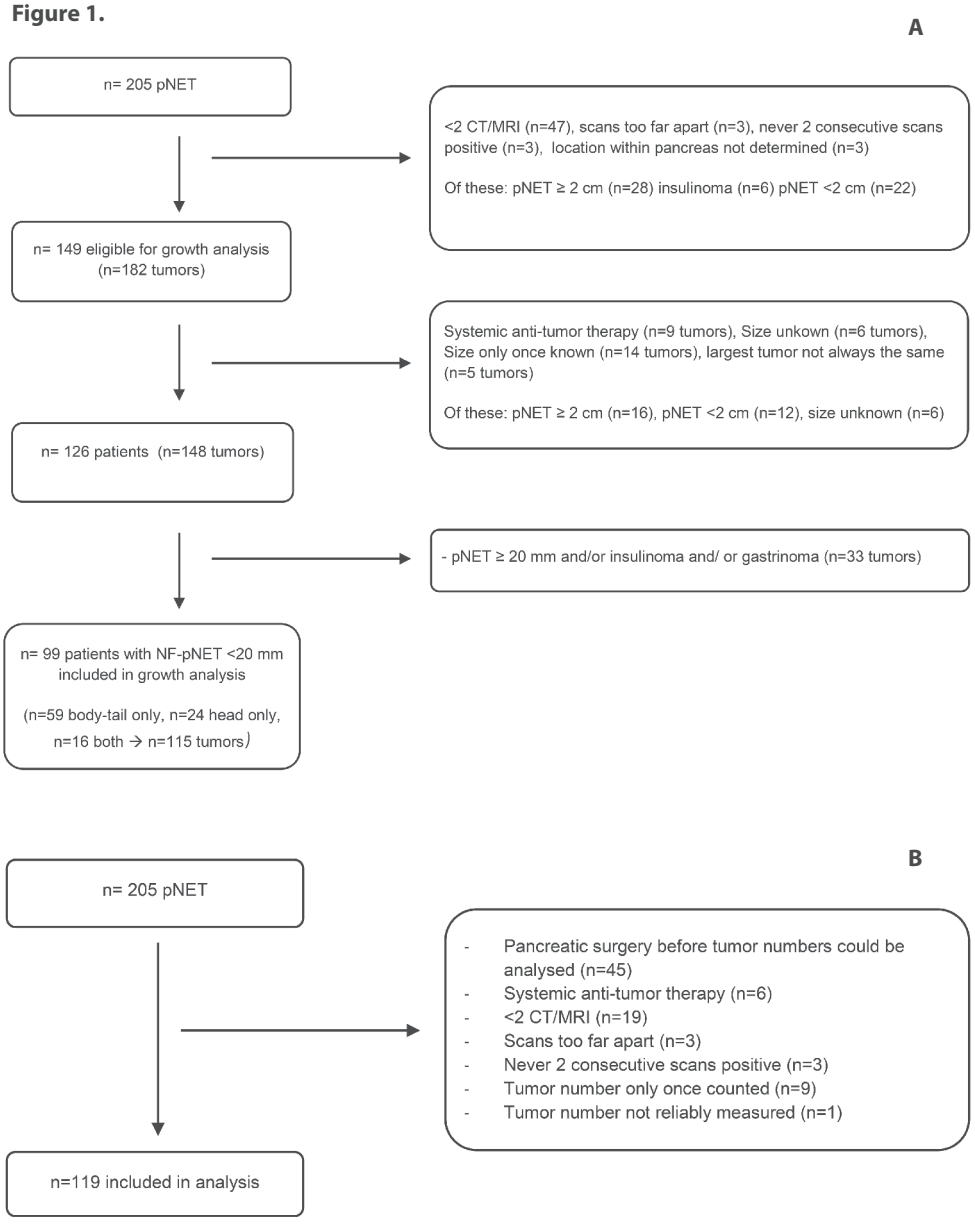
Strengths and limitations

This study represents the largest long-term follow-up study of small NF-pNETs in MEN1 patients. The DMSG MEN1 database includes >90% of the Dutch MEN1 patients population, reducing the chance of selection bias confounding results[13]. Data were collected per quarter according to a predefined protocol that was based on study questions formulated and agreed upon before the start of data collection, enabling reliable modeling of tumor size and minimizing incidental findings. Genotype-phenotype correlations were tested based on predicted effects on the MENIN protein and for those correlations previously reported, minimizing the risk of findings by multiple testing based on chance alone.

In this retrospective analysis, imaging studies were performed at the discretion of the treating physician and evaluation of scans was therefore not standardized. However, Dutch university medical centers are national referral centers for patients with MEN1 as well as for patients with pancreatic tumors, so CTs and MRIs were assessed by senior radiologists experienced in pancreatic imaging. The diagnosis of a pNET was pathologically confirmed in approximately 50%, in the other cases the diagnosis was made based on radiological imaging. Because stringent criteria for the diagnosis were used (consecutively identified pNET) and sensitivity and specificity of CT (73% and 96% respectively) and MRI (93% and 88%, respectively) are high, we are confident that the non-histologically confirmed cases represent true pNETs[13,22].

Because we aimed to analyze the natural course, patients who underwent less than two scans were excluded. Exclusion of tumors (size analysis) or patients (incidence analysis) that could not be followed because of surgical resection might

Figure 1.



CT computed tomography, *MRI* magnetic resonance imaging, *n* number, *NF* non-functional, *pNET* pancreatic neuroendocrine tumor.
Fig. 1A Flowchart growth analysis NF-pNETs <2 cm
Fig. 1B Flowchart incidence analysis

have led to bias. However, because our focus was on NF-pNETs < 2 cm and especially larger tumors are resected in daily clinical practice, we do not believe this is a major concern. On the other hand, one might argue that only very small tumors were included, which might have influenced the results. However, given median tumor size was 10 mm, half of the tumors was between 1 and 2 cm per definition. Because the subgroup of patients with progressive tumors is small, results should be interpreted with caution, because not all effect modifiers might be detectable. To assess the effect of mutations in the JUND and CHES1 interacting domain and missense vs nonsense/frameshift mutations on growth rate, only tumors with germline missense, nonsense or frameshift mutations were included, which might have led to a selection of patients.

Comparison with previous literature

In contrast to the few other small studies reporting on growth rate of NF-pNETs in MEN1, we used CT/MRI as the basis for size evaluation instead of endoscopic ultrasound (EUS). In the previous EUS studies, the reported growth rates were more concurrent with the modeled growth from progressive tumors in our study than with the growth rate of the total group of tumors, underscoring the fact that patients assessed by EUS might represent a selected group of patients[23-26]. A more recent EUS study on the growth of small pNETs, in which some patients of the present study were also included, showed a slower growth rate compared with our study (annual growth rate of 0.1 mm/ y; 95% CI=0.02 to 0.19), which might be due to their smaller baseline size (median 5 mm) [27].

Our results confirm the recently published results of the French Groupe d'étude des tumeurs endocrines, in which 28 of the 39 patients with small NF-pNETs who did not undergo surgery showed stable disease over an average follow-up of ten years[28].

Because of our long-term follow-up and high data density, we were able to asses tumor development over time by multilevel analysis, obtain a model of tumor growth, and assess determinants of growth, which had not been undertaken in other studies.

In the current study we show that in the subgroup of patients with growing tumors, growth rate is correlated with *MEN1* genotype. In this subgroup, missense mutations were associated with a higher growth rate than nonsense/frameshift mutations. Furthermore, nonsense/frameshift mutations in exons 2,9,10 and mutations in the CHES1 interacting domain were associated with slower growth. The latter can be explained by the absence of missense mutations in these subgroups (in the first subgroup by definition; in the second subgroup no missense mutations were present in our cohort). The results contradicted our assumption that in patients with missense mutations a milder phenotype might be seen, because some functioning MENIN is still present[19]. However, we did observe that the prevalence of a pNET was lower in patients with missense mutations when compared

Table 1. Characteristics of Patients With a pNET Included in the Growth Analysis and Analysis of Incidence

	Patients With a pNET Included in the Growth Analysis (*n*=99)	Patients With a pNET Included in the Incidence Analysis (*n*=119)
Gender n (%)		
Male	42 (42)	53 (45)
Female	57 (56)	66 (55)
Age (mean ± SD) at		
MEN1 diagnosis	36 ± 16	37 ± 16
First pNET diagnosed	41 ± 16	42 ± 16
End FUP[1]	48 ± 15	46 ± 15
MEN1 status n (%):		
Mutation negative[2]	3 (3)	3 (3)
Mutation positive	83 (84)	101 (85)
Mutation in family	13 (13)	15 (13)
Genotype n (%)		
Nonsense/frameshift 2,9,10[3]		
Yes	30 (30)	43 (36)
No	65 (66)	72 (61)
JUND interacting domain[4]		
Yes	33 (33)	38 (32)
No	31 (31)	44 (37)
CHES1 interacting domain[5]		
Yes	16 (16)	22 (18)
No	48 (48)	60 (50)
Missense or nonsense/frameshift[6]		
Missense	19 (19)	25 (21)
Nonsense/frameshift	45 (45)	57 (48)
Other NET n (%):		
Duodenal	11 (11)	12 (10)
Gastric	5 (5)	7 (6)
Thymus	0 (0)	3 (3)
Lung	19 (19)	24 (20)
Size median mm (IQR):		Not applicable
Largest tumor	14 (9-18)	
Largest tumor in analysis	13.2 (9-17)	
Largest tumor outside analysis	11.0 (7.2-16.5)	
Functional dpNET[7] n (%):		
Insulinoma	6 (6)	6 (5)
Gastrinoma	21 (21)	28 (24)
Pancreatic Surgery n (%)	29 (39)	31 (26)
Once	24	30
Twice	5	1
pNET-related LM n (%)	3 (3)	10 (8)
pNET-related mortality n (%)	0	7 (6)
FUP in y median (IQR)	13 (7-23)	11 (6-20)

Table 1.

[1] If alive at the end of follow-up

[2] Patients who had two out of three main MEN1 manifestations but negative mutation analysis

[3] All mutations included. Genotype dichotomized to nonsense and frameshift mutations in exons 2,9, and 10 vs other mutations

[4] Only patients with germline nonsense, frameshift and missense mutations included. JUND interacting domain: codons 1–40, 139–242 and 323–428.

[5] Only patients with germline nonsense, frameshift and missense mutations included. CHES1 interacting domain: codons 428–610.

[6] Only patients with germline nonsense, frameshift and missense mutations included.

[7] Prevalence of functional tumors among these patients; functional tumors were not included in the analysis of tumor size. So the tumor included in the growth analysis of these patients was a non--functional tumor.

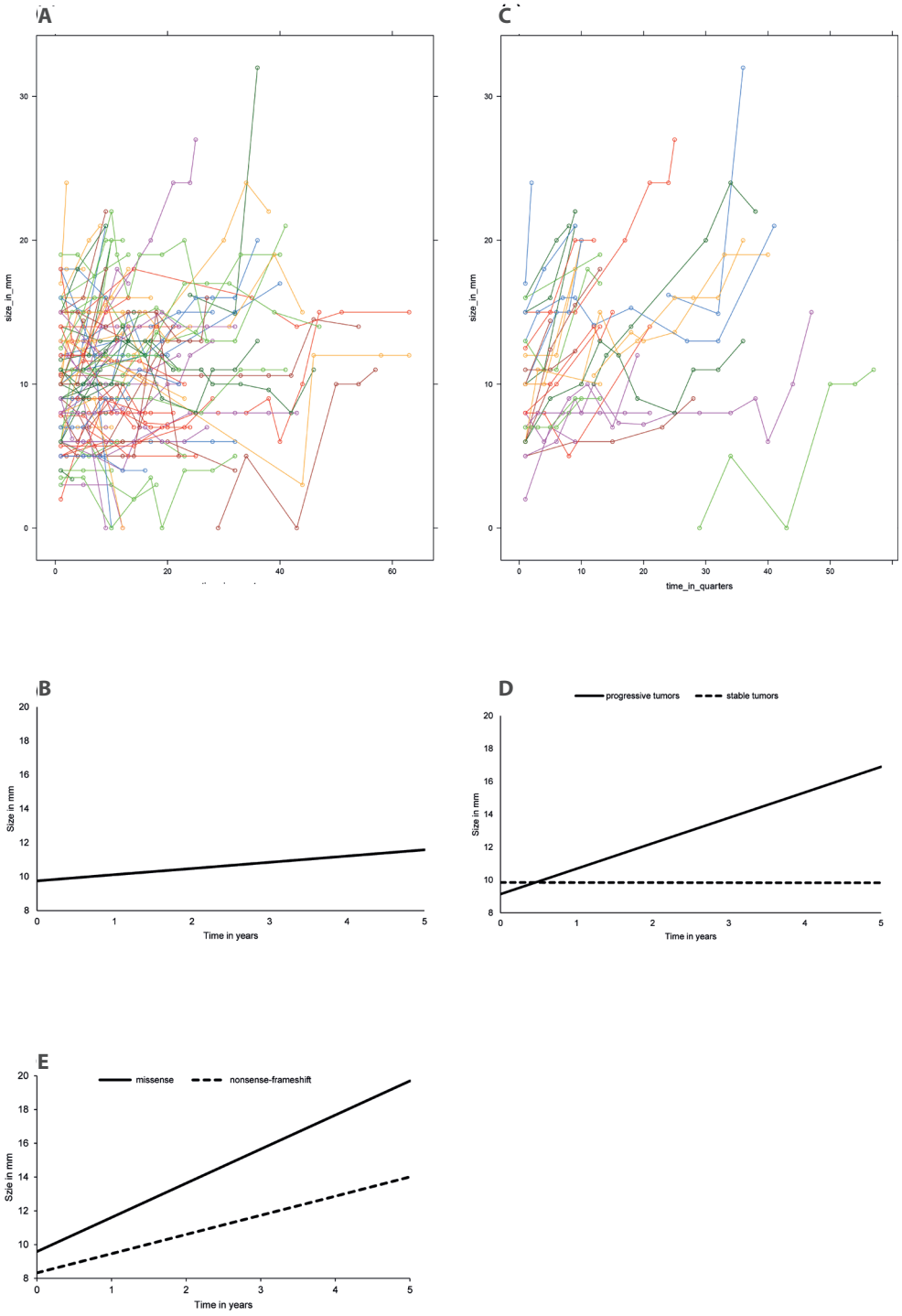
dpNET, duodenopancreatic neuroendocrine tumor; *FUP,* follow-up; *LM,* liver metastases;

Table 2 . Characteristics of NF-pNETs <2 cm Included in the Growth Analysis (115 Tumors From 99 Patients)

	n=115
Location n (%)	
Head	40 (35)
Body-tail	75 (65)
Baseline size mm mean (± SD)	10 (± 4)
Newly diagnosed tumor *n* (%)	
Yes (negative scans before detection)	62 (54)
No (first scan positive)	53 (46)
FUP in y median (IQR)	
of the tumor in the analysis	3 (2-6)
After the first scan, including FUP after analysis of size	5 (3-8)
Number of size measurements median (IQR)	4 (3-6)
2 measurement n (%)	20 (17)
3 - 5 measurements n (%)	62 (54)
≥ 6 measurement n (%)	33 (38)
Surgery of the tumor n (%)	16 (14)
Size largest tumor at pathology in mm median (IQR)	17 (14-27)
WHO 1 | WHO 2 | WHO 3 *n*	12 | 4 | 0
LN metastases no | yes | NA *n*	2 | 4 | 10
Use of SSAS during analysis *n* (%)	18 (16)
Age in y (mean ± SD)	
First measurement	43 ± 16
Last measurement	47 ± 15
Biochemical gastrinoma present n (%)	16 (14)

FUP, follow-up, *LN,* lymph node, *NA,* not available, *WHO,* world health organization.

Figure 2.


A
size_in_mm
C
time_in_quarters
B
Size in mm
Time in years
D
progressive tumors
stable tumors
E
missense
nonsense-frameshift

to patients with nonsense/frameshift mutations. Genotype was not statistically different between patients with growing vs stable tumors. Tumor growth was not associated with increase in tumor numbers, and there were patients with both stable and growing tumors. We therefore speculate that in the multistep process of MEN1 pNET development, tumor initiation and subsequent tumor growth represent distinct steps. In this multistep tumorigenesis process, tumor initiation may be driven by the severity of the effect of the *MEN1* germline mutation on the MENIN protein. However, for growth and progression additional genetic changes could take over as driving factors, and the severity of *MEN1* mutations may be inversely correlated with tumor growth. In larger tumors, mutations in the *DAXX* and *ATRX* genes might constitute these growth-driving events. De Wilde *et al*.[29] showed that *DAXX* and *ATRX* expression was lost in 25% of MEN1-related pNETs larger than 3 cm, but in none of the pNETs less than 3 cm and none of the microadenomas. Thus, it could be that in the setting of growth and progression of MEN1-related pNETs the absence of the MENIN protein is a factor associated with a milder natural course. In previous studies nonsense/frameshift mutations in exon 2, 9, and 10 were reported to be associated with a more aggressive pNET phenotype in a small series that included mostly functional tumors, but also with fewer recurrences after minimal invasive parathyroid surgery[18, 30]. More recently mutations in the CHES1 interacting domain were found to be associated with a more aggressive pNET phenotype also in a mixed group of tumors[20]. These reports may seem contradictory to the present findings but could also reflect many tissue-specific and growth stage-specific aspects of the role of the *MEN1* gene in tumor development.

Figure 2.
A Spaghetti plot: size of NF-pNETs < 2 cm over time
B Modelled growth of NF-pNETs < 2 cm
Growth curve (NF-pNETs) <2 cm. Coefficients derived from linear mixed models analysis. Mathematic representation of the model: tumor size in mm = 9.7 + (0.37*time in years).
C Spaghetti plot: size of progressive NF-pNETs <2 cm over time
D Growth curve of progressive vs stable NF-pNETs <2 cm. Coefficients derived from linear mixed models analysis.
Mathematic representation of the model:
Progressive: tumor size in mm = 9.1 + (1.55 * time in years).
Stable: tumor size in mm = 9.9 + (-0.01 * time in years).
E Growth curve of progressive NF-pNETs <2 cm stratified by genotype. Coefficients derived from linear mixed models analysis. *P*-value for interaction 0.09.
Mathematical representation of the model:
Missense: tumor size in mm = 9.6 + (2.02 *time in years)
Nonsense/frameshift: tumor size in mm = 8.3 + (1.14 *time in years)

Table 3. Results of the Growth Analysis of NF-pNETs <2 cm

	All Tumors *n*=115	Progressive Tumors *n*=35	Stable Tumors *n*=80
Growth mm/quarter (95% CI)	0.09 (0.04-0.15) *P*=0.002	0.39 (0.28 – 0.49) *P*<0.001	-0.001 (-0.03-0.03) *P*=0.9
Effect modifiers (*P*-value for interaction):			Not tested
Gender	*P*=0.7	*P*=0.8	
Male β (SE, 95% CI)	0.08 (0.04, 0.01-0.16)	0.37 (0.08, 021-0.54)	
Female β (SE, 95% CI)	0.11 (0.04, 0.02-0.19)	0.40 (0.07, 0.26-0.54)	
Age	*P*=0.7	*P*=0.4	
< median β (SE, 95% CI)	0.08 (0.04, 0.01-0.16)	0.35 (0.07, 0.21-0.49)	
≥ median β (SE, 95% CI)	0.11 (0.04, 0.02-0.19)	0.43 (0.08, 0.27-0.59)	
Presence of biochemical gastrinoma	*P*=1	Model failed to converge	
Yes β (SE, 95% CI)	0.09 (0.08, -0.08-0.25)	-	
No β (SE, 95% CI)	0.09 (0.03, 0.03-0.15)	-	
Newly diagnosed Tumor	*P*=0.8	*P*=0.7	
Yes β (SE, 95% CI)	0.10 (0.04, 0.02-0.18)	0.40 (0.07, 0.26-0.54)	
No β (SE, 95% CI)	0.09 (0.04, 0.00-0.17)	0.37 (0.08, 0.20-0.53)	
SSA	*P*=0.4	*P*=0.4	
Yes β (SE, 95% CI)	NA	NA	
No β (SE, 95% CI)	NA	NA	
Genotype missense vs nonsense/ frameshift[1]	*P*=0.2	*P*=0.09	
missense β (SE, 95% CI)	0.19 (0.07, 0.04-0.34)	0.51 (0.11, 0.28-0.73)	
nonsense/ frameshift β (SE, 95% CI)	0.08 (0.04, -0.00-0.16)	0.28 (0.05, 0.16-0.40)	
Nonsense/ frameshift exons 2,9,10[2]	*P*=0.4	*P*=0.02	
Yes β (SE, 95% CI)	0.12 (0.05, 0.02-0.21)	0.24 (0.06, 0.10-0.38)	
No β (SE, 95% CI)	0.07 (0.03, 0.00-0.14)	0.48 (0.07, 0.34-0.61)	
JUND interacting domain[3]	*P*=0.6	*P*=0.2	
Yes β (SE, 95% CI)	0.12 (0.05, 0.02-0.22)	0.42 (0.08, 0.26-0.59)	
No β (SE, 95% CI)	0.08 (0.05, -0.02-0.19	0.27 (0.07, 0.12-0.42)	
CHES1 interacting domain[4]	*P*=0.9	*P*=0.08	
Yes β (SE, 95% CI)	0.12 (0.07, -0.03-0.26)	0.22 (0.08, 0.05-0.39)	
No β (SE, 95% CI)	0.10 (0.04, 0.02-0.18)	0.41 (0.06, 0.27-0.54)	

Table 3.

β stands for the coefficient from the Linear Mixed Models analysis, denoting growth in mm/quarter.
[1]Only patients with germline nonsense, frameshift and missense mutations included.
[2]All mutations included. Genotype dichotomized to nonsense and frameshift mutations in exons 2,9 and 10 versus other mutations
[3]Only patients with germline nonsense, frameshift and missense mutations included. JUND interacting domain: codons 1–40, 139–242 and 323–428.
[4]Only patients with germline nonsense, frameshift and missense mutations included. CHES1 interacting domain: codons 428–610.
CI confidence interval, *SE* standard error, *SSA* somatostatin analogues.

Clinical implications

The current MEN1 guidelines recommend imaging studies once a year, whereas the recently updated ENETS guidelines recommend follow-up every 3-12 months in sporadic NF-pNETs < 2 cm.[9, 31] Taking into account the slow growth rate of MEN1-related NF-pNETs < 2 cm and the low number of adverse events, one might consider a less frequent radiological surveillance schedule for these tumors, for example every 2-3 years, possibly with the exception of patients carrying *MEN1* missense mutations. At the moment, no known clinical characteristics can predict the growth of individual tumors, which hampers tailored patient care.
The slow growth of MEN1-related NF-pNETs as shown in the current study combined with the fact that recent studies in independent cohorts show that surgery is not beneficial for NF-pNETs < two centimeters leads to the conclusion that from now on, surgery should not be standard treatment for MEN1-related NF-pNETs less than two centimeters[23-28, 32].

More than 40% of the sporadic pNETs harbor *MEN1* mutations[11]. Aberrant MENIN expression seems to be an early event in tumorigenesis of sporadic pNETs[33], whereas (as in MEN1-related pNETs) loss of *ATRX/DAXX* seem to be relatively late events[29, 33]. Therefore, at least a subset of sporadic pNETs share some genetic changes with MEN1-related pNETs, making data generated in MEN1-related pNETs relevant for this group as well. Although care should be taken in extrapolating hypotheses based on data of MEN1-related NF-pNETs to sporadic pNETs, it is interesting to note that, in a small series of sporadic NF-pNETs used for exome sequencing, tumors harboring mostly inactivating *MEN1* gene mutations showed a trend towards less aggressive behavior compared with patients with tumors without *MEN1* mutations[11]. This is in line with our thought that the absence of MENIN could be beneficial in later stages of tumorigenesis.

Treatment decisions regarding small NF-pNETs in MEN1 are currently based on "simple" clinical characteristics, such as tumor size and growth, which do not seem sufficient. Future research should therefore focus on finding driving factors for growth of MEN1-related pNETs as well as factors identifying patients at risk of future liver metastases to enable personalized cancer care. The results of our study underscore the previously recognized need of circulating multianalyte bio-

Table 4. Characteristics of Progressive vs Stable NF-pNETs <2 cm

	Progressive Tumors (*n*=35)	Stable Tumors (*n*=80)	*P*
Location: *n* (%)			0.29
Head	15 (43)	25 (31)	
Body-tail	20 (57)	55 (69)	
Stable tumor also, *n* (%)	8 (24)	-	
Baseline size mm mean (± SD)	9.9 (3.9)	10.1 (4.1)	0.80
n of size measurements, median (IQR)	4 (3-6)	4 (3-6)	0.86
Germline mutation *n*			
Nonsense/frameshift exons 2,9,10 yes/no	12/20	22/57	0.37
JUND interacting domain, yes/no	12/11	26/24	1
CHES1 interacting domain, yes/no	7/16	10/40	0.38
missense | nonsense/ frameshift	7/16	14/36	1
Presence of biochemical gastrinoma, *n* (%)	3 (9)	12 (15)	0.55
FUP analysis median (IQR)	3 (2-6)	4 (2-6)	0.75
FUP patient after t1, median (IQR)	5 (3-8)	5.5 (3-8)	0.95
Tumor surgically removed, *n* (%)	10 (29)	6 (8)	0.01
Age y mean (± SD)			
first measurement	41 (± 15)	44 (± 16)	0.34
last measurement	45 (± 15)	48 (± 15)	0.25

FUP follow-up, *IQR* interquartile range, *n* number, *SD* standard deviation

markers and the clinical use of microRNA and circulating tumor cells that would allow for accurate characterization of the evolution of these tumors[34].

Conclusion

In general, small NF-pNETs in MEN1 have a slow growth rate, and most tumors remain stable over time. Liver metastases were identified in only one percent of the patients after a median follow-up of five years. Thirty percent of the tumors were growing, but clinical and genetic characteristics cannot distinguish these tumors from stable tumors. Patients with *MEN1* missense germline mutations had faster growing tumors. A more intensive imaging regimen may be appropriate for such patients. Results of our study suggests that genetic or epigenetic events additional to the *MEN1* germline mutation are required for progression of pNETs. Future studies should be aimed at identifying these driving events as this could result in the development of novel biomarkers for the follow-up and management of these tumors.

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Disclosure Summary
W.W. de H. received honoraria from Ipsen and Novartis, has received research funds to his institution from Ipsen and Novartis, has travelled with Ipsen and Novartis. K.M.A.D. has a consulting/advisory role with Eisai. P.H.B. has a consulting/advisory role with Ipsen. B.H., has travelled with Ipsen. G.D.V. has received research funds to his institution from Ipsen and has travelled with Ipsen. The remaining authors have nothing to disclose.

Supplemental Materials and Methods

DutchMEN1 Study Group (DMSG) Database

The DMSG database contains longitudinal data from 1990-2014. For the main Multiple Endocrine Neoplasia type 1 (MEN1) manifestations [neuroendocrine tumors (NET), primary hyperparathyroidism (pHPT), pituitary adenomas (PIT) and adrenal cortical adenomas (ADR)] data of all identified patients regarding laboratory values, imaging results, surgical procedures and pathology results were collected for every quarter of every available year of follow-up. For patients with a follow-up starting before 1990, cross-sectional data were collected on whether manifestations were present, the quarter of diagnosis and the therapies received.

The following definitions were applied to identify the presence of MEN1 manifestations.

For all imaging studies the original reports were used, no review of images was performed.

Pancreatic NET

1990-2014:

A pNET is present

- when pathology results (surgical/biopsy) are positive and/or
- positive Computed Tomography (CT) or Magnetic Resonance imaging (MRI) or endoscopic ultrasound (EUS) confirmed at least once on subsequent imaging within three years. No negative imaging is allowed between the first two positive imaging studies. An imaging finding is disregarded as a pNET if two positive imaging studies are followed by two negative studies within three year without surgical resection.
- If only one imaging study was available and no pathology was available, this was score as a pNET if the imaging report was unequivocal and both the first and last author (CP, GV) agreed.

The time of diagnosis is the first of two consecutive positive imaging studies or the moment of positive pathology; whichever comes first.

<1990: If so noted in the cross-sectional data.

Insulinoma. Abnormal 72-h fast.

Duodenal NET (irrespective of functional status)

1990-2014:

A duodenal NET is present:

- When pathology results (surgery /biopsy) are positive.
- If no positive pathology results are available and positive esophagogastroduodenoscopy (EGD; or rarely CT/MRI) is confirmed at least once within three years (without negative EGD/CT/MRI in between and not followed by two consecutive negative imaging studies without surgical intervention) and somatostatin receptor scintigraphy (SRS) is positive on this location, this is also considered positive for dNET.

The time of diagnosis is the first of two consecutive positive imaging studies or the moment of positive pathology; whichever comes first.

<1990: When pathology results are positive.

Gastrinoma

1990-2014:

A gastrinoma is present if

- gastrin is >10 times the upper limit of normal (ULN) OR
- gastrin is >2 times the ULN twice consecutive in the absence of proton pump inhibitor use (no value <2ULN allowed in between) and not followed by two consecutive measurements <2ULN without surgery or start of systemic anti-tumor therapy OR

- gastrin is >5 times the ULN twice consecutive in the presence of proton pump inhibitor use (no value <5ULN allowed in between) and not followed by two consecutive measurements <5ULN without surgery or start of systemic anti-tumor therapy.
- A duodenal, pancreatic or gastric NET with positive immunohistochemistry for gastrin is considered as a pathologically proven gastrinoma as well as lymph node or liver metastases that are positive for gastrin on immunohistochemistry.

The time of diagnosis is the first of two consecutive elevated gastrin levels according to the definition or the moment of positive pathology; whichever comes first.

<1990: If documented in the cross-sectional data based on a combination of symptoms, elevated serum gastrin and evidence of increased acid secretion.

Gastric NET
1990-2014:
A gastric NET is present if:
- Pathology results (surgery/biopsy) are positive.
- If no positive pathology results are available and positive EGD (or rarely CT/MRI) is confirmed at least once within three years (without negative imaging studies in between and not followed by two consecutive negative imaging studies without surgical intervention) and SRS is positive on this location, this is also considered positive for gastric NET.

The time of diagnosis is the first of two consecutive positive imaging studies or the moment of positive pathology; whichever comes first.

<1990: If so noted in the cross-sectional data.

Thymic NET
1990-2014:
A thymic NET is present if:
- Surgical pathology results are positive and/or
- a thNET has been observed twice on consecutive CT/MRI scans within three years (no negative imaging allowed in between) and not followed by twice consecutive negative imaging without surgery.

The time of diagnosis is the moment of positive pathology or the first of two consecutively positive imaging studies whichever comes first.

<1990: If so noted in the cross-sectional data.

Primary lungNET
1990-2014:
A primary lungNET is present if:
Pathology (surgical/biopsy) results are positive and/or
- A pulmonary nodule consistent with a lungNET according to radiology reports is seen twice consecutively on CT/MRI within three years (no negative imaging in between) and is not followed by two consecutive negative imaging studies without surgery.
- If only one imaging study was available and no pathology was available, this was scored as a lungNET if the imaging report was unequivocal and both the first and last author (CP, GV) agreed.
- If no pathology results are available and the patient also has a pancreatic NET with liver metastases (LM) or the patient has a concurrent thymic NET, the lung nodules are not considered lungNETs because no certain distinction can be made between a primary lungNET and metastases of a thymic or pancreatic NET. Long nodules were also not considered lungNETs in patients who had a non-MEN1 related malignant tumor with distant metastases or a non-MEN1 related malignant tumor which preferentially metastasizes to the lung.

The time of diagnosis is the first of two consecutive positive imaging studies or the moment of positive pathology; whichever comes first.

<1990: If so noted in the cross-sectional data.

Pituitary adenoma
1990-2014:
A pituitary adenoma (PIT) is present if:
- Pathology results are positive and/or
- Radiological examination is compatible with the presence of a PIT and at least confirmed once by consecutive imaging or by elevated hormonal levels (>2 times the standard deviation of the reference values) reflecting hormonal hypersecretion.

<1990: If so noted in the cross-sectional data.

Primary Hyperparathyroidism
1990-2014:
pHPT is present if hypercalcemia is documented combined with elevated or inappropriately non-suppressed parathormone (PTH) levels in two consecutive measurements.

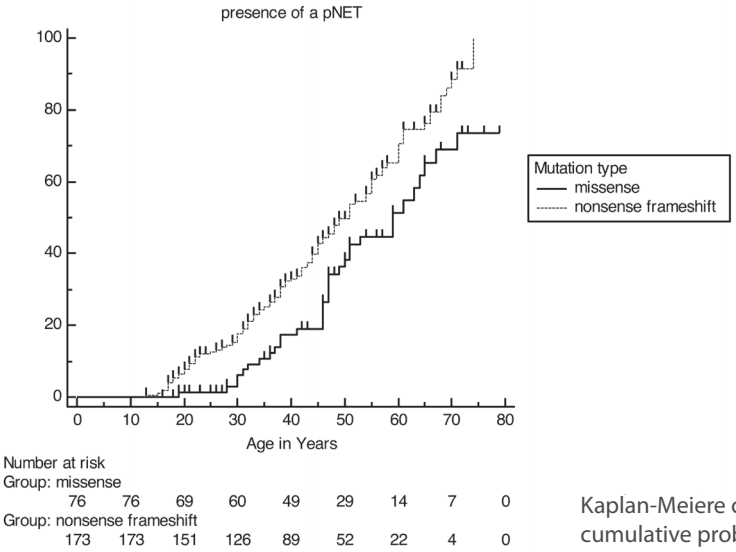
<1990: If so noted in the cross-sectional data.

Metastases of a NET
1990-2014:
Metastases of a NET to the lymph nodes, liver, peritoneal surface, bone and other locations were all defined in the same manner.
- Metastases would be considered present if pathology (either surgical or biopsy) was positive.
- If not, metastases had to be twice consecutively seen on CT/MRI/EUS within three years (without negative imaging studies in between and not followed by two consecutive negative imaging studies without intervention).
- If only the last imaging at the end of follow-up unequivocally showed one of the above mentioned metastases, this would also be considered as 'present' if the first and last author (CP, GV) agreed.

Origin of liver metastases
In a given MEN1 patient it might be difficult to determine which NET is responsible for the liver metastases that are present. To determine this, an expert panel was set up. For each patient with NET-related liver metastases a case file was made including information on symptoms, laboratory test, imaging, surgery and pathology over time. These case files were reviewed by the expert panel (WH, MV, GV) and the most likely NET to have caused these liver metastases was identified or it was classified as unkown.

<1990 no data were available on metastatic disease before 1990.

Supplemental Figure 1.



Kaplan-Meiere curve showing the cumulative probability of having a pancreatic neuroendocrine tumor (pNET) by mutation type.

Supplemental Table 1.

pedigree[1]	mutation[2]	mutation type[3]	nonsense/frameshift exon 2,9,10[4]	JUND interacting domain[5]	CHES1 interacting domain[6]	missense (ms) vs nonsense/frameshift (ns/fs)[7]	patient[8]	age (yr) at diagnosis first pNET[9]	largest tumor during follow-up (mm)[10]	included in growth analysis[11]	reason exclusion[12]	largest size tumor in growth analysis (mm)[13]
16	c.683T>C(p.Leu228Pro)	missense	no	yes	no	ms	16.1	65	55	no	size ≥ 20	NA
16	c.683T>C(p.Leu228Pro)	missense	no	yes	no	ms	16.3	47	unkown	no	size unknown	NA
16	c.683T>C(p.Leu228Pro)	missense	no	yes	no	ms	16.4	31	0	no	never two consecutive scans positive	NA
35	c.482G>A(p.Gly161Asp)	missense	no	yes	no	ms	35.1	46	77	no	systemic anti-tumor therapy	NA
42	c.958G>T(p.Asp320Tyr)	missense	no	no	no	ms	42.2	51	24	no	size ≥ 20	NA
45	c.965A>G(p.His322Arg)	missense	no	no	no	ms	45.2	64	40	no	size only once known	NA
55	c.1067A>G(p.Tyr356Cys)	missense	no	yes	no	ms	55.1	47	32	no	less than two CT/MRI	NA
59	c.482G>T(p.Gly161Val)	missense	no	yes	no	ms	59.1	28	25	no	less than two CT/MRI	NA
59	c.482G>T(p.Gly161Val)	missense	no	yes	no	ms	59.2	50	26	no	scans too far apart	NA
60	c.116T>G(p.Leu39Trp)	missense	no	yes	no	ms	60.1	46	9	no	insulinoma	NA
69	c.506C>A(p.Ala169Asp)	missense	no	yes	no	ms	69.1	46	17	no	less than two CT/MRI	NA
77	Ala541Thr	missense	no	no	yes	ms	77.1	41	40	no	less than two CT/MRI	NA
89	c.1267G>A(Asp423Asn)	missense	no	yes	no	ms	89.1	34	35	no	size unknown	NA
92	c.1046C>G p.(Thr349Arg)	missense	no	yes	no	ms	92.1	46	39	no	less than two CT/MRI	NA
17	c.-110-?_1848+?del(p.?)	deletion entire gene	no	NA	NA	NA	17.1	64	86	no	systemic anti-tumor therapy	NA
68	c.-110-?_1848+?del(p.?)	deletion entire gene	no	NA	NA	NA	68.1	50	13,2	no	less than two CT/MRI	NA
68	c.-110-?_1848+?del(p.?)	deletion entire gene	no	NA	NA	NA	68.3	74	unkown	no	less than two CT/MRI	NA
33	c.-110-?_669+?del(p.?)	deletion exon 1,2 and 3	no	NA	NA	NA	33.2	20	16	no	size only once known	NA
91	c.461-?_1848+?del	deletion exon 3 - 10	no	NA	NA	NA	91.1	46	0	no	never two consecutive scans positive	NA
4	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	4.1	61	20	no	size ≥ 20	NA
4	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	4.3	40	unkown	no	location within pancreas not determined	NA
5	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	5.1	32	9	no	less than two CT/MRI	NA
5	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	5.2	27	20	no	size ≥ 20	NA
5	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	5.5	50	25	no	less than two CT/MRI	NA
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.3	55	70	no	size ≥ 20	NA
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.4	52	unkown	no	largest tumor not always the same	NA
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.6	19	12	no	location within pancreas not determined	NA
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.8	41	12	no	less than two CT/MRI	NA
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.12	55	27	no	less than two CT/MRI	NA
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.15	15	8	no	less than two CT/MRI	NA
51	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	51.3	31	21	no	location within pancreas not determined	NA
51	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	51.4	34	22	no	size only once known	NA
52	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	52.2	54	50	no	less than two CT/MRI	NA
53	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	53.1	45	35	no	size ≥ 20	NA
57	c.834_835delinsATGACC(p.Glu279X)	in-frame deletioninsertion	no	NA	NA	NA	57.1	30	30	no	less than two CT/MRI	NA
57	c.834_835delinsATGACC(p.Glu279X)	in-frame deletioninsertion	no	NA	NA	NA	57.2	34	65	no	systemic anti-tumor therapy	NA
23	mutation analysis negative	NA	NA	NA	NA	NA	22.3	38	unkown	no	less than two CT/MRI	NA
29	mutation analysis negative	NA	NA	NA	NA	NA	29.1	23	unkown	no	less than two CT/MRI	NA
64	mutation analysis negative	NA	NA	NA	NA	NA	64.1	61	51	no	systemic anti-tumor therapy	NA
75	mutation analysis negative	NA	NA	NA	NA	NA	75.1	48	40	no	less than two CT/MRI	NA
76	mutation analysis negative	NA	NA	NA	NA	NA	76.1	63	11	no	less than two CT/MRI	NA
78	mutation analysis negative	NA	NA	NA	NA	NA	78.1	57	44	no	systemic anti-tumor therapy	NA
81	mutation analysis negative	NA	NA	NA	NA	NA	81.1	82	12	no	less than two CT/MRI	NA
85	mutation analysis negative	NA	NA	NA	NA	NA	85.1	45	38	no	less than two CT/MRI	NA
88	mutation analysis negative	NA	NA	NA	NA	NA	88.1	56	unkown	no	systemic anti-tumor therapy	NA
80	c.1064+1G>A (IVS7+1G>A)	splice	no	NA	NA	NA	80.1	35	20	no	size ≥ 20	NA
84	c.799-9G>A(p.?)	splice	no	NA	NA	NA	84.1	15	13	no	less than two CT/MRI	NA
56	c.113C>T(p.Ser38Phe)	unclassified variant	NA	NA	NA	NA	56.1	47	70	no	systemic anti-tumor therapy	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.1	36	8,2	no	less than two CT/MRI	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.3	13	unkown	no	less than two CT/MRI	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.4	33	21	no	insulinoma	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.8	21	22	no	size only once known	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.10	43	10	no	scans too far apart	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.11	66	unkown	no	scans too far apart	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.12	38	26	no	size ≥ 20	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.13	48	22	no	size ≥ 20	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.14	60	0	no	less than two CT/MRI	NA
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.15	55	35	no	size only once known	NA
10	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	10.1	41	20	no	size ≥ 20	NA
11	c.1677_1684dup8(p.Lys562fs)	frameshift	yes	no	yes	ns/fs	11.2	38	8	no	less than two CT/MRI	NA
12	c.1430dupG(p.Glu478fs)	frameshift	yes	no	yes	ns/fs	12.1	26	55,5	no	size ≥ 20	NA
15	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	15.1	57	9,4	no	less than two CT/MRI	NA
19	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	19.1	46	6,1	no	less than two CT/MRI	NA
19	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	19.4	55	44	no	size ≥ 20	NA
19	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	19.5	49	40	no	never two consecutive scans positive	NA
20	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	20.3	44	27	no	size ≥ 20	NA
20	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	20.4	57	3	no	less than two CT/MRI	NA
20	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	20.5	39	69	no	size ≥ 20	NA
21	c.1430dupG(p.Glu478fs)	frameshift	yes	no	yes	ns/fs	21.1	42	86,4	no	size ≥ 20	NA
21	c.1430dupG(p.Glu478fs)	frameshift	yes	no	yes	ns/fs	21.2	17	8	no	size only once known	NA
21	c.1430dupG(p.Glu478fs)	frameshift	yes	no	yes	ns/fs	21.3	39	37	no	less than two CT/MRI	NA
22	c.1430dupG(p.Glu478fs)	frameshift	yes	no	yes	ns/fs	22.1	48	7	no	size only once known	NA
22	c.1430dupG(p.Glu478fs)	frameshift	yes	no	yes	ns/fs	22.2	43	unkown	no	largest tumor not always the same	NA
25	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	25.2	36	40	no	size ≥ 20	NA
27	c.375_376delAT(p.Ile125fs)	frameshift	yes	no	no	ns/fs	27.1	40	80	no	less than two CT/MRI	NA
31	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	31.2	51	38	no	less than two CT/MRI	NA
31	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	31.3	18	98	no	less than two CT/MRI	NA
41	c.1397_1419del(p.Glu466fs)	frameshift	yes	no	yes	ns/fs	41.4	19	7	no	insulinoma	NA
49	c.1520del(p.Lys507fs)	frameshift	yes	no	yes	ns/fs	49.1	48	160	no	less than two CT/MRI	NA
50	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	50.1	38	60	no	less than two CT/MRI	NA
61	c.517del(p.Leu173fs)	frameshift	no	yes	no	ns/fs	61.2	56	76	no	systemic anti-tumor therapy	NA
61	c.517del(p.Leu173fs)	frameshift	no	yes	no	ns/fs	61.3	21	27	no	size ≥ 20	NA
65	c.378delG(p.Trp126X)	frameshift	yes	no	no	ns/fs	65.1	30	30	no	size ≥ 20 and gastrinoma	NA

pedigree[1]	mutation[2]	mutation type[3]	nonsense/frameshift exon 2,9,10[4]	JUND interacting domain[5]	CHES1 interacting domain[6]	missense (ms) vs nonsense/frameshift (ns/fs)[7]	patient[8]	age (yr) at diagnosis first pNET[9]	largest tumor during follow-up (mm)[10]	included in growth analysis[11]	reason exclusion[12]	largest size tumor in growth analysis (mm)[13]
67	c.207dup(p.Asp70fs)	frameshift	yes	no	no	ns/fs	67.1	20	8	no	less than two CT/MRI	NA
82	c.1555_1556delinsA p.(Pro519fs)	frameshift	yes	no	yes	ns/fs	82.1	47	60	no	less than two CT/MRI	NA
83	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	83.1	45	35	no	less than two CT/MRI	NA
87	c.839G>A p.(Arg280Lys)	frameshift	no	no	no	ns/fs	87.1	61	120	no	size ≥ 20	NA
90	c.696del(p.(lys233fs))	frameshift	no	yes	no	ns/fs	90.1	55	10	no	less than two CT/MRI	NA
7	c.1099A>T(p.Lys367X)	nonsense	no	yes	no	ns/fs	7.4	21	7	no	less than two CT/MRI	NA
7	c.1099A>T(p.Lys367X)	nonsense	no	yes	no	ns/fs	7.5	30	50	no	size ≥ 20	NA
26	c.1323G>A(p.Trp441X)	nonsense	yes	no	yes	ns/fs	26.1	33	25	no	less than two CT/MRI	NA
32	c.1594C>T(p.Arg532X)	nonsense	yes	no	yes	ns/fs	32.1	51	6	no	less than two CT/MRI	NA
32	c.1594C>T(p.Arg532X)	nonsense	yes	no	yes	ns/fs	32.3	17	16	no	size only once known	NA
32	c.1594C>T(p.Arg532X)	nonsense	yes	no	yes	ns/fs	32.4	60	26	no	systemic anti-tumor therapy	NA
34	c.810G>A(p.Trp270X)	nonsense	no	no	no	ns/fs	34.1	34	10	no	less than two CT/MRI	NA
34	c.810G>A(p.Trp270X)	nonsense	no	no	no	ns/fs	34.2	37	unkown	no	less than two CT/MRI	NA
36	c.1074C>G(p.Tyr358X)	nonsense	no	yes	no	ns/fs	36.2	51	73	no	size only once known	NA
36	c.1074C>G(p.Tyr358X)	nonsense	no	yes	no	ns/fs	36.3	46	50	no	size ≥ 20	NA
36	c.1074C>G(p.Tyr358X)	nonsense	no	yes	no	ns/fs	36.5	69	10	no	less than two CT/MRI	NA
36	c.1074C>G(p.Tyr358X)	nonsense	no	yes	no	ns/fs	36.6	22	9	no	less than two CT/MRI	NA
36	c.1074C>G(p.Tyr358X)	nonsense	no	yes	no	ns/fs	36.7	60	50	no	size ≥ 20	NA
58	c.1192C>T(p.Gln398X)	nonsense	no	yes	no	ns/fs	58.1	34	unkown	no	largest tumor not always the same	NA
66	c.1594C>T(p.Arg532X)	nonsense	yes	no	yes	ns/fs	66.1	45	12	no	less than two CT/MRI	NA
70	c.1258C>T(p.Arg420X)	nonsense	yes	yes	no	ns/fs	70.1	42	57	no	less than two CT/MRI	NA
70	c.1258C>T(p.Arg420X)	nonsense	yes	yes	no	ns/fs	70.2	71	28	no	size ≥ 20	NA
70	c.1258C>T(p.Arg420X)	nonsense	yes	yes	no	ns/fs	70.3	31	30	no	size ≥ 20	NA
1	c.552G>T(p.Glu184Asp)	missense	no	yes	no	ms	1.1	36	7	yes	NA	7
8	c.1169C>T(p.Ala390Val)	missense	no	yes	no	ms	8.1	67	13	yes	NA	13
8	c.1169C>T(p.Ala390Val)	missense	no	yes	no	ms	8.2	47	27	yes	NA	27
8	c.1169C>T(p.Ala390Val)	missense	no	yes	no	ms	8.3	63	18	yes	NA	18
16	c.683T>C(p.Leu228Pro)	missense	no	yes	no	ms	16.2	37	6	yes	NA	6
16	c.683T>C(p.Leu228Pro)	missense	no	yes	no	ms	16.5	32	9	yes	NA	9
28	c.1024G>C(p.Ala342Pro)	missense	no	yes	no	ms	28.1	30	11	yes	NA	11
37	c.362T>A(p.Val12Asp)	missense	no	yes	no	ms	37.1	71	21	yes	NA	21
38	c.718G>A(p.Glu240Lys)	missense	no	yes	no	ms	38.1	53	20	yes	NA	20
40	c.3G>C(p.Met1?)	missense	no	yes	no	ms	40.1	51	14	yes	NA	14
42	c.958G>T(p.Asp320Tyr)	missense	no	no	no	ms	42.1	47	15	yes	NA	15
45	c.965A>G(p.His322Arg)	missense	no	no	no	ms	45.1	38	13	yes	NA	13
46	c.545T>C(p.Leu182Pro)	missense	no	yes	no	ms	46.1	38	13	yes	NA	13
46	c.545T>C(p.Leu182Pro)	missense	no	yes	no	ms	46.2	49	22	yes	NA	22
46	c.545T>C(p.Leu182Pro)	missense	no	yes	no	ms	46.3	19	14	yes	NA	14
54	c.1067A>G(p.Tyr356Cys)	missense	no	yes	no	ms	54.1	61	4	yes	NA	4
55	c.1067A>G(p.Tyr356Cys)	missense	no	yes	no	ms	55.2	59	9	yes	NA	9
63	c.550G>C(p.Glu184Gln)	missense	no	yes	no	ms	63.1	30	35	yes	NA	14
89	c.1267G>A(Asp423Asn)	missense	no	yes	no	ms	89.2	59	9	yes	NA	9
17	c.-110-?_1848+?del(p.?)	deletion entire gene	no	NA	NA	NA	17.2	37	12,3	yes	NA	12,3
44	c.-110-?_1848+?del(p.?)	deletion entire gene	no	NA	NA	NA	44.1	33	9	yes	NA	9
44	c.-110-?_1848+?del(p.?)	deletion entire gene	no	NA	NA	NA	44.2	60	14	yes	NA	11
68	c.-110-?_1848+?del(p.?)	deletion entire gene	no	NA	NA	NA	68.2	35	14	yes	NA	14
47	-110-?_460del+?del(p.?)	deletion exon 1 and 2	no	NA	NA	NA	47.1	27	13	yes	NA	13
33	c.-110-?_669+?del(p.?)	deletion exon 1,2 and 3	no	NA	NA	NA	33.1	32	15	yes	NA	9
4	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	4.2	37	12	yes	NA	12
5	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	5.3	55	15	yes	NA	15
5	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	5.4	18	5	yes	NA	5
5	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	5.6	47	16	yes	NA	16
5	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	5.7	46	20	yes	NA	20
5	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	5.8	15	11	yes	NA	11
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.1	34	8	yes	NA	8
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.2	25	14	yes	NA	14
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.5	45	14	yes	NA	14
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.7	43	14	yes	NA	14
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.9	25	18	yes	NA	18
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.10	33	18	yes	NA	16
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.11	39	21	yes	NA	21
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.13	23	6	yes	NA	6
13	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	13.14	14	13,5	yes	NA	13,5
24	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	24.1	36	10	yes	NA	10
39	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	39.1	39	19	yes	NA	19
39	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	39.2	44	8	yes	NA	8
51	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	51.1	34	29	yes	NA	20
51	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	51.2	20	12	yes	NA	12
52	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	52.1	66	10	yes	NA	10
52	c.358_360del(p.Lys120del)	in-frame deletion	no	NA	NA	NA	52.3	37	16	yes	NA	16
30	mutation analysis negative	NA	NA	NA	NA	NA	30.1	25	21	yes	NA	21
86	mutation analysis negative	NA	NA	NA	NA	NA	86.1	26	15	yes	NA	15
93	unknown	NA	NA	NA	NA	NA	93.1	56	10	yes	NA	10
9	c.799-9G>A(p.?)	splice	no	NA	NA	NA	9.1	52	8	yes	NA	8
9	c.799-9G>A(p.?)	splice	no	NA	NA	NA	9.2	76	11,6	yes	NA	11,6
14	c.670-6C>G(p.?)	splice	no	NA	NA	NA	14.1	64	6	yes	NA	6
72	c.1365G>C(p.Gln455His)	unclassified variant	NA	NA	NA	NA	72.1	54	24	yes	NA	24
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.2	18	24	yes	NA	24
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.5	55	9	yes	NA	9
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.6	44	14,5	yes	NA	14,5
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.7	19	17	yes	NA	17
2	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	2.9	70	16	yes	NA	16
6	c.112delT(p.Ser38fs)	frameshift	yes	yes	no	ns/fs	6.1	15	15	yes	NA	15
6	c.112delT(p.Ser38fs)	frameshift	yes	yes	no	ns/fs	6.2	45	6	yes	NA	6
11	c.1677_1684dup8(p.Lys562fs)	frameshift	yes	no	yes	ns/fs	11.1	51	19	yes	NA	19

pedigree[1]	mutation[2]	mutation type[3]	nonsense/frameshift exon 2,9,10[4]	JUND interacting domain[5]	CHES1 interacting domain[6]	missense (ms) vs nonsense/frameshift (ns/fs)[7]	patient[8]	age (yr) at diagnosis first pNET[9]	largest tumor during follow-up (mm)[10]	included in growth analysis[11]	reason exclusion[12]	largest size tumor in growth analysis (mm)[13]
12	c.1430dupG(p.Glu478fs)	frameshift	yes	no	yes	ns/fs	12.2	17	13,7	yes	NA	11
19	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	19.2	37	16	yes	NA	16
19	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	19.3	30	21	yes	NA	21
20	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	20.1	65	13,2	yes	NA	13,2
20	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	20.2	29	7,8	yes	NA	7,8
25	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	25.1	66	15	yes	NA	15
31	c.249_252del(p.Ile85fs)	frameshift	yes	no	no	ns/fs	31.1	68	11	yes	NA	11
41	c.1397_1419del(p.Glu466fs)	frameshift	yes	no	yes	ns/fs	41.1	33	19	yes	NA	3
41	c.1397_1419del(p.Glu466fs)	frameshift	yes	no	yes	ns/fs	41.2	49	15	yes	NA	15
41	c.1397_1419del(p.Glu466fs)	frameshift	yes	no	yes	ns/fs	41.3	44	20	yes	NA	20
48	c.631del(p.Arg211fs)	frameshift	no	yes	no	ns/fs	48.1	17	8	yes	NA	8
48	c.631del(p.Arg211fs)	frameshift	no	yes	no	ns/fs	48.2	21	15	yes	NA	15
61	c.517del(p.Leu173fs)	frameshift	no	yes	no	ns/fs	61.1	20	18	yes	NA	18
67	c.207dup(p.Asp70fs)	frameshift	yes	no	no	ns/fs	67.2	22	18	yes	NA	18
67	c.207dup(p.Asp70fs)	frameshift	yes	no	no	ns/fs	67.3	54	7	yes	NA	7
73	c.1561dup(p.Arg521fs)	frameshift	yes	no	yes	ns/fs	73.1	32	9	yes	NA	9
79	c.1579_1580delGT(p.Val527fs)	frameshift	yes	no	yes	ns/fs	79.1	42	22	yes	NA	22
3	c.1192C>T(p.Gln398X)	nonsense	no	yes	no	ns/fs	3.1	22	33	yes	NA	7
3	c.1192C>T(p.Gln398X)	nonsense	no	yes	no	ns/fs	3.1	25	32	yes	NA	32
7	c.1099A>T(p.Lys367X)	nonsense	no	yes	no	ns/fs	7.1	31	6	yes	NA	6
7	c.1099A>T(p.Lys367X)	nonsense	no	yes	no	ns/fs	7.2	58	11	yes	NA	6
7	c.1099A>T(p.Lys367X)	nonsense	no	yes	no	ns/fs	7.3	32	9	yes	NA	9
7	c.1099A>T(p.Lys367X)	nonsense	no	yes	no	ns/fs	7.6	38	11	yes	NA	11
7	c.1099A>T(p.Lys367X)	nonsense	no	yes	no	ns/fs	7.7	61	18	yes	NA	18
18	c.322C>T(p.Arg108X)	nonsense	yes	no	no	ns/fs	18.1	35	14,4	yes	NA	14,4
32	c.1594C>T(p.Arg532X)	nonsense	yes	no	yes	ns/fs	32.2	60	7	yes	NA	7
32	c.1594C>T(p.Arg532X)	nonsense	yes	no	yes	ns/fs	32.5	28	4	yes	NA	4
32	c.1594C>T(p.Arg532X)	nonsense	yes	no	yes	ns/fs	32.6	23	6	yes	NA	6
36	c.1074C>G(p.Tyr358X)	nonsense	no	yes	no	ns/fs	36.1	16	18	yes	NA	18
36	c.1074C>G(p.Tyr358X)	nonsense	no	yes	no	ns/fs	36.4	45	19	yes	NA	19
43	c.1074C>G(p.Tyr358X)	nonsense	no	yes	no	ns/fs	43.1	68	15	yes	NA	15
62	c.810G>A(p.Trp270X)	nonsense	no	no	no	ns/fs	62.1	61	18	yes	NA	18
62	c.810G>A(p.Trp270X)	nonsense	no	no	no	ns/fs	62.2	27	10	yes	NA	10
66	c.1594C>T(p.Arg532X)	nonsense	yes	no	yes	ns/fs	66.2	74	5	yes	NA	5
70	c.1258C>T(p.Arg420X)	nonsense	yes	yes	no	ns/fs	70.4	32	17	yes	NA	17
71	c.377G>A(p.Trp126X)	nonsense	yes	no	no	ns/fs	71.1	52	9	yes	NA	9
74	c.377G>A(p.Trp126X)	nonsense	yes	no	no	ns/fs	74.1	30	42	yes	NA	7

In this table every patient from our database with a pNET is shown (n=205).
Every row in this table corresponds to a patient.
The table is sorted by whether the patient is included in the growth analysis or not.

1. This column denotes the pedigree the patients belongs to, numbers are random
2. This column shows the mutation in theMEN1 gene for each pedigree

Columns 3 through 9 provide additional information on the mutation type.

3. This column specifies the mutation type for each pedigree.
4. This column specifies whether the mutation is a nonsense/frameshift mutation in exon 2,9,10 (yes) or not (no)
5. This column specifies whether the mutation is in the JUND interacting domain (yes) or not (no)
6. This column specifies whether the mutation is in the CHES1 interacting domain (yes) or not (no)
7. This columns specifies whether the mutation is a missense (ms) mutation or nonsense/frameshift (ns/fs) or neither (NA)
8. Column eight shows the individual patients, numbering being the pedigree number followed by a patient number in random orde
9. This column specifies the age at which the first pNET was diagnosed in each patient

Column 10 through 13 provide information on tumor size and inclusion in the growth analysis.

10. This column provides the size in mm of the largest pNET during follow-up detected in this patient
11. This column specifies if the patient was included in the growth analysis for size
12. This column specifies the reason for exclusion if the patient was not included in the growth analysis
13. This column specifies the largest size of the tumor (mm) from this patient that was included in the growth analysis. In patients not included in the growth analysis it says "NA".

NA not applicable

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Chapter 9

General Discussion

General Discussion

Introduction

Recently the gene causing Multiple Endocrine Neoplasia type 1 (MEN1) celebrated its 20th birthday. From a co-occurrence of endocrine tumours that runs in the family (an inherited syndrome) to a genetic disease for which gene and gene product are known. Although one might say that in 2018 MEN1 has come of age, the management of MEN1 is still challenging[1]. In recent years tremendous progress has been made in understanding the structure and function of menin, the protein encoded by the *MEN1* gene, and its role in the pathogenesis of MEN1. Still, more work is needed for example to understand the endocrine tissue specificity of MEN1 and the pathways involved in the different MEN1 manifestations[2-4]. Regarding the clinical management of patients with MEN1, many questions remain as well. The current guidelines (2012) open with the remark that "there is currently a lack of evidence from controlled clinical trials that specifically evaluate methods of diagnosis and screening for the tumours or treatment of MEN1"[5]. In this thesis we provide evidence on several aspects of the natural course and long-term outcome in MEN1. Detailed knowledge of the natural course is necessary to be able to give evidence based recommendations for screening and therapy. Knowledge of determinants of natural course may guide further research into novel treatment strategies as well as lead to more personalized management of the disease. This thesis also shows how observational research in a rare disease can provide high quality evidence based answers to important clinical questions.

MEN1 in the era of genetic diagnosis

The discovery of the *MEN1* gene in 1997 heralded a new era in the follow-up and treatment of patients with MEN1[6]. Presymptomatic diagnosis was now possible and asymptomatic mutation carriers could be periodically screened for early detection and treatment of manifestations. Shortly thereafter the first clinical consensus guideline was published[7]. The question arose if an early genetic diagnosis with subsequent clinical screening for disease manifestations leads to a better patient outcome[8]. In addition, one might ask if the phenotype of genetically diagnosed patients is different from that of clinically diagnosed patients. In **chapter 3** we therefore investigated if a genetic diagnosis leads to a better outcome compared to a clinical diagnosis[9]. In addition we compared the prevalence of manifestations in both groups of patients.

Patients with MEN1 treated at the University Medical Center (UMC) Utrecht (1978-2007) were included. A total of 58% of the included patients was clinically diagnosed and 41% was diagnosed by presymptomatic genetic screening. In our cohort we found that the point prevalence for manifestations was lower than the prevalence reported in the then recent literature, which was caused by patients who were still free of clinical manifestations at the end of follow-up[10-15]. At the moment MEN1 was diagnosed, although 63% was still free of clinical manifestations,

37% of the genetically diagnosed patients already had manifestations. At the end of follow-up, patients who were diagnosed by presymptomatic screening had less manifestations and no malignancy or death was seen in this group.
The difference in outcome between the two groups must however be viewed with caution since duration of follow-up differed between both groups and the clinically diagnosed group was older at the end of follow-up which in itself will lead to a higher prevalence of manifestations, as the penetrance of the different manifestations of MEN1 increases with age[16]. In addition lead time bias may play a role in these outcomes. However, in our cohort age of diagnosis did not differ between the two groups, indicating that by genetic testing also milder MEN1 phenotypes are discovered. The improved outcome observed in genetically diagnosed patients cannot, given the difference in follow-up, solely be credited to the genetic diagnosis. However, as this study shows, a genetic diagnosis can identify patients before manifestations occur and in these patients manifestations can therefore be diagnosed in early stages and malignancy prevented. Indeed, a recent study by Van Leeuwaarde *et al.* showed that there was a significant negative clinical impact for MEN1 family members who did not receive a timely genetic diagnosis after the diagnosis of the index patient[17].
We learned from this research that genetic screening can indeed identify patients before clinical manifestations occur and in our cohort led to the identification of milder MEN1 phenotypes. Early genetic diagnosis probably leads to a better clinical outcome, although this cannot be definitely concluded from the research we presented in **Part I**. The research in **Part I** also underscored the need to better understand the natural course of MEN1 in this new era and the need for large unselected populations with long-term longitudinal follow-up. This ultimately led to the foundation of the DutchMEN1 Study Group.

DutchMEN1 Study Group: lessons learned from the Dutch national MEN1 database

It is challenging to perform scientific research in rare diseases with high internal and external validity that provides answers of such a level of evidence that it can be used in day-to-day clinical practice. However, it is a challenge that can be met, as we have shown with the work done by the DutchMEN1 study group (DMSG).
Randomized controlled trials (RCT), which are regarded as the highest level of evidence, are difficult to perform in a disease as rare as MEN1. It is difficult and sometimes even impossible to recruit the necessary number of participants (or the time to do so would be so extended that the medical landscape between the end and beginning of the study will have substantially changed) and the event rate is generally low. Funding for such an undertaking would be difficult to pertain. Cohort studies are the next best level of evidence and if executed correctly can provide valuable information. However, they are prone to different forms of bias such as selection bias, information bias and confounding by indication and these issues need to be correctly dealt with[18].

Therefore, in 2008 the DMSG was founded to improve the care of MEN1 patients by performing high quality research addressing clinically important questions. To this end a national retrospective longitudinal database was constructed. Throughout the different stages of this work, we learned valuable lessons.

First and foremost patient participation is extremely valuable. The DMSG has recognized this from the beginning since it was formed as a collaboration between all UMCs and the Dutch MEN patient advocacy group (Belangengroep MEN). By involving patients from the start, research goals can be correctly prioritized, patient centred research questions can be formulated, patient involvement can be maximized and results be funnelled back to those with the highest need to know[19].

Research questions were then formulated, the first priority being more insight into the natural course of MEN1 and more insight in the usefulness of the periodical screening protocol.

The next step is the study population, which is crucial, especially in rare diseases. The study population greatly influences the external validity of a study[20]. In rare disease, it is likely that expert centres, which are the prime source of scientific research, only see patients with the more complex phenotypes of the disease or only patients in whom intervention is needed, thereby creating a selected population. This underscores the need for nationwide databases including all patients diagnosed with the disease in a particular country. Since in the Netherlands >90% of the MEN1 patients are treated in UMCs irrespective of the MEN1 phenotype and the DMSG is a collaboration of all the Dutch UMCs we were able to create a database including >90% of the Dutch MEN1 population, thereby minimizing the risk of selection bias. This also underscores the fact that research into a rare disease must be collaborative. Another factor that made the completeness of the database possible, is the collaboration with the patient advocacy group, which led to familiarity of our research amongst their members.

In the next stage, based on the formulated research questions, the database was designed as a web-based database in a secure environment, thus allowing data collection from any site in the Netherlands. To allow determining the natural course the database was designed as a longitudinal database, with data input every quarter of every available year of follow-up. Data were collected on laboratory results, results of radiological and nuclear imaging, surgical procedures, pathology results and medical treatment. To minimize the information bias only "raw data" were collected in the database eg in primary hyperparathyroidism (pHPT) the values of PTH and calcium were included, but there was no variable stating pHPT yes/no. The final dataset was established after discussion among DMSG members, again including the opinion of the patient representatives.

Data collection in retrospective databases is another important step in which potential bias can be introduced. Missing data, if related to the outcome or course of the disease, can be a source of bias. In retrospective databases, more time spent at data retrieval can maximize the completeness. Data collection itself is also a po-

tential source of information bias if the data is not collected in the same manner for every patient. If data are collected by the treating physicians its completeness and uniformity may depend on the time they are able to spend at data collection and the affinity they have with the project. Therefore a protocol was developed, which meticulously described for each variable how it should be collected. Data collection was performed by centrally appointed data-collectors, one or two at the time, which were either final year medical students or PhD students working on the MEN1 research database. This minimalized observer differences, allowed the data collectors to gain vast experience with this large database, provided the time needed to ensure data completeness and made sure that every researcher working with this database to answer research questions was fully aware of its (in) possibilities.

The final stage before data analysis to answer stipulated research questions is to prepare the data to be analysed. Since only raw data was collected, this is not a step to be taken lightly. From numerous scans, laboratory results and pathology results it needed to be established which patients had which manifestation at any given point in time. We therefore developed strict definitions, which by the construction of syntaxes for data analysis programs could be applied to the database. For example a pancreatic tumour was considered present in case of "positive Computed Tomography (CT) or Magnetic Resonance Imaging (MRI) or endoscopic ultrasound (EUS) confirmed at least once on subsequent imaging within three years", hereby including confirmation of a diagnosis over time in the 'gold standard'. No negative imaging is allowed between the first two positive imaging studies. An imaging finding is disregarded as a pNET if two positive imaging studies are followed by two negative studies within three years without surgical resection[21]. For complex decisions, for example whether liver metastases that were present originated from a gastrinoma/insulinoma or a concomitant non-functioning pNET, an expert panel was formed[22]. For laboratory values, which were determined in eight different laboratories over more than 20 years we chose to use "times the upper limit of normal" in the analysis to overcome differences in assay, units and reference values between institutions.

In summary, building a retrospective longitudinal database for MEN1 has taught us to involve the patients, be complete in the study population, make it a multicentre national collaboration, build the database based on predefined clinical questions, protocolize all steps, appoint dedicated data-collectors who are also the ones conducting the studies, use "raw data" as much as possible but plan in advance how to make the data fit for analysis.

The results from the DMSG database have already led to changes in clinical practice and even to new insights into the disease[23-28]. In addition, valuable contributions to the knowledge on the natural course and treatment of the most prevalent manifestations leading to the highest morbidity and even mortality were added, which are described below.

Surgical strategy in MEN1-related primary hyperparathyroidism

Primary hyperparathyroidism (pHPT) is by far the most common MEN1 manifestation and is a major cause of morbidity. Almost every MEN1 patient will be diagnosed with pHPT at some point in their lives[25]. That means that one of the most frequent decisions that has to be made by patients and their treating physicians is when and how to proceed with surgery for pHPT. However at the start of the work on this thesis, it was unclear what should be the preferred surgical procedure for MEN1-related pHPT[29]. In MEN1 all parathyroids are affected by the *MEN1* germline mutation and if given enough time all will develop hyperplasia or frank adenomas. So when looking at the risk of recurrence, removing all the parathyroid tissue would be the best option. However, extensive surgery leads to a higher rate of postoperative hypocalcaemia. Less extensive surgery on the other hand, might lead to persistent disease and early recurrence with the need for reoperation. To answer the question what should be the preferred surgical strategy for MEN1-related pHPT we first performed a systematic review and meta-analysis of the available literature and included the data of the patients operated in the UMC Utrecht (**chapter 4**). Our study is the only systematic review and meta-analysis on this subject to date. We included 12 studies for pooled analysis[29]. In this meta-analysis we show that persistent and recurrent disease are significantly higher after less than subtotal parathyroidectomy (SPTX; eg removing less than 3 out of the 4 parathyroid glands) but causes very few cases of hypoparathyroidism compared to the more extensive procedures. In addition we showed that the larger the amount of parathyroid tissue removed the smaller chance of recurrence of hyperthyroidism, at the cost of more postoperative hypoparathyroidism. Based on these results we recommended SPTX should be the surgical procedure of choice in MEN1-related pHPT. However, the data used for the pooled analysis were all from retrospective observational studies. Four of the 16 identified studies could not be used in the pooled analysis and data on postoperative hypoparathyroidism was not available in all the series. Still, from a medical point of view, based on the best available evidence at that point, SPTX seemed to offer the best balance between persistent or recurrent disease on the one hand and postoperative hypoparathyroidism on the other hand.

Next, as described in **chapter 5**, with the results and methodological shortcomings of the studies included in the systematic review in mind, we performed a cohort study with long-term follow-up using the data of the DMSG national MEN1 database. In this study we assessed the optimal surgical strategy for MEN1-related pHPT and described the course of postoperative hypocalcaemia in more detail[30]. In addition we examined a previously described genotype-phenotype correlation with regard to surgical strategy in pHPT[31]. The results of this study corroborate the findings of the meta-analysis and strengthen the recommendation of SPTX as the preferred surgical strategy. Still, postoperative hypoparathyroidism is frequent (56%) and no clinical characteristics other than the type of surgery predicts permanent hypoparathyroidism. In patients undergoing less than SPTX persisten-

ce or recurrent disease appeared to occur less often in patients with a nonsense or frameshift mutation in exon 2, 9 and 10. Therefore, if less than SPTX is considered, this procedure might be more suited for this subgroup of patients. Based on the results of the study, postoperative hypoparathyroidism lasting for more than six months should not be considered 'permanent' hypoparathyroidism since in more than half of the patients vitamin D and calcium could be tapered and eventually stopped after longer follow up. Interestingly, recurrence was rarely seen in patients with hypoparathyroidism lasting more than six months. This prognostic significance of postoperative hypoparathyroidism was confirmed in a recent study by Frysten *et al.* where they showed that not having postoperative hypocalcaemia was significantly associated with recurrent disease[32].

So has the debate regarding the optimal surgical procedure for MEN1-related pHPT been laid to rest seven years after the publication of our results? The answer is: No! To quote a recent review "Type of operation for parathyroid surgery in MEN1 patients is still controversial"[33]. In the past years, different studies have been published promoting again less aggressive surgery [eg minimal invasive parathyroidectomy (MIP) or unilateral clearance] in selected patients based on imaging[34,35]. However, others have indicated that this strategy is associated with a high failure rate and that current imaging techniques cannot reliably indicate single gland or unilateral disease[36].

What we need to move forward from here, is a different mind-set with regard to surgical treatment of MEN1-related pHPT. We need to realize that one ideal surgical strategy that fits every patient will probably not be found. And we need to reconsider the definition of an "optimal" surgical outcome. It is far to one dimensional to look only at persistence/recurrence rates and the amount of hypoparathyroidism we are willing to accept to accomplish these results.

What is missing when looking at it from a patients point of view, is how postoperative hypocalcaemia affects quality of life, what the chances are of recurrence after 1, 5 or 10 years with different procedures and if recurrent or persistent disease leads to symptomatic hypercalcaemia or more hypercalcaemic complications (hospitalization for hypercalcaemic crisis, fractures because of low bone mineral density, renal dysfunctions or symptomatic renal stones). Very little evidence is available to answers these questions. Indeed, information on the complications of longstanding pHPT and especially the effect on bones and renal function in robust cohort studies is missing. Recent studies show that MEN1-related pHPT leads to more bone and renal complications than sporadic pHPT and recovery after parathyroidectomy might be less or at least slower[37,38]. This is, however, based on small studies because in most MEN1-pHPT studies, including our own, no information is given on bone density or renal function. In addition, we do not know the effect of timing of surgery on these parameters. No evidence is available in the literature on patient perception of postoperative hypoparathyroidism. These knowledge gaps need to be filled with results from large national registries. And with those answers a decision aid should be created for patients. Because unless

there is clear medical harm associated with one strategy, patients may weigh the importance of different outcomes very differently than physicians do. For an individual patient the importance of different outcomes may even weigh differently in different stages of his or her live. This is the different mind-set that will lead to true well informed decision making of patients together with their doctors.

Natural course of MEN1-related NETs

Whereas pHPT is the most prevalent manifestation and responsible for most MEN1-related surgeries, metastasized neuroendocrine tumours (NET) in MEN1 are the most important cause of disease-related mortality[39,40]. Therefore in **chapter 6**, we started by performing a comprehensive review of what is known of the natural history of MEN1-related duodenopancreatic and thoracic NETs and the function of menin in tumorigenesis[41]. The results of this review showed important gaps in current knowledge. In duodenopancreatic NETs, very little is known with regard to prognostic factors for the occurrence of distant metastases and survival. In addition, due to early genetic diagnosis, implementation of screening programs and improved imaging techniques, the detection of small non-functioning pancreatic NETs (NF-pNETs) increases. Little is known of their natural history and clinical relevance. The same is true for MEN1-related pulmonary neuroendocrine tumours, which also seem to be more common than previously thought, but with largely unknown natural history and impact on survival. These questions and areas of uncertainty led to the two retrospective longitudinal cohort studies on respectively the natural course and survival of lung and thymus NETs and on the long-term natural course of small (<2cm) NF-pNETs[21,42] described in **chapters 7 and 8**.

This has taught us that thymus NETs are an important cause of death due to their aggressive behaviour, although the prevalence is low. No prognostic factors related to survival could be determined. Lung NETs are more prevalent than previously thought (prevalence of 13% in our study). Due to the unique properties of the DMSG national MEN1 database we were able to describe the natural course of lung NETs by assessing tumour size over time, showing that lung NETs in MEN1 have an indolent course with a tumour doubling time of 4.5 years. No lung NET-related mortality was seen. Interestingly tumours in male patients had a faster growth rate. With regard to small NF-pNETs we have shown that these tumours remain mostly stable during long-term follow-up and the occurrence of distant metastases was rare (1%). A subgroup of tumours was slowly growing, but no clinical characteristics related to growth could be identified. In the subgroup of progressive tumours, those with missense mutations demonstrated faster growth.

So, what do we learn from these insights into the natural course of MEN1-related NETs and how does this influence the care for patients with MEN1?

With regard to thymus NET, cohorts published after our study have confirmed the prevalence and poor prognosis of MEN1-related thymus NETs[43-45]. Even in incidentally diagnosed cases, the development of metastases is high[43]. Current guidelines recommend thoracic imaging every 1-2 years[5]. However, it is unclear if this leads to improved survival; given the very low incidence the number needed to screen to timely diagnose thymus NET is very high and radiation exposure has to be taken into account as well[46].
Recently Singh Ospina *et al.* proposed to perform a multicentre RCT into different screening strategies for lung and thymus NET in MEN1[47]. This is nowadays probably feasible, especially if international collaborations are undertaken. However, in the time it would take to recruit the necessary number of participants combined with the time to reach the desired outcome (overall survival), the diagnostic landscape will probably have changed drastically and results might not be applicable anymore. Prospective inception cohorts within existing registries, as Singh Ospina *et al.* also suggest, are certainly of value and this option should be pursued[47]. However, in addition, other directions should be explored. Are new biomarkers such as circulating tumours cells and microRNAs perhaps able to identify thymus NET early in its course[48]? When combining data from national registries, can we identify patients at risk for thymus NET development based on clinical characteristics or genotype? And is there perhaps a place for prophylactic total thymectomy during parathyroid surgery, since minimal invasive techniques might abolish the need for median sternotomy or thoracotomy[49]?

With regard to lung NETs, given the demonstrated slow doubling time and indolent course, the screening interval can probably be safely lengthened from the presently recommended 1-2 years to once every 2-3 years and perhaps there needs to be a gender specific screening interval, if the gender difference in tumours growth can be corroborated in other series. But more importantly, more information is needed with regard to the question if and when intervention is needed in MEN1-related lung NETs. Current guideline state that curative surgery, where possible, is the treatment of choice for lung NETs[5]. Our study showed that surgical resection did not improve survival and that the overall outcome of patients was favourable, however results must be viewed with caution since data are retrospective and the indication for surgery was set by the individual surgeons and internists[42]. In another recently published retrospective series every patient with a lung NET underwent surgical resection also leading to a favourable outcome[44]. Whether watchful waiting might also be an acceptable strategy seems a prudent question given what we now know regarding the indolent natural course of tumours.
It is also of vital importance that we consult with the patients regarding these questions: how do they weigh the burden of repeated screening and what impact does a watchful waiting strategy have on their quality of life. In addition, it is important to keep patients informed on what we learn regarding the natural history

of these thoracic NETs and the effect of screening strategies. Because there is still much uncertainty regarding follow-up and treatment of thoracic NETs in MEN1, this is essential for a meaningful discussion of harm and benefit and to make truly well informed shared decisions between patients and their treating physicians possible.

At the start of the work on this thesis the natural course of small (<2cm) NF-pNETs in MEN1 was largely unknown[41]. In recent years, several groups sought for genotype-phenotype correlations with regard to tumour behaviour. We now know that at meticulous long-term follow-up, NF-pNETs smaller than 2 cm in MEN1 grow very slowly and rarely lead to distant metastases[21]. We identified a subgroup of small NF-pNETs with tumour progression, albeit that tumour growth was still slow. Within this subgroup we found an association between previously suggested genotype-phenotype correlations and tumour growth. This warrants further attention since identifying these underlying mechanisms driving tumour growth might lead to ways of identifying which tumour will ultimately metastasize and which tumours will remain silent. In addition, up until today, no clinical markers other than size and WHO grade (based on the mitotic count) are prognostic for distant metastases[22, 50]. More research into novel biomarkers and pathways involved in MEN1-related tumorigenesis might lead to better ways to predict tumour behaviour.
For day-to-day practice, given the slow growth rate, radiological surveillance of small NF-pNETs might be done far less frequent than current guidelines advice, for example every 2-3 years. The indolent course of NF-pNETs also has consequences for the only curative therapy: pancreatic surgery. Especially because pancreatic surgery is associated with a significant amount of major early complications (33%) and approximately one in five patients will develop either exocrine (malabsorption) or endocrine (diabetes mellitus) pancreatic insufficiency[51]. Most importantly, no survival benefit has been demonstrated of surgical resection of small NF-pNETS in different independent cohorts[52-55]. Therefore, a watchful waiting approach seems safe with a low risk of disease associated mortality and given the now available evidence surgery should not be standard therapy for NF-pNETs smaller than 2 cm. In daily clinical practice, ultimately treatment advices regarding small NF-pNETs need to be based on discussion in tumour boards of experienced professionals in centers of expertise. The final treatment strategy must be based on shared decision making between the doctor and the patient, discussing which risks are acceptable to both, and assessing how the patient weighs different options in his or her personal situation.

Future directions
How should we proceed from this point? It is important not to consider RCTs the holy grail of rare disease research. These are difficult to organize, require multinational and multicentre approaches, need a lot of time to recruit the necessary

number of participants and require a very long follow-up to deliver meaningful results. Often well executed cohort studies in unselected populations provide workable answers much faster. RCTs shouldn't be excluded either, but long and hard thought should be given for which clinical questions an RCT will be the best way to proceed. An example of such a question might be surgery for NF-pNETs 2-3 cm in size. In rare diseases databases will remain an important source of research data and can provide the basis for methodological high quality research with the power to change daily practice. Most database presently in place are retrospective in nature. Prospective databases allow for further minimisation of missing data, enable collection of more baseline characteristics and comorbidities and allow for higher quality of data because protocols and tools for data collection, ideally automated within the course of daily patient care, are in place before data are generated. In addition the development of a prospective database offers the change to build in possibilities to connect with other national databases. Such an international collaboration is the way forward, but cannot be entered into lightly. It requires mutual trust and respect and solid agreements beforehand on data ownership, access and publication rights. In addition privacy of patients included must be protected at all times and national and international privacy and database laws should be upheld. It is also important to investigate beforehand if study populations in national databases are comparable. The need for international collaborations is widely recognized[56, 57]. Indeed the DMSG has already combined forces with the several international groups on different projects such as the insulinoma research and the breast cancer research[28]. In addition, since more insight is needed into the function of menin and the specific pathways involved in MEN1-related tumorigenesis, clinical databases should be combined with structured biobanking to accommodate translational research.

In areas of uncertainty it is of vital importance to understand the perspective of the patient. Therefore more research is needed into the way patients weigh different strategies and what they deem as important for their everyday quality of life. Those parameters should be collected for every therapeutic or diagnostic option we offer, so that patients and their physicians can make informed management decisions individualized to the patient's need.

Conclusion

In conclusion, the work of this thesis has shown that in rare diseases such as MEN1 it is important to start with identifying the clinical questions doctors and patients have. Next, a systematic review of literature and meta-analysis will provide a starting point and identify gaps that need to be filled to answer clinical questions. National databases, ideally including everyone with the rare disease in an area or country can provide an unbiased source of data for cohort studies to fill the gaps in knowledge and provide answers to clinical questions. Results will be funnelled back to doctors and patients alike, as patients remain involved throughout the scientific process. This enables making shared, informed and individualized deci-

sions on further management, based on estimated risks of withholding a therapy or diagnostic procedure balanced against benefits and potential harm.

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Chapter 10

Dutch summary/Nederlandse samenvatting

Dutch summery/Nederlandse samenvatting

Achtergrond

Het onderzoek dat in dit proefschrift is beschreven gaat over Multipele Endocriene Neoplasie type 1 (afgekort als MEN1). Het MEN1 syndroom is een erfelijke aandoening die ontstaat door een mutatie in het *MEN1* gen dat ligt op chromosoom 11. Mensen met een mutatie in dit gen hebben gedurende het leven risico op het ontwikkelen van tumoren van specifieke hormoon producerende organen. De drie belangrijkste klinische uitingen (of manifestaties) van dit syndroom zijn (1) primaire hyperparathyroïdie [adenomen (goedaardige tumoren) van de bijschildklieren, dit geeft een verhoogd calcium gehalte in het bloed met klachten en verschijnselen die daarbij passen], (2) neuroendocriene tumoren van de twaalfvingerige darm en de alvleesklier en (3) hypofyse adenomen. Daarnaast kunnen onder andere ook neuroendocriene tumoren van de thymus, de maag en de longen en goedaardige bijniertumoren ontstaan. De levensverwachting van mensen met het MEN1 syndroom is korter dan van de algemene populatie, meestal is dit het gevolg van kwaadaardige neuroendocriene tumoren van de thymus, twaalfvingerige darm en alvleesklier.

MEN1 is een zeldzaam ziektebeeld, in Nederland zijn er ongeveer 400 mensen met dit syndroom. Goed wetenschappelijk onderzoek naar de uitkomsten van verschillende aandoeningen binnen dit ziektebeeld is daarom uitdagend.

In 2008 werd de DutchMEN1 Study Group (DMSG) opgericht met als missie de verbetering van de zorg voor MEN1 patiënten door het verrichten van kwalitatief hoogwaardig wetenschappelijk onderzoek. De DMSG is een samenwerkingsverband van de afdelingen endocrinologie van alle academische ziekenhuizen in Nederland (ieder centrum is door één endocrinoloog vertegenwoordigd) en de Nederlandse MEN patiëntenvereniging (Belangengroep M.E.N.). Het belang van patiënten vertegenwoordiging in deze onderzoeksgroep kan niet genoeg benadrukt worden. Door de patiënten zelf te betrekken bij wetenschappelijk onderzoek kan worden gewaarborgd dat de vraagstellingen van het onderzoek ook echt relevant zijn voor patiënten, dat zij op de hoogte zijn van het onderzoek en gemotiveerd tot deelname en bijdrage en dat zij snel geïnformeerd worden over de uitkomsten van de onderzoeken. De DMSG werkt ook nauw samen met de endocrien chirurgen in Nederland. Door de DMSG is een nationale MEN1 database opgezet met gegevens van alle MEN1 patiënten in Nederland die vanaf 1990 in de verschillende academische centra behandeld zijn. In deze database zijn gegevens verzameld (zoals uitslagen van bloedonderzoek, beeldvormende onderzoeken en operaties) over de verschillende uitingen van het MEN1 syndroom. Deze gegevens zijn voor ieder kwartaal van ieder jaar dat we deze patiënten konden vervolgen, ingevuld. Door deze manier van dataververzameling kan goed onderzoek gedaan worden naar het beloop van het MEN1 syndroom.

Deel I:
MEN1 in het tijdperk van genetische diagnostiek

Het *MEN1* gen werd geïdentificeerd in 1997. Sindsdien is het mogelijk om dragers van een mutatie in het *MEN1* gen te identificeren voordat zij uitingen ontwikkelen van het ziektebeeld. Hierbij kan men zich afvragen of het vroegtijdig identificeren van mutatiedragers in families leidt tot een betere uitkomst op langere termijn. Daarnaast is het voorstelbaar dat door genetische diagnostiek ook een subgroep van MEN1 patiënten openbaar wordt met weinig manifestaties van het syndroom die anders niet geïdentificeerd zou worden. Dit proefschrift begint in **deel I** met een overzichtsartikel over het MEN1 syndroom waarbij van de verschillende endocriene manifestaties het beloop, de diagnostiek en de behandeling wordt beschreven (**hoofdstuk 2**). In **hoofdstuk 3** beschrijven we onderzoek dat verricht is onder patiënten met het MEN1 syndroom die in het UMC Utrecht onder behandeling zijn (geweest). In dit onderzoek hebben we terugkijkend (retrospectief) vastgelegd welke uitingen van het syndroom deze patiënten hadden en of er verschil was tussen mensen die de diagnose MEN1 gekregen hebben op klinische gronden (op basis van uitingen van het syndroom) of door genetische screening (omdat het bij hen in de familie voorkwam). In dit onderzoek zijn 74 patiënten beschreven die gemiddeld 5,5 jaar gevolgd zijn. Van deze mensen hadden 43 een klinische diagnose en 30 een genetische diagnose. In deze totale groep was primaire hyperparathyreoïdie de meest voorkomende manifestatie (bijna 80%), gevolgd door neuroendocriene alvleeskliertumoren (bijna 50%) en hypofyse adenomen (bijna 40%). De gemiddelde leeftijd bij de eerste uiting van het MEN1 syndroom was 32 jaar. In de groep patiënten met een genetische diagnose had 63% op het moment van diagnose nog geen uiting van het ziektebeeld, en 18% was aan het einde van de volgperiode nog steeds vrij van manifestaties. Bij vergelijking van de patiënten die klinisch gediagnosticeerd zijn met hen die genetisch gediagnosticeerd zijn, valt op dat de patiënten met een genetische diagnose minder uitingen van het ziektebeeld hebben en dat overlijden of kwaadaardige MEN1-gerelateerde tumoren in deze groep niet gezien werden. Nu moeten hier een paar kanttekeningen bij geplaatst worden. De groep die genetisch gediagnosticeerd werd hebben we minder lang kunnen vervolgen en was aan het eind van de volgperiode jonger dan de groep die klinisch gediagnosticeerd werd. Het is dus mogelijk dat de uitingen nog moeten komen in deze groep. Maar aan de andere kant moet ook opgemerkt worden dat er geen significant verschil was tussen beide groepen in de leeftijd waarop de diagnose MEN1 werd gesteld. Wij concluderen uit deze bevindingen dat je met genetische diagnostiek de manifestaties vóór kunt zijn, dat je waarschijnlijk ook mensen met een milder ziektebeeld identificeert en dat het aannemelijk is dat door genetische screening de lange termijn uitkomsten gunstig beïnvloed worden. Dit laatste kan op basis van ons onderzoek niet met zekerheid worden gesteld, daarvoor moeten we lange termijn uitkomsten afwachten.

Deel II:

Chirurgische behandeling van primaire hyperparathyroïdie bij MEN1

Primaire hyperparathyroïdie (afgekort als pHPT) is de meest voorkomende uiting van het MEN1 syndroom en verantwoordelijk voor de meeste operaties die patiënten met het MEN1 syndroom ondergaan. pHPT heeft daardoor een belangrijke invloed op de kwaliteit van leven. In **deel II** van het proefschrift onderzoeken we wat de optimale chirurgische behandeling van pHPT bij MEN1 patiënten zou moeten zijn. In **hoofdstuk 4** beschrijven we een groep van 52 MEN1 patiënten met pHPT die in het UMC Utrecht behandeld werden van 1967-2008. Hierbij hebben we gekeken naar de uitkomsten van de bijschildklierchirurgie die deze mensen ondergingen als behandeling van hun pHPT. We hebben drie verschillende chirurgische technieken met elkaar vergeleken: totale parathyreoïdectomie (afgekort als TPTX), subtotale parathyreoïdectomie (afgekort als SPTX) en minder dan subtotale parathyreoïdectomie (afgekort als <SPTX). De resultaten van de behandeling hebben we gecategoriseerd als persisterende ziekte (pHPT komt binnen 6 maanden terug), recidief ziekte (pHPT komt terug maar na 6 maanden) en postoperatieve hypoparathyreoïdie (een te laag calcium door het niet goed functioneren van de achtergebleven of ge-auto-transplanteerde bijschildklieren). Ook hebben we onze resultaten in een meta-analyse gebundeld met andere onderzoeken. De resultaten laten zien dat na TPTX en STPX het risico op persisterende ziekte en recidief beduidend lager is dan na <STPX. Het risico op hypoparathyreoïdie is het hoogst na TPTX en het laagst na <SPTX. Wij concluderen hieruit dat SPTX de beste chirurgie behandeling is voor MEN1-gerelateerde pHPT.

In een vervolgstudie hebben we gekeken naar lange termijn uitkomsten van chirurgische behandeling van pHPT bij MEN1 patiënten; dit is beschreven in **hoofdstuk 5**. Wij hebben het beloop bestudeerd van alle MEN1 patiënten uit het UMC Utrecht en het Radboud MC in Nijmegen die geopereerd zijn aan de bijschildklieren. Bij de uitkomsten hebben we gekeken naar persisterende of recidief ziekte, maar ook in meer detail naar de postoperatieve hypoparathyreoïdie. Daarnaast hebben we gekeken of het beloop afhangt van het type mutatie in het *MEN1* gen. De resultaten van dit onderzoek bevestigen dat SPTX de gunstigste balans biedt tussen genezing (83%) en postoperatieve hypoparathyreoïdie (39%). Bij TPTX was er bij 19% sprake van recidief/persisterende ziekte en bij 66% postoperatieve hypoparathyreoïdie. Bij <SPTX was er bij 53% sprake van recidief/ persisterende ziekte en bij 24% postoperatieve hypoparathyreoïdie. Daarnaast laat dit onderzoek zien dat postoperatieve hypoparathyreoïdie weliswaar zeer langdurig kan zijn (gemiddelde duur 1,5 jaar), maar dat het in de meeste gevallen toch herstelt. Dit onderzoek heeft ook aangetoond dat mensen met een bepaald type mutatie in het *MEN1* gen na <SPTX (dus een relatief kleine operatie) minder vaak een terugkerende ziekte hebben dan mensen met andere typen mutaties. Wij concluderen hieruit dat als een kleine operatie overwogen wordt, dit mogelijk meer geschikt is voor een subgroep van de patiënten die dit specifieke mutatietype hebben.

Deel III:
Natuurlijk beloop van neuroendocriene tumoren in de borstholte en de alvleesklier bij MEN1

Bij mensen met het MEN1 syndroom kunnen neuroendocriene tumoren (afgekort als NET) ontstaan in de twaalfvingerige darm, de alvleesklier, de maag, de longen en de thymus (zwezerik). Vooral de NETs in de alvleesklier (afgekort als pNET) en de thymus kunnen kwaadaardig zijn en deze kwaadaardige NETs zijn tegenwoordig de belangrijkste MEN1 gerelateerde doodsoorzaak. **Deel III** begint in **hoofdstuk 6** met een overzichtsartikel over wat er bekend is van het natuurlijke beloop van NETs van de longen, thymus en alvleesklier. Daarnaast gaat dit artikel meer in detail in op de functie van menine. Menine is het eiwit waar het *MEN1* gen voor codeert. Het eiwit menine is in de cel betrokken bij het reguleren van de gentranscriptie. Dit is het proces waarbij het DNA wordt afgelezen, wat uiteindelijk leidt tot de productie van een specifiek eiwit in de cel. Menin is zowel betrokken bij remming als bij activatie van gentranscriptie.

In **hoofdstuk 7** beschrijven we het voorkomen en het beloop van neuroendocriene tumoren van de long en thymus bij patiënten in de Nederlandse MEN1 database. Daaruit blijkt dat NETs van de thymus relatief zeldzaam zijn; slechts bij elf (3.4%) van de 323 patiënten, voornamelijk bij mannen (10 van de 11 patiënten) werd dit gezien. Van deze elf patiënten overleden er 7 aan de gevolgen van het thymus NET, de gemiddelde 10-jaars overleving van patiënten met een thymus NET was 25%.

Van NETs van de long bij MEN1 patiënten werd lange tijd gedacht dat ook dit relatief zeldzaam was, ons onderzoek laat zien dat deze NETs vaker voorkomen dan werd aangenomen. Bij 42 (13%) van de patiënten werd een long NET vastgesteld. De prognose van deze tumoren was in ons onderzoek gunstig, geen van de patiënten overleed als gevolg van deze tumor. De gemiddelde 10-jaars overleving van patiënten met een long NET was 71%. Opvallend in dit onderzoek was dat de long NETs bij mannen harder groeiden dan bij vrouwen, de gemiddelde verdubbelingstijd van de tumor was 2.5 jaar bij mannen en 5.5 jaar bij vrouwen.

Het onderzoek dat beschreven is in **hoofdstuk 8** gaat over de groei van kleine (<2 cm) niet functionerende neuroendocriene tumoren in de alvleesklier (afgekort als NF-pNETs). Onderzoek van deze kleine NF-pNETs is belangrijk, omdat deze tumoren steeds vaker opgemerkt worden doordat beeldvormende technieken de laatste jaren sterk verbeterd zijn. Artsen en patiënten worden daarom steeds vaker met deze kleine NF-pNETs geconfronteerd, waarbij dan de vraag is hoe hiermee om te gaan. NF-pNETs maken geen actieve hormonen die tot een klinisch syndroom kunnen leiden en werden traditioneel dan ook laat ontdekt; een deel van de patiënten had ook al uitzaaiingen op het moment van diagnose. Operatieve verwijdering geldt als voorkeursbehanderling voor NF-pNETs, maar het is de vraag of dit ook nodig is voor de tumoren kleiner dan 2 cm. Wij hebben de groei

van 115 NF-pNETs < 2 cm van 99 patiënten gedurende een mediaan van drie jaar (interkwartielafstand twee tot zes jaar) gevolgd. De gemiddelde groeisnelheid van deze kleine NF-pNETs bleek zeer laag namelijk 0.4 mm per jaar. Bij 70% van de tumoren bleek er zelfs helemaal geen groei op te treden in de periode dat we deze tumoren vervolgden. Bij de 30% van de tumoren waar wel groei optrad was de gemiddelde groei 1.6 mm per jaar. Geen enkel klinisch kenmerk bleek te voorspellen welke tumoren zouden groeien en welke niet. Wel bleek in de groep van groeiende tumoren dat een bepaald type mutatie geassocieerd was met snellere groei. In onze studie werd geen overlijden gezien ten gevolge van deze kleine NF-pNETs, wel werden er in één geval uitzaaiingen naar de lever gevonden. Wij concluderen dan ook dat, gezien de lage groeisnelheid, het lage percentage uitzaaiingen en overlijden, en het feit dat andere studies laten zien dat operatieve verwijdering van deze kleine NF-pNETs geen overlevingsvoordeel geeft, men niet in eerste instantie voor chirurgie hoeft te kiezen omdat deze kleine NF-pNETs heel goed gemonitord kunnen worden.

Hoofdstuk 9 bespreekt de relevantie voor de dagelijks praktijk van het in dit proefschrift beschreven onderzoek en de implicaties daarvan voor de toekomst.

Verklarende woordenlijst

Bijschildklieren: dit zijn kleine orgaantjes die achter de schildklier liggen, meestal zijn er 4 bijschildklieren (links en rechts boven achter de schildklier en links en rechts onder achter de schildklier), maar soms komen er meer voor.

Hypoparathyreoïdie: niet functionerende bijschildklieren leidend tot een te laag calcium gehalte in het bloed. Dit kan klachten geven zoals tintelingen, spierkrampen en in ernstiger gevallen ook hartritmestoornissen en ademhalingsproblemen door spasmen van de spieren rond het strottenhoofd.

Interkwartiel afstand: de afstand tussen het 25ste en 75ste kwartiel, gebruikt om de spreiding rond de mediaan aan te geven.

Mediaan: Die waarde waar 50% van de metingen voor en 50% van de metingen achter ligt, de middelste waarde van alle metingen. Gebruikt voor variabelen die geen normale verdeling hebben en waar het gemiddelde dus geen goede weergave is van het midden. Bij een normale verdeling komen mediaan en gemiddelde overeen.

Meta-analyse: Een systematisch overzicht van de wetenschappelijke literatuur waarbij de resultaten van vergelijkbare onderzoeken worden gebundeld en herberekend waardoor je een meer betrouwbare uitspraak kunt doen over het effect van een behandeling.

Minder dan subtotale parathyreoïdectomie (<SPTX): Een resectie van minder dan 3 bijschildklieren. Meestal betreft dit de verwijdering van twee bijschildklieren links of rechts of een selectieve verwijdering van één aangedane bijschildklier.

Neuroendocriene tumor (NET): tumor die ontstaat uit neuroendocriene cellen. Deze cellen bevinden zich onder andere in het maagdarmkanaal en de alvleesklier maar ook in de longen en de thymus (zwezerik). Deze tumoren gedragen zich anders dan de "gewone" darmkanker (darmcarcinoom) of alvleesklierkanker (pancreascarcinoom).

Niet functionerende neuroendocriene tumoren van de alvleesklier (NF-pNETs): neuroendocriene tumoren van de alvleesklier (pancreas) die niet gepaard gaan met uitscheiding van actieve hormonen. Neuroendocriene tumoren waarbij wel uitscheiding is van actieve hormonen worden functionerende neuroendocriene tumoren van de alvleesklier genoemd. Dit zijn bijvoorbeeld insulinomen (neuroendocriene tumoren die insuline produceren en daardoor leiden tot lage bloedsuikers) of VIPomen (neuroendocriene tumoren die vaso-actief intestinaal peptide (VIP) produceren wat leidt tot een syndroom met hevige diarree).

Primaire hyperparathyreoïdie (pHPT): Dit is een aandoening waarbij er goedaardige tumoren (ook wel adenomen genoemd) ontstaan in één of meerdere

bijschildklieren. Dit leidt tot overproductie van het bijschildklierhormoon PTH (parathyroïd hormoon) en dat geeft een verhoogd calcium in het bloed. Klassiek worden de klachten van te hoog calcium benoemd als "moans (somberheid, stemmingsveranderingen), stones (risico op nierstenen), groans (pijn in de buik, obstipatie) en bones (risico op botontkalking).

Subtotale parathyreoïdectomie (SPTX): hierbij worden alle 4 de bijschildklieren geïdentificeerd tijdens de operatie en worden er 3 of 3,5 verwijderd)

Thymus: ook wel zwezerik genoemd, een klein orgaantje hoog achter het borstbeen dat belangrijk is in de ontwikkeling van het immuun stelsel. Bij volwassenen is dit orgaantje doorgaans verschrompeld.

Totale parathyreoïdectomie (TPTX): hierbij worden alle 4 de bijschildklieren tijdens de operatie geïdentificeerd en verwijderd en wordt de minst aangedane bijschildklier middels een autotransplantatie teruggeplaatst in een spier van de arm of in de hals.

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"En weest dankbaar" (Kol. 3:15)

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Geachte professor Valk, beste Gerlof. Eindelijk is het dan zover! Na zoveel jaar en vele kilometers verder dan toch een boekje. Ik ben je dankbaar dat ik samen met jou aan het begin mocht staan van dit prachtige onderzoeksproject. Ik heb bewondering voor de visie die je hebt en hoe je erin slaagt dit te verwezenlijken met enorm enthousiasme, hard werk en doorzettingsvermogen. Ik zie nog dat handgeschreven A4'tje met de outline van de database, resultaat van een treinreis naar de ENETS in Parijs. De rest is history zullen we maar zeggen. Je hebt een enorme betrokkenheid bij de patiëntengroep die je begeleidt en je wetenschappelijk werk wordt gedreven door het verlangen de zorg voor deze patiënten naar het hoogst mogelijk niveau te tillen. Jouw grote epidemiologische kennis heb je vrijelijk met mij gedeeld, de laatste tijd reageren we dan ook vaak met dezelfde commentaren bij het bekijken van wetenschappelijk werk van anderen. Jouw gave om mensen samen te brengen en te enthousiasmeren heeft geleid tot het ontstaan van de DMSG. Als promotor was je ongelofelijk betrokken bij het werk, ik geloof dat er weinig promotoren zijn die zoveel van de ins en outs van het dagelijks werk weten als jij. Onze reis van de afgelopen jaren laat zich misschien het beste samenvatten als "van Delphi naar Utrecht". Ik ben je dankbaar voor alle kansen die je mij gegeven hebt en spreek de hoop uit dat dit geen punt in onze samenwerking is maar een bijzondere komma. Om af te sluiten met onze favoriete quote 'I love it when a plan comes together'.

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Diezelfde gedrevenheid leg je aan de dag in het wetenschappelijk werk, waarbij je ook veel aandacht hebt voor de basale wetenschap, respect daarvoor. En wat hebben we ontzettend gelachen, onder andere in Gubbio met 'gruppa Pieterman' een term van jou die op de een of andere manier is blijven hangen. Ik verheug me op de voortzetting van onze samenwerking.

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List of Publications

List of Publications

Pieterman CR, Schreinemakers JM, Koppeschaar HP, Vriens MR, Rinkes IH, Zonnenberg BA, van der Luijt RB, Valk GD. Multiple endocrine neoplasia type 1 (MEN1): its manifestations and effect of genetic screening on clinical outcome. *Clin Endocrinol (Oxf)*. 2009 Apr;70(4):575-81.

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Curriculum vitae

Curriculum vitae

Carolina Rebecca Christina (Carla) Pieterman was born on the 3rd of August 1983 in Amersfoort, the Netherlands. After graduating from grammar school at the "Corderius College" in Amersfoort in 2001, she studied Medicine at the University of Utrecht, where she obtained her medical degree in 2008. In the final years of her medical training she performed scientific internships on Multiple Endocrine Neoplasia type 1 at the University Medical Center Utrecht under supervision of Prof. dr. G.D. Valk and she participated in the foundation of the DutchMEN1 Study Group. Most of the work described in this thesis was performed with this collaborative research group consisting of endocrinologists from all University Medical Centers in the Netherlands. From 2008 onwards she worked as a PhD student at the University Medical Center Utrecht under supervision of Prof. dr. G.D. Valk. In 2011 she started her Residency Internal Medicine at the Meander Medical Center in Amersfoort under supervision of Dr. R. Fijnheer and Dr. R. Bosma. From September 2015 she continued her Residency at the University Medical Center Utrecht under supervision of Prof. dr. H.A.H. Kaasjager and dr. J.J. Oosterheert. She started her endocrinology training under supervision of Prof. dr. G.D. Valk and Dr. A.M.E. Stades in September 2016. From 2014-2017 she served as chair of the Dutch Multiple Endocrine Neoplasia patient organization (Belangengroep M.E.N.), for which she currently serves as advisor to the Board. She was co-chair of the organizing committee of the 15th international workshop on Multiple Endocrine Neoplasia and other rare endocrine tumours held in Utrecht, the Netherlands in 2016.