10 Swiss kindreds with multiple endocrine neoplasia type 1: assessment of screening methods

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disease characterised by the combined occurrence of tumours of the parathyroid glands, the enteropancreatic neuroendocrine system and the anterior pituitary gland. The genetic defect has been mapped to the long arm of chromosome 11q13, and the MEN1-gene was recently identified by positional cloning. Genetic screening for MEN1 germline mutations allows the identification of gene carriers in affected kindreds. Biochemical and radiological screening for MEN1 tumours allows an earlier diagnosis and treatment, and, thus may reduce morbidity and mortality. Since there is no consensus about the frequency and the extent of the necessary screening investigations, evaluation of proposed screening programs is of importance.

Methods: The aims of our study were to identify the MEN1-gene mutations and to detect the gene-carriers in 10 Swiss MEN1 families, as well as to assess biochemical and radiological screening methods. The study included 45 members from 10 MEN1 families.

Results: Every family had a different type of MEN1-gene mutation. Thirty out of 45 family members were gene mutation carriers. Twenty-two MEN1-gene carriers had typical MEN1 tumours: parathyroid, enteropancreatic and pituitary tumours were found in 21, 14 and 1 patients, respectively. Applying a defined screening program the following manifestations in asymptomatic MEN1-gene carriers were detected: 9 primary hyperparathyroidism, 3 nonfunctioning pancreatic tumours, 1 gastrinoma, 1 nonfunctioning microadenoma of the pituitary and 1 macronodular adrenal hyperplasia.

Conclusions: The genetic screening facilitates the identification of individuals who carry MEN1-gene mutations, and allows one to exclude non-mutant gene carriers from further investigations. The prospective biochemical and radiological screening of gene mutation carriers allows the earlier detection of MEN1-associated tumours. Therefore, it might be expected that morbidity and mortality of the MEN1 could be reduced.

Key words: multiple endocrine neoplasia type 1; germline mutation; genetic screening; chromosome 11; genotype; phenotype; primary hyperparathyroidism; enteropancreatic tumours; pituitary tumours; morbidity

Introduction

Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant disorder characterised by the combined occurrence of tumours of the parathyroid glands, the enteropancreatic neuroendocrine system and the anterior pituitary gland [1]. Other manifestations include adrenocortical lesions, carcinoid tumours and cutaneous tumours such as angiofibromas, lipomas and collagenomas [2–5]. The first description of combined familial occurrence of nephrolithiasis, intestinal ulcers and endocrine tumours dates back more than 60 years [6].
result in a loss of function of menin with a diminished tumour-suppressor activity leading to the development of tumours in MEN1.

Although more than 260 different MEN1 germline mutations have been identified [14] there is no evidence of a genotype-phenotype correlation. Between and within kindreds a wide variety exists concerning age of onset, clinical presentation and natural history.

Genetic screening for MEN1 germline mutations allows the identification of gene carriers who will develop MEN1 tumours. Biochemical and radiological screening for MEN1 tumours allows an earlier diagnosis and treatment, and may reduce morbidity and mortality [15, 16]. In the last years biochemical and radiological techniques for the diagnosis of MEN1-associated tumours improved and their availability increased. These diagnostic tests have been used for investigations of MEN1 families to a different extent by various study groups [15, 17–19]. However, the importance as well as the implications of some investigations for the clinical management of MEN1 patients are not known.

We investigated 10 Swiss MEN1 families regarding genotype, phenotype and natural history. Since there is no consensus about the frequency and the extent of the necessary screening investigations, evaluation of proposed screening programs is of importance. We therefore present and discuss our experience with 10 MEN1 families in the context of the published literature, local facilities for diagnostic investigations and financial resources.

**Methods**

**Patients**

The study included 45 members from 10 MEN1 families living in the eastern and central part of Switzerland (16 women, 29 men; age between 15 and 80 years). All of them were genetically investigated for MEN1–gene mutations with the exception of three patients with MEN1 who had died before 1997. In these three patients, the diagnosis of MEN1 was based on the presence of two or more MEN1-associated tumours according to the Stockholm criteria. The type of germline mutation was established retrospectively by the proof of MEN1–gene mutation in at least one of their offspring.

**Genetic analysis**

Since 1997, blood samples for DNA testing were taken and analysed at the Department of Pathology of the University Hospital in Zurich. Informed consent was obtained before genetic analysis was started. In the case of minor family members, their parents gave the consent.

The blood-derived DNA was examined for germline mutations in the complete coding sequence of the MEN1–gene using non-radioactive PCR-based single strand conformation polymorphism (SSCP) analysis, and heteroduplex gel electrophoresis followed by automated non-radioactive sequencing of PCR products from exons with aberrant band patterns [5, 20].

**Biochemical and radiological screening program**

Until 1997, almost all family members were enrolled in the screening program. After the introduction of the genetic analysis in 1997, only mutation gene carriers were followed. Usually, the screening investigations were performed annually. The clinical part of the screening program consisted of a medical history taken of all affected family members with a detailed questionnaire and a physical examination. Routine biochemical investigations included the assessment of total calcium, intact parathyroid hormone, glucose, insulin, gastrin, prolactin and insulin-like growth factor I (IGF-I) in the fasting state. In some cases, the biochemical workup was completed with the determination of the pancreatic polypeptide (PP), vasoactive intestinal polypeptide (VIP), chromogranin A and glucagon serum levels. Rarely, these were followed by a calcium and secretin stimulation.

Radiological imaging consisted of magnetic resonance imaging (MRI) of the pituitary and CT-scan of the duodenum, pancreas and adrenals. Endosonography of the duodenum and pancreas was performed in some cases.

**Cost-assessment of the screening investigations**

As a basis for the cost analysis of the proposed annual screening program, we used the ambulatory price list currently valid for the year 2000 of the institutions involved. The annual expenditures for the biochemical and radiological screening amount to about SFr 522. The costs for the genetic screening amount to SFr 1500. The costs of additional exceptional investigations such as basal levels of PP, chromogranin, VIP, glucagon and the stimulated levels of these hormones after calcium and secretin stimulation, and alternative imaging procedures were not included in this cost assessment.

**Results**

**Genotype**

Since 1997 we analysed the blood samples of 10 different MEN1 families with 42 members for germline mutations in the MEN1–gene. Twenty-seven of them were found to have a mutation. Every family exhibited its own type of mutation suggesting no common ancestry. We found the following mutations: 3 missense mutations (one polymorphism included), 3 frameshift deletions/insertions, 2 stop codon mutations, 1 intron deletion and 1 splice acceptor mutation. Most mutations were located in the 5' coding region of the gene (table 1).

**Phenotype**

Twenty-two of the 30 affected individuals (3 of 30 without DNA analysis who died before 1997)
exhibited clinical or biochemical manifestations of the MEN1 syndrome. So far, the remaining 8 mutation carriers showed no evidence of any disease (median age 22 years, range 15–40).

The 22 patients showed the following pattern of manifestations (table 2, figure 1):

**Primary hyperparathyroidism (pHPT)**
Twenty-one patients had pHPT (median age at diagnosis 39 years, range 23–78). In 14 of the 21, diagnosis was made between the age of 20 and 40 years. Eight of them had no hypercalcemia-associated symptoms and were considered asymptomatic. Nine newly diagnosed cases of pHPT were found due to the family screening program; five of them were asymptomatic at the time of diagnosis.

To date 13 patients have been operated and three will undergo parathyroidectomy soon. Three patients have refused cervical exploration and two died prior to surgery.

**Enteropancreatic neuroendocrine tumours**
Fourteen patients out of 22 had neoplasias of the duodenum or the pancreas (median age at diagnosis 47 years, range 24–73). Nine of them suffered from a gastrinoma; malignancy was shown in 3 patients. One patient had an insulinoma and 4 had clinically nonfunctioning neuroendocrine tumours. Four enteropancreatic neoplasms were detected due to screening.

Five patients underwent pancreatic or duodenopancreatic tumour resection and one will have an operation in the near future. Three of the four patients with clinically nonfunctioning tumours are being observed regularly and have not yet been operated. One patient with Zollinger-Ellison-syndrome declined a surgical intervention. Four patients died before surgery.

**Pituitary tumours**
Until now, only one of the gene carriers developed a pituitary tumour, a nonfunctioning microadenoma (8 mm in diameter), at the age of 34.

**Other MEN1-associated tumours**
We found in 7 patients MEN1-associated neoplasms such as adrenocortical tumours (3), malignant thymic carcinoid (1), carcinoid of the lung (1), ependymoma (1) and cutaneous tumours (3) such as lipomas and angiofibromas (median age at diagnosis 39 years, range 23–49).

**Mortality**
To date 5 of the 30 investigated patients have died (mean age 60 ± 12, range 47–81). Only one of the deaths was related to MEN1; this patient died of a local recurrence of a malignant carcinoid of the thymus at the age of 55. The other patients died of gastric carcinoma, hepatocellular carcinoma, pneumonia with multiorgan failure, and in a car crash. At the time of death 4 patients had pHPT and 4 patients suffered from gastrinoma.

### Table 1
Genotype.

<table>
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<th>Family</th>
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<th>type of mutation</th>
<th>exon</th>
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<th>carriers</th>
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<td>intronic splice donor site mutation</td>
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* Other MEN1-associated manifestations: carcinoid of the lung / thymus, adrenocortical tumors, ependymoma, angiofibromas, lipomas

### Table 2
Phenotype.

<table>
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</table>

* Other MEN1-associated manifestations: carcinoid of the lung / thymus, adrenocortical tumors, ependymoma, angiofibromas, lipomas
Discussion

We have investigated 10 MEN1 families with provenance from the central and eastern parts of Switzerland. Every kindred had a distinct type of mutation of the \textit{MEN1}-gene, therefore, a common ancestry seems to be unlikely. The genetic analysis revealed 6 new mutations (Exon 2: 2493ins5, Exon 3: W183C, Intron 4: 5178-9G\textbackslash A, Exon 5: 5186del9, Exon 10: 7766insC, 7656delG) [14, 20, 21]. One family exhibited a R17IQ amino acid change in exon 3 which is believed to be a polymorphism. The frequency of the allele in the general population is 5%. However, it remains to be seen whether this genetic alteration really represents a harmless polymorphism or whether it has to be regarded as a point mutation with low penetrance MEN1 phenotype. Since the family clinically exhibits a MEN1 phenotype according to the Stockholm criteria, it might be that we have missed a germline mutation or another alteration of the MEN1 gene with our method. Thus, rare families with large germline deletions of the MEN1 gene have been reported in the literature [22]. Loss of heterozygosity or fluorescence in situ hybridization studies on blood cells of this family are necessary to clarify this point. However, allelic loss of large parts of the MEN1 gene are unlikely in this family since the R17IQ amino acid change as well as polymorphism L432L in exon 9 was present in a heterozygous state in the DNA of clinically affected family members.

PHPT is the first and the most common feature in MEN1 and occurs in 95 - 99% of all MEN1 patients [2, 18, 22, 23]. In our families 95% (21/22 patients) of all patients with MEN1 had pHPT. PHPT was in 77% (17/22 patients) the first manifestation of MEN1. However, the prevalence of pHPT in all known gene carriers was 70%, which is due to the early detection of \textit{MEN1}-gene carriers by genetic analysis in members of known MEN1 families. Clinical onset of pHPT typically occurred in the third and fourth decade, which is in line with the literature (figure 1) [2, 18]. However, in members of the family with the presumed polymorphism, pHPT was diagnosed at an advanced age (70–80 years) in a still asymptomatic stage. It remains to be seen whether a polymorphism of the \textit{MEN1}-gene causes an attenuated and delayed clinical manifestation of the MEN1 syndrome as the example of our family might suggest.

The incidence of enteropancreatic neuroendocrine tumours in our MEN1 patients was 64%, with gastrinomas representing the majority of the tumours. These findings are comparable to previous reports [2, 22]. In contrast to published data just one of our MEN1-gene carriers was found to have a pituitary tumour (nonfunctioning) (4.5%). Almost all MEN1-gene carriers (88%) have been investigated biochemically (prolactin, IGF-I). According to the literature, only about 10% of the pituitary tumours are nonfunctioning [2, 24]. Therefore, we should have detected most of the clinically relevant pituitary tumours by biochemical screening. However, we might have missed a nonfunctioning tumour because of an incomplete screening of our cohort by MRI.

In 32% of our patients with MEN1, we also found MEN1-associated tumours (adrenocortical tumours (3), malignant thymic carcinoid (1), carcinoid of the lung (1), ependymoma (1) and cuta-
neous tumours (3) such as lipomas and angiofibromas.

A number of reports suggest that no obvious correlation between genotype and phenotype exists in the MEN1 syndrome [25]. In accordance, our data show no genotype-phenotype correlation either.

Screening for MEN1 in patients consists in the determination of the germline genetic state (gene carrier or normal) and the early diagnosis of tumours. The cloning of the MEN1-gene allows one to identify individuals with germline mutations who are at risk of developing the MEN1 syndrome, whereas a DNA test without a mutation in the MEN1-gene will exclude a family member from further clinical, biochemical and radiological investigations [15]. We performed genetic screening only within kindreds where a family member already had manifestations of the MEN1 syndrome. In our population (10 families) we detected 30 of 45 individuals with germline mutations. The remaining 15 family members showed no mutations and hence could be excluded from the prospective screening program for tumour manifestations.

The costs for the genetic screening amount to a one-time cost of SFr 1500. The annual expenditures for biochemical and radiological screening are capital-intensive. At our institutions these costs amount to at least SFr 522 per year. At least the latter costs are saved for the 15 non-mutant gene carriers, i.e. 33% of our potential MEN1 candidates, due to genetic screening. Therefore, genetic screening in families with a known MEN1-patient saves costs by exclusion of non-mutant gene carriers from annual biochemical and radiological screening investigations. The ideal age for carrying out genetic investigations in young family members is not clearly defined. The earliest manifestations of MEN1 tumours have been detected between 5 and 10 years of age [2, 26]. Thus, genetic screening at about the age of 10 could be recommended [27–30]. Our young family members were investigated genetically between the age of 10 and 20 years. None of the newly diagnosed MEN1-gene carriers had evidence of a MEN1 tumour. However, the decision for genetic investigation during childhood must be done carefully. It should be based on the advantages of early testing, specific wishes of the parents and possible adverse psychological consequences for the investigated young individual. Prior to any genetic screening, careful counselling by an experienced clinician or a human geneticist is mandatory. Genetic counselling should include information about possible manifestations of MEN1, the various treatment strategies of MEN1-associated tumours, the possible advantages of early diagnosis of family members, and the psychosocial consequences that may arise from a pathologic result.

Individuals identified to be carriers of a mutation should be monitored clinically and in particular biochemically and radiologically for the development of tumours. This allows one to detect tumour manifestations at a preclinical asymptomatic stage, and to initiate earlier treatment. By means of the proposed screening program we found a previously unknown pHPT in four newly identified gene carriers.

As soon as a MEN1-gene carrier is identified, biochemical screening should be performed annually. The extent of the biochemical analysis is comparable between different study groups and should include specific tests for pHPT, gastrinoma, insulinoma and pituitary tumours [31–33]. This biochemical screening program allowed us to detect nine patients with pHPT (four of them newly diagnosed gene carriers), one case of gastrinoma but no patient with an endocrine active pituitary tumour. Our findings strongly support the generally accepted recommendation that the measurement of calcium and gastrin must be an indispensable part of the minimal annual biochemical screening program. Up to now, we did not diagnose any new insulinoma, prolactinoma or acromegaly in our screening program. Biochemical screening allows the detection of MEN1-associated-tumours one to two decades prior to clinically overt disease [15, 17]. Therefore, medical or surgical treatment will start earlier and may reduce the rate of morbidity and mortality (e.g. treatment of gastrinomas with proton pump inhibitors reduces the risk of peptic ulcers). However, there is still no consensus about the operative management and timing of surgery for some MEN1-associated tumours (e.g. gastrinoma).

Our radiological screening program for enteropancreatic tumours consists of an abdominal CT scan every three years in gene carriers without any clinical or biochemical signs of a tumour. When there was biochemical evidence of an enteropancreatic tumour, the radiological investigations were done immediately. This strategy is in line with the recommendations of other groups [32, 33], however, other authors prefer yearly imaging studies [31, 34]. By means of the radiological screening of the abdomen we found nonfunctioning pancreatic tumours in three patients and a macronodular adrenal hyperplasia in another.

Although the incidence of pituitary tumours ranged from 15% to 90% in different MEN1 kindreds, in our series pituitary imaging did not appear to pay off since only one nonfunctioning microadenoma was detected, a finding of comparable prevalence in the overall population and of little or unknown clinical relevance. Regular screening for pituitary tumours with MRI may reveal more nonfunctioning microadenomas, and cause new management dilemmas and additional costs. Therefore, we consider clinical and biochemical screening for pituitary tumours more important than MRI of the pituitary gland.

In summary, our screening program applied to a cohort of 30 MEN1-gene carriers revealed pHPT in nine, nonfunctioning pancreatic neoplasias in three, and gastrinoma, non-functioning
microadenoma of the pituitary and macronodular adrenal hyperplasia in one patient each. Thanks to the screening strategy we were able to establish these diagnoses earlier and start specific treatment sooner, potentially reducing morbidity and mortality from MEN1. However, further studies are necessary to prove that screening strategies decrease morbidity or mortality caused by MEN1.

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