Clinical and Prognostic Implications of the Genetic Diagnosis of Hereditary NET Syndromes in Asymptomatic Patients

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- neuroendocrine tumor
- hereditary tumor
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Abstract

Neuroendocrine tumors (NETs) can be sporadic or they can arise in complex hereditary syndromes. Patients with hereditary NETs can be identified before the development of tumors by performing genetic screenings. The aim of the study was to evaluate the clinical and prognostic impact of a preclinical genetic screening in subjects with hereditary NET syndromes. 46 subjects referred for hereditary NET syndrome [22 MEN1, 12 MEN2, 12 Familial Paragangliomatosis (FPGL)] were enrolled and divided in 2 groups (group A, 20 subjects with clinical appearance of NET before the genetic diagnosis; group B, 26 subjects with genetic diagnosis of hereditary NET syndromes before the clinical appearance of NETs). The main outcome measures were severity of disease, prognosis, and survival. The rate of surgery for MEN1-, MEN2-, FPGL4-related tumors was 90% in group A and 35% in group B ($p<0.01$). Both symptoms related to tumors and symptoms related to therapies were significantly less frequent in group B than in group A ($p<0.05$). Tumor stage was locally advanced or metastatic in 50% of group A and in no one of group B ($p<0.01$). The mortality rate was 25% in group A and 0% in group B ($p<0.05$). An early genetic screening for hereditary NET syndromes results in an improvement in clinical presentation and morbidity. A potential impact of the genetic screening on the mortality rate of these subjects is suggested and needs to be investigated in further and more appropriate studies.

Introduction

Neuroendocrine tumors (NETs) are rare and heterogeneous neoplasms with variable biological behavior. The estimated incidence of NETs is about 1–5 cases/100 000/year. Recent data show a progressive increase of the incidence in the last years and a high increase of their prevalence and survival [1]. NETs can be sporadic or can arise in complex hereditary endocrine disorders such as Multiple Endocrine Neoplasias (MENs), Familial Paragangliomatosis (FPGLs), Neurofibromatosis type 1 (NF1), von Hippel–Lindau Disease (VHL), Tuberous Sclerosis, and Carney Complex [2]. It has been estimated that hereditary NETs represent 10–30% of these tumors but this rate seems to be an underestimation [2]. Recently, new imaging procedures have been developed, such as 68Ga-DOTATOC PET, which was reported to be highly sensitive in detecting NETs [3].

Patients with hereditary NET syndromes inherit the susceptibility to develop multiple endocrine neoplasias, which can be associated with nonendocrine tumors and/or nontumor lesions. Some NET syndromes, such as MENs, are characterized by germline mutations usually inherited as an autosomal dominant disease according to the Knudson's "two-hits hypothesis" [4]. Compared to the sporadic forms, hereditary NETs present an earlier age at onset, multiple tumor localizations, and higher secretory activity.

For subject identified as asymptomatic mutant carriers for MENs, clinical, biochemical, and instrumental workups have been elaborated, in order to early detect tumors and to start a precocious treatment. No workups are available for mutant carriers for FPGLs [5–7].

Multiple Endocrine Neoplasias (MENs)

There are different kinds of MEN syndrome: MEN1, MEN2, and the recently discovered MEN4. MEN1 or Wermer's Syndrome (OMIM #131100) is an autosomal dominant disorder characterized by high penetrance, variable inter- and intra-

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familial expressivity and genetic anticipation [8,9]. The prevalence of MEN1 is estimated to be 1:30 000 individuals, with the same distribution between males and females [10]. The clinical diagnosis of MEN1 is based on the concomitant occurrence of at least 2 of the following tumors: parathyroid adenoma, pituitary adenoma, and gastroenteropancreatic (GEP)-NET. Familial MEN1 is defined as at least one MEN1-related NET plus at least one first-degree relative with at least one of the 3 classical tumors or a known germline *MEN1* mutation [5]. Typically, NETs associated to MEN1 rise up 2 decades before the sporadic ones. They are generally benign; however, both GEP-NETs and carcinoids can be malignant.

MEN2 or Sipple's Syndrome (OMIM #171400) is an autosomal dominant disease resulting from germline mutations of the *RET* proto-oncogene, with an estimated prevalence of 1:30 000 subjects [5]. RET is a receptor tyrosine kinase, which results to be activated in MEN2 [11]. MEN2 is divided in 3 clinical variants: a) MEN2A (medullary thyroid cancer, pheochromocytoma, primary hyperparathyroidism and cutaneous lichen amyloidosis); b) familial medullary thyroid cancer (FMTC); and c) MEN2B (medullary thyroid cancer, mucosal and intestinal ganglioneuromatosis, marfanoid habitus) [5,12,13]. About 56% of cases belong to the subtype 2A, while the subtype 2B is the most aggressive with an elevated morbidity and mortality [12–14]. MEN2 is characterized by a genotype-phenotype correlation [12].

MEN4 is a recently discovered hereditary NET syndrome caused by mutations in the cyclin dependent kinase inhibitor (CDKI) gene *CDKN1B/p27*^{Kip1}. The affected animals exhibited phenotypic overlap of both MEN1 and MEN2 in an autosomal recessive pattern of inheritance. The phenotype of the CDKN1B mutation-positive subjects is still unclear and more affected cases need to be identified before any conclusions can be drawn and clinical management measurements can be taken [15,16].

Familial Paragangliomatosis (FPGLs)

FPGLs are a family of hereditary syndromes of susceptibility to multiple neuroectodermal tumors. Paragangliomas arise from adrenal medulla (pheochromocytomas) or extra-adrenal ganglia (paragangliomas) and are characterized by high vascularization and slow growth. In 10–50% of the cases, paragangliomas and pheochromocytomas are not sporadic and arise within genetic disorders such as FPGLs, MEN2, NF1, VHL, and Carney Complex [17–19].

There are 4 subtypes of FPGLs; only 3 of them (FPGL1, FPGL3, and FPGL4) are associated to known germline mutations of the genes encoding subunits of succinate dehydrogenase (SDH) [20].

The genetic origin influences the natural history of NETs: this is particularly evident in MEN1 where the diagnosis is made around the sixth decade of life in sporadic tumors while it is anticipated of about 3 decades in hereditary tumors [5,8]. The identification of hereditary NET syndromes is relevant to achieve a precocious diagnosis of the tumors and this may be important to prevent severe complications and unfavorable outcome. In the last years, it has been possible to identify a number of genes and molecular pathways involved in the development of NETs. Of consequence, some patients with an apparently sporadic tumor have been reclassified as carriers of a hereditary NET with relevant implications on the clinical course of disease and quality of life. Recently, the genetic screening has been reported to determine a more favorable outcome in asymptomatic subjects with MEN1 syndrome [21,22].

The aim of this study was to evaluate the clinical and prognostic impact of a preclinical genetic diagnosis in patients with different hereditary NET syndromes (MEN1, MEN2, FPGL4).

Patients and Methods

Patients

Between January 2003 and December 2009, 62 subjects were evaluated for hereditary NET at the Department of Molecular and Clinical Endocrinology and Oncology of the "Federico II" University Hospital of Naples. They included subjects with clinical, biochemical, and morphological features suggestive for a hereditary NET and first-degree relatives of patients with hereditary NETs. In details, the genetic analysis was performed in 9 patients with clinical diagnosis of MEN1 and 21 asymptomatic subjects first-degree relatives of patients with MEN1; 5 patients with clinical diagnosis of medullary thyroid carcinoma and 11 asymptomatic subjects first-degree relatives of patients with MEN2; 6 patients with clinical diagnosis of paraganglioma and 10 asymptomatic subjects first-degree relatives of patients with FPGL4.

Among the whole group genetically tested, 46 subjects (18 males, 28 females) were diagnosed as hereditary NET: 22 (9 males, 13 females), belonging to 9 families, were affected with MEN1; 12 (3 males, 9 females), belonging to 5 families, were affected with MEN2 (FMTC); and 12 (5 males, 7 females), belonging to 6 families, were affected with FPGL4.

All women included were in premenopausal age. No patients have been treated with corticosteroids or other drugs known to induce alterations in bone mineral density.

We did not observe phenocopies among the first-degree relatives of patients with MEN1, MEN2, or FPGL who were negative at the genetic test and so considered not affected with MEN1, MEN2, or FPGL.

The 46 subjects with hereditary NETs were divided in 2 groups: group A (20 subjects), patients with clinical onset of disease, and group B (26 subjects), carriers of gene mutations without clinical symptoms at the time of the genetic diagnosis.

All the 46 subjects with hereditary NET syndromes were clinically, biochemically, and morphologically evaluated to detect the presence of tumors and related manifestations according to the specific NET syndrome. A clinical follow-up was performed every 6 months, a biochemical and morphological follow-up yearly (unless there were clinical or biochemical signs of disease onset or modification). In MEN1 patients, the follow-up also included a duodeno-pancreatic echoendoscopy to detect non-functioning endocrine tumors early.

The total duration of the follow-up was 49.41 ± 3.35 (17–111) months for all the 46 subjects, 52.25 ± 5.7 (17–111) months for group A, and 47.23 ± 4.04 (21–81) months for group B.

Genetic analysis

Written informed consent was obtained from all subjects undergoing genetic test. Genomic DNA of patients of group A was extracted from peripheral blood leukocytes and the specific exons for MEN1, MEN2, or hereditary pheochromocytoma/paraganglioma genes (*RET*, *SDH*, *VHL*) were analyzed according to standard protocols [23–25]. In subjects of group B, the first-degree relatives of patients with genetic diagnosis of hereditary

NET, genetic test was performed to detect the known mutation in the family.

Among the 30 subjects genetically screened for the *MEN1* gene mutations, 22 of them (73%) were positive, 8 (27%) first-degree relatives of MEN1 patients were negative, and considered not affected with MEN1. Through *MEN1* mutation analysis the disease-causing mutations identified were frameshift 317delC (exon 2), frameshift 335delA (exon 2), missense Trp220Arg (exon 4), frameshift 1061delC (exon 7), nonsense Arg527Stop (exon 10), frameshift 1671del11 (exon 10).

Among the 16 subjects genetically screened for the *RET* proto-oncogene mutations, 12 of them (75%) were positive, 4 (25%) first-degree relatives were negative and considered not affected with MEN2. Through *RET* mutation analysis the disease-causing mutation identified was Glu768Asp (exon 13) in all unrelated families.

Among the 16 subjects genetically screened for the hereditary pheochromocytoma/paraganglioma genes (*RET*, *VHL*, *SDHB, SDHC, SDHD*) mutations, 12 of them (75%) were positive for *SDHB* mutations, and 4 (25%) first-degree relatives were negative and considered not affected with FPGL. Through *SDHB* mutation analysis the disease-causing mutations identified were nonsense Q30X (exon 2), c.183T > G (exon 2), c.423+1G > A (exon 4), 536–538delCAG (exon 6), c.603G > A (exon 6).

Statistical analysis

The statistical analysis was performed by SPSS for Windows version 15.0 (SPSS, Inc., Chicago, IL). Data are reported as mean ± SEM. The significance was set at 5%, $p < 0.05$. The comparison between the numerical data was performed by the analysis of variance (ANOVA test). The comparison between the categorical data was performed by χ^2-test with Yates correction and Fisher exact test as appropriate.

Results

Prevalence

In group A, the prevalence of at least one tumor associated to MEN1, MEN2, or FPGL4 syndrome was by definition 100%, while, in group B, the prevalence of at least one tumor associated to the syndrome was 50% ($p < 0.001$) (**Table 1**). The distribution of each type of NET in group A and in group B is shown in **Table 1**.

Table 1 Prevalence of tumors in MEN1, MEN2, and FPGL4 patients with clinical (group A) and pre-clinical (group B) diagnosis

	Group A n of affected pts/ total pts (%)	Group B n of affected pts/ total pts (%)	Total n of affected pts/ total pts (%)
MEN1			
Parathyroid	8/9 (88.9)	7/13 (53.8)	15/22 (68.2)
Pituitary	7/9 (77.8)	5/13 (38.5)	12/22 (54.5)
PRLoma	6/9 (66.7)	2/13 (15.4)	8/22 (36.4)
GHoma	0/9 (0)	0/13 (0)	0/22 (0)
GH-PRLoma	1/9 (11.1)	0/13 (0)	1/22 (4.5)
ACTHoma	0/9 (0)	0/13 (0)	0/22 (0)
TSHoma	0/9 (0)	0/13 (0)	0/22 (0)
NF	0/9 (0)	3/13 (23.1)	3/22 (13.6)
GEP tract	8/9 (88.9)	5/13 (38.5)**	13/22 (59.1)
Gastrinoma	7/9 (77.8)	2/13 (15.4)	9/22 (40.9)
Insulinoma	1/9 (11.1)	0/13 (0)	1/22 (4.5)
NF	2/9 (22.2)	4/13 (30.8)	6/22 (27.3)
Carcinoids	0/9 (0)	0/13 (0)	0/22 (0)
Thymus	0/9 (0)	0/13 (0)	0/22 (0)
Bronchus	0/9 (0)	0/13 (0)	0/22 (0)
Other site	5/9 (55.6)	0/13 (0)*	5/22 (22.7)
Adrenal adenoma	4/9 (44.4)	0/13 (0)**	4/22 (18.2)
Meningioma	2/9 (22.2)	0/13 (0)	2/22 (9.1)
MEN2 (FMTC)			
MTC	5/5 (100)	5/7 (71.4)	10/12 (83.3)
Other tumors	0/5 (0)	0/7 (0)	0/12 (0)
FPGL4			
Paraganglioma	6/6 (100)	0/6 (0)*	6/12 (50)
Cervical	1/6 (16.7)	0/6 (0)	1/12 (8.3)
Mediastinic	0/6 (0)	0/6 (0)	0/12 (0)
Abdominal	3/6 (50)	0/6 (0)	3/12 (25)
Retroperitoneal	1/6 (16.7)	0/6 (0)	1/12 (8.3)
Pelvic	1/6 (16.7)	0/6 (0)	1/12 (8.3)
Other tumors	0/6 (0)	0/6 (0)	0/12 (0)

*$p < 0.01$ (group A vs. group B); **p 0.05 (group A vs. group B)

Clinical features

Clinical features, therapies and outcome of patients with MEN1, MEN2, and FPGL4 are shown in **Table 2**. As a whole, for patients with hereditary NET syndrome with clinical onset (group A), the percentage of patients who underwent surgery for MEN1-, MEN2- or FPGL4-related tumors was 90% while for carriers of gene mutations (group B) it was 35% ($p < 0.01$).

At the last follow-up, tumor stage was locally advanced or metastatic in 50% of patients of group A and in none of the subjects of group B ($p < 0.01$). Disease activity was characterized by progression in 50% of patients of group A and in 4% of subjects of group B ($p < 0.01$). Tumor-associated symptoms were in 70% of patients of group A and 27% of subjects of group B ($p < 0.01$). Symptoms related to medical and/or surgical therapies were in 45% of patients of group A and 12% of subjects of group B (**Table 2**).

The mortality rate was 25% in group A and 0% in group B ($p < 0.05$) (**Table 2**).

MEN1

The mean age at diagnosis was 45.4±3.1 years in group A and 24.2±3.8 years in group B ($p < 0.001$). In group A, the first MEN1-related manifestation was nephrolithiasis in 67% of cases, osteoporosis in 11% of cases, peptic ulcer in 33% of cases, headache and visual defects in 11%, and pituitary hyperfunction in 22%. In group B, the first MEN1-related manifestation was nephrolithiasis in 23% of cases, peptic ulcer in 15% of cases, pituitary hyperfunction in 8% of cases; in 62% of cases no MEN1-related clinical manifestation was observed (**Fig. 1**). Hypogonadism was only reported in 2 MEN1 patients, one male with a GH/PRL-secreting macroadenoma (group A) and one female affected with a macroPRLoma (group B). All patients with osteoporosis/osteopenia were affected with primary hyperparathyroidism: they were 2 patients in group A, both affected with osteoporosis, both harboring a PRL-secreting pituitary adenoma and one being affected with hypogonadotropic hypogonadism and 3 patients in group B, all affected with osteopenia, with neither PRL-secreting adenomas nor hypogonadism.

Surgery and symptoms were more frequent in group A than in group B ($p < 0.05$). In particular, 5 subjects in group A and 2 subjects in group B underwent parathyroidectomy, which was total in 3 subjects in group A and in 1 in group B, and near total in 2 in group A and in 1 in group B. In 4 of 6 cases that had undergone parathyroid surgery, partial thyroidectomy was contextually performed because of the presence of thyroid nodules. 2 subjects in group A

Table 2 Clinical features, therapy and outcome in MEN1, MEN2, and FPGL4 patients with clinical (group A) and preclinical (group B) diagnosis

	MEN1		MEN2		FPGL4		Total	
	Group A n of affected pts/ total pts (%)	Group B n of affected pts/ total pts (%)	Group A n of affected pts/ total pts (%)	Group B n of affected pts/ total pts (%)	Group A n of affected pts/ total pts (%)	Group B n of affected pts/ total ts (%)	Group A n of affected pts/ total pts (%)	Group B n of affected pts/ total pts (%)
Surgery	7/9 (77.8)	3/13** (23.1)	5/5 (100)	5/7 (71.4)	6/6 (100)	0/6* (0)	18/20 (90)	8/26* (30.8)
Tumor stage#								
Localized	5/9 (55.6)	13/13** (100)	1/5 (20)	7/7** (100)	4/6 (66.7)	6/6 (100)	10/20 (50)	24/26* (92.3)
Advanced	4/9 (44.4)	0/13** (0)	4/5 (80)	0/7** (0)	2/6 (33.3)	0/6 (0)	10/20 (50)	0/26* (0)
Disease activity#								
Remission	0/9 (0%)	4/13 (30.8)	1/5 (20)	2/7 (28.6)	4/6 (66.7)	6/6 (100)	5/20 (25)	12/26 (46.2)
Progression	4/9 (44.4)	1/13 (7.7)	4/5 (80)	0/7** (0)	2/6 (33.3)	0/6 (0)	10/20 (50)	1/26* (4)
Stability	5/9 (55.6)	8/13 (61.5)	0/5 (0)	3/7 (42.9)	0/6 (0)	0/6 (0)	5/20 (24)	11/26 (42.3)
Symptoms#								
Related to tumor	8/9 (88.9)	7/13 (53.8)	3/5 (60)	0/7 (0)	3/6 (50)	0/6 (0)	14/20 (70)	7/26* (27)
Related to therapy	2/9 (22.2)	0/13** (0)	4/5 (80)	3/7 (42.9)	3/6 (50)	0/6 (0)	9/20 (45)	3/26 (11.5)
No symptoms	0/9 (0%)	5/13** (38.5)	0/5 (0)	2/7 (28.6)	4/6 (66.7)	6/6 (100)	4/20 (20)	13/26 (50)
Mortality	4/9 (44.4)	0/13** (0)	1/5 (20)	0/7 (0)	0/6 (0)	0/6 (0)	5/20 (25)	0/26** (0)

*p<0.01 (group A vs. group B); **p<0.05 (group A vs. group B); #at the last follow-up

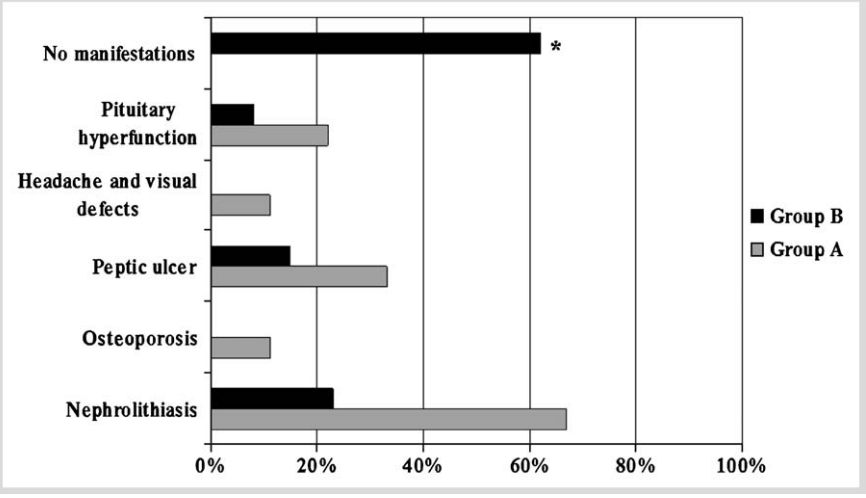

Fig. 1 Clinical manifestations at onset in MEN1 patients with clinical (group A) and pre-clinical (group B) diagnosis. *p<0.01 (group A vs. group B).

and 2 in group B underwent pancreatic surgery, which was 1 body-tail pancreatectomy in group A and 2 in group B and 1 duodeno-head pancreatectomy in group A. One subject in group A and none in group B underwent pituitary surgery, which was performed by transsphenoidal approach. Tumor stage and mortality were worse in group A than in group B (p<0.05) (**Table 2**). Pituitary tumor was more frequently a macroadenoma in group A than in group B (p<0.05) and GEP-NETs had more frequent complications in group A than in group B (p<0.01) (**Table 3**).

The mortality rate was 44% in group A and 0% in group B (p<0.05) (**Table 2**).

MEN2

The mean age at diagnosis was 47.8±1.9 years in group A and 38.9±4.6 years in group B. In group A, the first clinical manifestation was cervical swelling, associated to elevated calcitonin (>10ng/l) and CEA (>4ng/ml) serum concentrations in 100% of cases and lymph node metastases in 60% of cases. In group B, the first MEN2-related manifestation was elevated calcitonin in 43% of cases, associated to thyroid nodules in 71% of cases; there were neither thyroid nodules nor elevated calcitonin in 29% of cases (**Fig. 2**). Tumors were at a more advanced stage and more progressive in group A than in group B (p<0.05) (**Table 2**).

The mortality rate was 20% in group A and 0% in group B (**Table 2**).

FPGL4

The mean age at diagnosis was 28.7±3.5 years in group A and 32.3±5.0 years in group B. In the patients of group A, the first clinical manifestation was a hypertensive crisis in 33% of cases, acute abdomen in 17% of cases; 50% of patients were asymptomatic and were diagnosed on the basis of radiologically evident lesions. In the subjects of group B, there were no signs of FPGL occurrence (**Fig. 3**). The percentage of subjects who underwent surgery for FPGL4-related tumors was higher in group A than in group B (p<0.05).

The mortality rate was 0% both in group A and in group B (**Table 2**).

Discussion

The reason for a delay in the clinical diagnosis of hereditary NETs and the consequent frequent presentation at the time of diagnosis with a locally advanced or metastatic disease is that NETs are generally indolent, have a low proliferative index, and remain

Tumor site	Group A	Group B
Parathyroid		
Number of adenomas	2.5 ± 0.3 (2–4)	2.0 ± 0.2 (1–3)
Parathyroid	**n of affected pts/total pts (%)**	**n of affected pts/total pts (%)**
Complications	5/9 (55.6)	5/13 (38.5)
Nephrolithiasis	5/9 (55.6)	2/13 (15.4)
Osteopenia/osteoporosis	2/9 (22.2)	3/13 (23.1)
Renal failure	1/9 (11.1)	0/13 (0)
Pituitary	**n of affected pts/total pts (%)**	**n of affected pts/total pts (%)**
Macroadenoma	5/9 (55.6)	1/13 (7.7)**
Microadenoma	2/9 (22.2)	4/13 (30.8)
Complications	7/9 (77.8)	5/13 (38.5)
Loco-regional features#	3/9 (33.3)	1/13 (7.7)
Pituitary hypersecretion##	7/9 (77.8)	2/13 (15.4)*
GEP tract	**n of affected pts/total pts (%)**	**n of affected pts/total pts (%)**
Number of neoplasias	4.4 ± 1.8 (1–16)	2.5 ± 0.9 (1–7)
Hyperfunctioning syndrome	7/9 (77.8)	2/13 (15.4)*
Complications	7/9 (77.8)	2/13 (15.4)*
Peptic ulcer	7/9 (77.8)	2/13 (15.4)*
Hypoglycemia	1/9 (11.1)	0/13 (0)
Tumor mass	4/9 (44.4)	0/13 (0)**

*p < 0.01 (group A vs. group B); **p < 0.05 (group A vs. group B); # Headache, visual defects; ## Hyperprolactinemia, acromegaly

Table 3 Clinical features in MEN1 patients with clinical (group A) and preclinical (group B) diagnosis

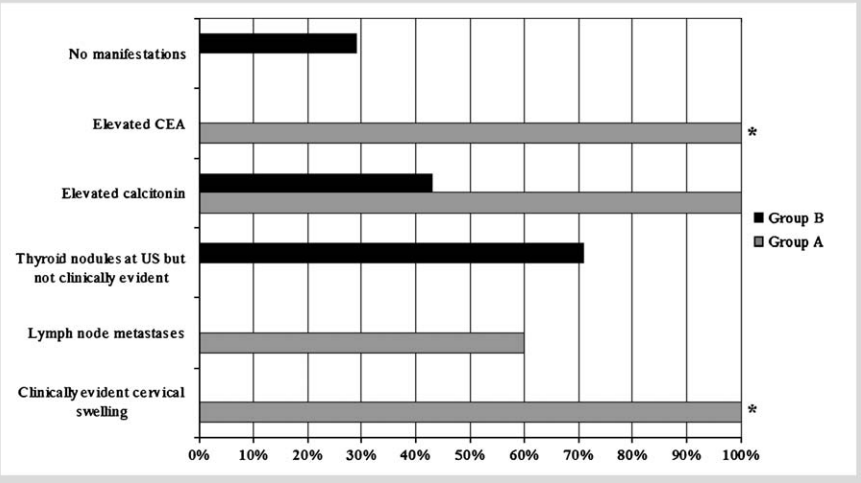

Fig. 2 Clinical manifestations at onset in MEN2 patients with clinical (group A) and preclinical (group B) diagnosis. *p < 0.01 (group A vs. group B).

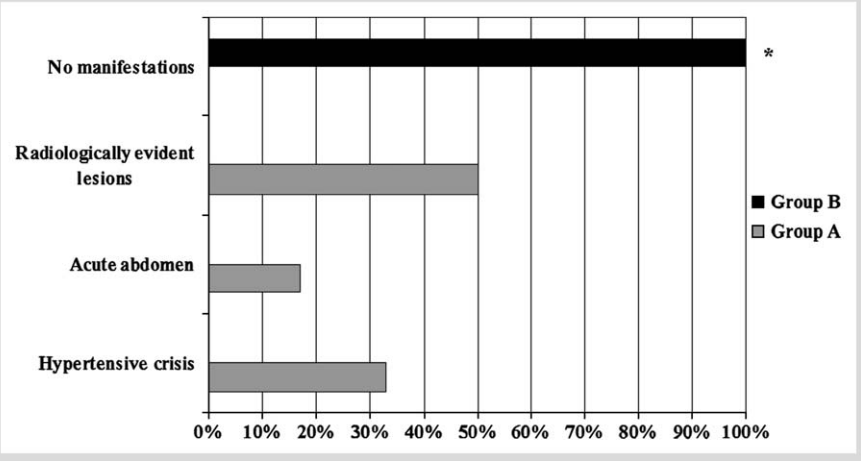

Fig. 3 Clinical manifestations at onset in FPGL4 patients with clinical (group A) and preclinical (group B) diagnosis. *p < 0.01 (group A vs. group B).

asymptomatic for long time. Nowadays, the improved knowledge on NET biology and genetics offers the possibility to achieve an early diagnosis of hereditary NETs, through the genetic screening, thus preventing the evolution toward a clinically, biochemically, or radiologically evident tumor [26–31]. In this attempt, periodical evaluations in reference health centers are necessary in subjects with preclinical hereditary NETs, especially young subjects, to detect tumors early and prevent complications and risk of malignant transformation [5, 8, 20, 32].

In the last years, the growing number of subjects with hereditary NET who is diagnosed at a preclinical stage is changing the clinical picture of these tumors. Until now the clinical features and natural history of hereditary NETs were based on data from patients with clinical evidence of disease. With the identification of specific genes responsible for the development of hereditary NETs, more and more subjects are recognized to be carriers of NET-related gene mutations. Preclinical genetic screening in asymptomatic first-degree relatives of patients with hereditary NET syndromes leads to detect these neoplasias at an early stage, even when subjects are still asymptomatic.

The impact of genetic screening on the management and outcome of asymptomatic subjects genetically diagnosed for MEN1 has been recently evaluated in 2 studies [21,22]. These studies highlighted that genetic screening in asymptomatic MEN1 subjects before the clinical appearance of NETs is associated with an improvement of the long-term outcome. However, these conclusions have been achieved only for one of the hereditary syndromes associated with NETs. There are no studies evaluating the impact of genetic screening in patients with hereditary NET syndromes as a whole group, including different types of syndrome.

The current study has evaluated patients with MEN1, MEN2, and FPGL highlighting that in patients with hereditary NET syndromes, regardless from the type of syndrome, an early diagnosis and treatment of potentially malignant tumors is necessary to reduce tumor-related morbidity. The rationale for grouping different patients with different hereditary NETs is to emphasize the role of genetic screening in hereditary NET syndromes, which have all in common the tendency to develop NETs along the life and the possibility to identify the syndrome before the clinical appearance of the disease.

The mortality rate was significantly higher in subjects with clinical appearance of NET than in those with genetic diagnosis of hereditary NET syndromes before the clinical appearance of NETs. Although the evaluation of mortality is limited by the fact that subjects in the former group were older than those in the latter group, these data suggest that the genetic screening potentially impacts on mortality in hereditary NET syndromes. This represents a relevant point for further and more appropriate investigations.

Patients clinically diagnosed require more aggressive and hazardous therapies, more frequent and invasive medical examinations than those who were identified at the genetic screening in the absence of clinical evidence of disease. In addition, the former group has worse outcome and lower rate of cure than the former one.

Another relevant point is that patients with hereditary NET syndromes need to be followed in experienced centers which should represent a landmark for this pathology in a specific geographic region. This is because MEN1, MEN2, FPGL as well as other NET, and non-NET genetic syndromes may vary in gene penetrance and expressivity between populations from different geographic areas [5,13,17,25]. About 6 million inhabitants of the Campania region (including Naples and surrounding areas) represent the population which refers to our center for patients with NET, which has already identified 46 patients with hereditary NET syndrome since the start of its activity in 2003. The series of patients described here reveals some differences as compared to data reported in previous studies concerning tumor distribution in patients with MEN1 and FPGL4 but not in those with MEN2. In MEN1, the prevalence of pituitary adenomas and GEP-NETs observed in the current study was higher than that previously observed. Then the prevalence of carcinoids (thymic or bronchial) was lower while the prevalence of adrenal cortical adenomas and meningiomas was higher than in previous studies [5,8,21,22]. Hyperparathyroidism was the first manifestation in the majority of cases. Anyway a pancreatic NET, 4 cm in size, was the first manifestation in a young totally asymptomatic woman, daughter of a MEN1 index case. Of consequence, the periodical evaluation for hyperparathyroidism onset only in MEN1 subjects without clinical manifestations at the diagnosis is not sufficient and these patients need to be more extensively evaluated in the course of their periodical follow-up, confirming previous observations [5,22]. The impairment of bone mineral density was consistent with osteoporosis in group A and osteopenia in group B. It is likely that hyperprolactinemia may in part explain the severity of loss in bone mineral density in patients of group A, however the duration of hyperparathyroidism, which was higher in group A than in group B, is the most reliable explanation. In FPGL4 patients, the peculiar trait of the present series was the absence of adrenal paragangliomas (pheochromocytomas) in association with extra-adrenal paragangliomas.

In conclusion, this study evaluated the clinical and prognostic impact of genetic screening in patients with hereditary NETs associated to MEN1, MEN2, and FPGL4, highlighting that an early genetic diagnosis in asymptomatic subjects is recommended to identify subjects at risk to develop one of the above mentioned syndromes as early as possible before the occurrence of clinical manifestations, in order to improve their long-term outcome and to ensure a survival and quality of life similar to that observed in the general population. Based on data from large series of patients followed in experienced centers, diagnostic and therapeutic algorithms have to be elaborated to improve the management of patients with hereditary NET syndromes.

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