CLINICAL SCIENCES

THE IMPACT OF CLINICAL AND GENETIC SCREENINGS ON THE MANAGEMENT OF THE MULTIPLE ENDOCRINE NEOPLASIA TYPE 1

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Lourenco Jr DM, Toledo RA, Coutinho FL, Margarido LC, Siqueira SAC, Cortina MA, Montenegro FL, Machado MC, Toledo SP. The impact of clinical and genetic screenings on the management of the multiple endocrine neoplasia type 1. CLINICS. 2007;62(4):465-76.

PURPOSE: To perform clinical and genetic screening for multiple endocrine neoplasia type 1 (MEN1) in patients at the Academic Hospital of the University of São Paulo School of Medicine, and to analyze its impact on clinical management of patients with MEN1.
METHODS: The clinical diagnosis of MEN1 was made in accordance with the Consensus on multiple endocrine neoplasias. Mutation analysis of the entire *MEN1* tumor suppressor gene and genetic screening of at-risk family members were performed by direct sequencing. To analyze the implementation of genetic diagnosis, the studied patients were separated into 3 groups: MEN1 index cases (group I), clinically diagnosed MEN1 cases (group II), and genetically diagnosed MEN1 cases (group III).
RESULTS: In total, 154 individuals were clinically and genetically studied. We identified 12 different *MEN1* mutations. Fifty-two MEN1 cases were identified: 13 in group I, 28 in group II, and 11 in group III. The mean age in group III (27.0 years) was significantly lower than in groups I (39.5 years) and II (42.4 years; $P = 0.03$ and $P = 0.01$, respectively). Patients in groups I and II mostly presented 2 or 3 MEN1-related tumors, while 81.8% of those in group III presented 1 or no MEN1-related tumor. Additionally, in group III, 45.4% of cases were asymptomatic, and no metastasis or death was verified. Surveillance for *MEN1* mutations allowed the exclusion of 102 noncarriers, including a case of MEN1 phenocopy.
CONCLUSION: Our data supports the benefits of clinical and genetic screening for multiple endocrine neoplasia type 1 in the management of this syndrome.

KEYWORDS: Multiple endocrine neoplasia. MEN1. *MEN1* gene. Screening. Genetic diagnosis.

INTRODUCTION

Clinical Aspects of the MEN1 syndrome

Multiple endocrine neoplasia type 1 (MEN1; OMIM 131100) is an autosomal dominant inherited tumor syndrome mainly characterized by parathyroid, endocrine pancreas, and pituitary tumors.[1,2] According to the MEN Consensus (2001), the diagnosis of this condition is based on the concomitant occurrence in a patient of at least 2 of these 3 major MEN1-related tumors.[1]

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Received for publication on July 05, 2007
Accepted for publication on July 07, 2007

Hyperparathyroidism in MEN1

Primary hyperparathyroidism (HPT) is the most common clinical feature of MEN1, and it occurs in 73% to 100% of cases (Table 1). HPT is usually (80%) the first clinical manifestation of MEN1.[3] Primary hyperparathy-

roidism due to MEN1 (HPT/MEN1) differs from sporadic primary HPT (sHPT) in several aspects, as it presents, a) multiglandular parathyroid hyperplasia or adenoma; b) age-onset 2 decades earlier than sHPT (20 vs 40 years of age); c) sex ratio of 1:1 in contrast to the 1:3 for sHPT; and d) higher recurrence rates of HPT after parathyroidectomy.[4,5] Although HPT/MEN1 tends to be less aggressive than sHPT, usually presenting parathyroid hormone (PTH) ranging from 2 to 3 times over the upper normal limits and moderately high calcium levels,[6] "moans, groans, and stones" and renal insufficiency may occur in advanced stages of this disease. In late diagnosed cases, renal dialysis and kidney transplantation may be necessary.[5]

Table 1 - Prevalence (%) of clinical features in MEN1 disease*

Endocrine Tumors	
Parathyroid adenoma (73-100)	Anterior pituitary tumor (20-40)
	Prolactinoma (62-76)
Enteropancreatic tumor (30-80)	NS (14-24)
Gastrinoma ** (30-75)	Co-secreting (10)
Insulinoma (10-30)	GH-secreting (9)
	ACTH-secreting (4)
Carcinoids ** (> 10)	TSH-secreting (rare)
	Adrenal cortex NS (12-40)
Nonendocrine Tumors	
Facial angiofibroma (40-80)	

*References [1,3,11,14,18–22]
**tumor with malignant potential (> 25%); NS, nonsecreting.
MEN1 = multiple endocrine neoplasia type 1

Surgical management for HPT/MEN1 includes subtotal or total parathyroidectomy followed by an autograph to the forearm, clearly differing from the adenomectomy recommended for sHPT. Furthermore, in MEN1 cases, preventive thymectomy is recommended in association with parathyroidectomy to prevent thymic carcinoids.[1,7–10] These facts underline the importance of performing the diagnosis of MEN1 for better management of HPT in these patients.[7–10]

The penetrance of HPT in MEN1 in several series has been reported as high as 95% at 50 years of age, so it has been considered the main clinical feature for the diagnosis of MEN1. Thus, screening for other MEN1-related diseases was initially considered less necessary for recognition of MEN1. Screening for HPT in MEN1 was usually performed only until 50 years of age. However, recent series have shown lower prevalences (50%-70%) of HPT as the first clinical characteristic in MEN1 and lower HPT penetrance (70%) than previously reported. These findings indicated that HPT surveillance should be performed for longer periods.[11,12]

Endopancreatic tumors in MEN1

Pancreatic/duodenal endocrine tumors (PETs) occur in up to 30% to 80% of patients with MEN1.[3,13,14] Gastrinoma in MEN1 is the most prevalent functioning PET, seen in 30% to 75% of MEN1 cases[5]. The gastrinomas in MEN1 mostly occur in the duodenum (up to 90%) and is frequently multifocal, in contrast to unifocal sporadic gastrinomas.[5] Furthermore, up to 25% of all gastrinomas are related to MEN1.[1,15] Surgical procedures in gastrinoma associated with MEN1 are usually more extensive than those applied to its sporadic counterpart. Enucleation of multiple nodules of the duodenum associated with subtotal (80%-85%) pancreatectomy is recommended for patients with MEN1, whereas enucleation of the unique small tumor of the duodenum or pancreas and duodenectomy or partial pancreatectomy for bigger tumors are usually performed for sporadic gastrinomas.[16,17] Thus, the presurgical MEN1 diagnosis offers the surgeon important information for a better approach regarding PETs in MEN1 cases.

A similar statement was also applied to insulinoma associated with MEN1, which is the second most prevalent functioning PET in MEN1 (10%-30%). For patients with insulinoma due to MEN1, subtotal pancreatectomy combined with enucleation of possible nodules of the head of pancreas is the recommended surgical approach .[1,5] Enucleation of the unique nodule of the pancreas or partial pancreatectomy are the most common surgical approaches to sporadic insulinomas.

Pituitary tumors in MEN1

Pituitary adenomas have been documented in up to 40% of MEN1 cases reported in extended series.[18–20] However, lower prevalences (18%-21%) have been also documented by others.[21,22] In 17% of MEN1 cases, a pituitary adenoma may be the initial lesion.[18] Interestingly, age at the diagnosis of pituitary tumors is similar (~37 yrs) for MEN1, familial isolated pituitary adenomas, and sporadic pituitary tumors.[18,23] Pituitary adenomas in MEN1 are usually more aggressive and larger than in its sporadic counterpart. Thus, up to 85% of pituitary tumors associated with MEN1 are macroadenomas (32% invasive), in contrast to 42% in non-MEN1 cases.[18]

Prolactinoma is the most frequent pituitary disease in MEN1; 62% to 76% of patients with MEN1 having pituitary disease present with prolactinoma, although nonsecreting pituitary adenomas are also frequent (14%-24%).[18,21] Co-secreting, GH-, and ACTH-secreting pituitary tumors are less frequent (10%, 9%, and 4%, respectively),[18] whereas FSH and TSH-secreting tumors are both very rare in MEN1.[24]

In addition to the major features of MEN1 mentioned above, up to 20 other endocrine and nonendocrine tumors have been described in association with MEN1 (Table 1).[13] Due to its extended and widely variable phenotype, MEN1 is presently considered a complex, multisystemic disorder, and its clinical diagnosis may turn to be a difficult task.[13]

Thymic carcinoid tumors and gastrinoma are the major causes of death in MEN1 patients.[25,26] More than 90% of MEN1-associated thymic carcinoids are malignant;[25–27] however, these tumors are relatively rare. On the other hand, although gastrinomas are usually less malignant (60%) than thymic carcinoids,[28] they can be detected in up to 75% of MEN1 cases depending on the series (Table 1). Malignancies are responsible for significant lowering ages of death verified for MEN1 (55.4 years for men and 46.8 years for women), as compared to life expectation in the general population (> 70 years).[29]

No preventive surgical approach has been shown to significantly improve the outcome of MEN1.[1] However, it is accepted that the earlier the identification of MEN1 neoplasias, the better the clinical management of this disease.[30,31] As recommended by the MEN Consensus, the suggested approach for patients with MEN1 is based on periodical surveillance of MEN1-related neoplasias that should begin as early as 5 to 20 years of age.[1] Surveillance for MEN1 neoplasia is a time-consuming, laborious, expensive, and lifelong procedure that includes clinical, biochemical, and imaging investigations. However, it has been proven to be efficient in the identification of MEN1 tumors and in the reduction of morbidity[30] and mortality of patients with MEN1.[25,26,31]

Therefore, the establishment of a structured and long-term follow-up program focused on screening for MEN1 would be a worthwhile effort.[29]

Genetic Aspects of MEN1

Familial MEN1 is an autosomal dominantly inherited disease presenting almost complete penetrance.[13] The gene responsible for MEN1 (*MEN1*) was identified at 11q13 by two different research groups, one from NIH[32] and another from the European Consortium.[33] Since genetic screening for *MEN1* became available, more than 400 germline and somatic mutations have been identified in this gene.[13,34] Most *MEN1* mutations are inactivating, nonsense, or frameshift variants. Although splicing mutations represent only 5% of the overall mutations identified in the *MEN1* gene,[34] some of these disease-causing variants can be frequently found.[35] Furthermore, several missense *MEN1* mutations have been identified, mostly occurring in evolutionary conserved sites, predicted to be related to retained relevant functions (Toledo RA et al., Clin Endocrinol (Oxf). 2007 Jun 6; [Epub ahead of print] PMID: 1755549, in press).

No hotspots have been found in the *MEN1* gene; however, patients from different genetic backgrounds have shown recurrent mutations in GC-rich regions that are prone to slippage, suggesting mutational "warm-spot" areas in the *MEN1* gene.[33] To date, no relevant genotype-phenotype correlation has been reported.[32] Also, interfamilial and intrafamilial phenotype variability has been demonstrated.[32,33] Thus, relatives of individuals with MEN1 who harbor the same disease-causing mutation may present different clinical pictures.[36]

It has been reported that *MEN1* mutation could not be found in up to 30% of familial MEN1 cases genetically tested.[13] Technical limitations or the presence of mutations in the gene promoter region (which is not usually accessed in genetic screenings) may explain the finding of genetically negative MEN1 cases.[13] Such a *MEN1* mutation profile results in a laborious routine genetic investigation. At present, genetic screening for *MEN1* abnormalities is mostly available in developed countries.

The *MEN1* gene codifies for a 610-amino acid protein named menin.[37] Several tumor suppressor roles of menin have been disclosed so far, such as, a) cell cycle and cell growth control, b) transcription regulation, c) DNA repair, d) genome stability, e) apoptosis regulation, and f) endocrine cell proliferation.[37,38] A *MEN1* germline mutation predisposes the genome to a second mutational event concerning MEN1-associated glands, causing loss of heterozygosity (LOH) of the 11q13 locus. The inactivation of menin is predicted to disrupt its tumor suppressor molecular pathways, thus leading to MEN1 tumorigenesis.[37] These findings are consistent with the Knudson's 2-hit hypothesis for tumor suppressor genes.[39]

State of art of MEN1 in Brazil

To date, little information is known about the clinical and genetic profile of patients with MEN1 in Brazil. As occurs for other syndromes, such as sporadic HPT, for which specific screening programs are not performed in Brazil,[40] it is likely that most patients with MEN1 present as symptomatic, late-diagnosed cases. To our knowledge, no public hospital other than Hospital das Clínicas in Brazil is routinely offering genetic testing for *MEN1* gene mutations.

During the last 10 years, patients with MEN1 (and also MEN2) have been followed at the Disciplina de Endocrinologia of the Hospital das Clínicas, University of São Paulo, School of Medicine, at no charge through the Brazilian National System of Health (*Sistema Único de Saúde, SUS*). Our unit has developed expertise in diagnosis, management, and treatment of MEN1, and it has become one of the reference centers for this disease in Brazil.[41–46]

In this study, we report the results of clinical and genetic screenings of patients with MEN1. We also evaluated the impact of *MEN1* genetic screening on the diagnosis and management of patients with MEN1, before and after its implementation. As far as we know, this is the first systematic genetic screening for MEN1 disease performed in South America.

PATIENTS AND METHODS

This study was approved by local ethics committee. Written informed consent was obtained from all patients undergoing genetic testing.

Briefly, the clinical screening of patients with MEN1 at our hospital started in 1997 and comprised the following 3 phases: phase 1—systematic clinical identification of patients with MEN1 at this institution was performed; phase 2—MEN1 genealogies were expanded, and clinical screening was performed; and phase 3—the genetic testing for *MEN1* was routinely incorporated into the clinical practice in our unit. The studied patients were followed for 10 years.

Phase 1 – Identification of index cases of MEN1

The criterion for the diagnosis of MEN1 was the presence of at least 2 MEN1-related tumors in the index cases.[1] Hyperparathyroidism (HPT) was identified based on the presence of hypercalcemia associated with inappropriately elevated/borderline serum levels of parathyroid hormone (PTH). Prolactinoma was recognized by consistently high serum prolactin concentrations, menstrual irregularities, and/or hypogonadism associated with pituitary adenoma shown by MRI. Insulinoma was verified by clinical signs and symptoms of hypoglycemia associated with abnormal insulin/glucose ratios. Gastrinoma was documented by the presence of repetitive gastro-duodenal ulcers, gastric acid hypersecretion, hypergastrinemia, and endoscopic ultrasound findings. Somatotrophinoma was diagnosed through measurements of GH and IGF1; pituitary MRI was also performed. Cushing syndrome was diagnosed by clinical findings, ACTH/cortisol measurements, and image studies. Other rare involvements such as carcinoid tumors were also actively sought using standard clinical, biochemical, and image procedures.

Phase 2 – Clinical screening of relatives at-risk for MEN1

Familial MEN1 was defined when at least 1 MEN1-related tumor was found in a first-degree relative. At-risk family members were invited to come to the hospital for exams. Clinical screening for MEN1-related neoplasias was performed, as recommended by the MEN Consensus.[1,13] Annual biochemical exams and a tri-annual imaging investigation were performed. Those who presented biochemical or/and imaging abnormalities consistent with MEN1 and who had complaints related to MEN1 symptoms were diagnosed as affected. Relatives who had MEN1-related features at screening but no clinical symptoms were considered asymptomatic MEN1 cases. Relatives with no complaint and normal biochemical and imaging results were considered "not conclusive" and were invited to participate in the annual clinical screening.

Phase 3 – *MEN1* genetic analysis

The optimization and standardization of genetic protocols for *MEN1* mutation analysis were established in 2004. Laboratory procedures included, a) genomic DNA extraction from peripheral blood and oral swabs; b) amplification of the entire *MEN1* coding region (exons 2-10) and also exon/intron boundaries by polymerase chain reaction (PCR); c) automated DNA sequencing, and d) *MEN1* mutation analysis.

Optimization and standardization of genetic protocols for *MEN1*

DNA extraction

After obtaining written informed consent, 10 mL of peripheral blood were collected (in 2 EDTA-containing tubes) from the 13 MEN1 probands. Genomic DNA was extracted according to a standard salting-out protocol or GFX Genomic Blood DNA Kit (Amersham Bioscience, Piscataway, NJ, USA). DNA from oral swab samples was obtained using the Chelex 100® (BioRad).

MEN1 mutation analysis - Optimized protocol

To perform the PCR amplification of the entire coding region (exons 2-10) and intron/exon boundaries of *MEN*1, we used specific primers as previously reported.[32,47] To optimize PCR conditions, we used cycling temperatures and $MgCl_2$ gradient assays in a MJ PTC-200 thermocycling apparatus (MJ Research). Best results were obtained using 200 ng of genomic DNA, 1X reaction buffer, 2.5 mM $MgCl_2$, 0.5 mM of each dNTP, 0.5 pmol of each primer and 1.25 U of *Taq* DNA polymerase (Invitrogen, São Paulo) in a total volume of 30 µL. Thermocycling conditions for the PCR were optimized as follows: 3 min at 94°C, followed by 30 cycles of 1 min at 94°C, 45 s with a 65°C-to-60°C touchdown annealing temperature program (minus 1ºC per cycle until 60ºC was reached), and 1 min at 72°C, followed

by 10 min of final extension at 72°C. The PCR products were confirmed by electrophoresis on a 1.5% agarose gel and purified using a Concert Rapid PCR Purification System kit (Life Technologies, Bethesda, MD).

Sequencing reactions were directly performed from purified PCR products using internal primers for both strands and Big Dye Terminator v3.1 (Applied Biosystems, Foster City, CA). Sequencing was carried on an automated sequencer (ABI Prism 310 DNA Analyzer, Applied Biosystems, Foster City), according to the manufacturer's recommendations. The homology of generated sequences was obtained using BLAST (http://www.ncbi.nlm.nih.gov/BLAST). Two sequencing editor software programs (Gene StudioTM Professional Edition, Suwanee; and Mutation Surveyour, Softgenetics, PA, USA) were used to identify DNA abnormalities.

After the identification of the disease-causing *MEN1* mutation in an index case, DNA samples from relatives who participated in the clinical screening (phase 2) were analyzed. First-degree relatives who did not participate in phase 2 were contacted and invited to come to the hospital and undergo the screening. To family members living far from São Paulo and claiming not to be able to travel, oral swabs were sent by mail. Furthermore, during the period of the project, several local visits were made by a physician (DML) and a social assistant from our group. They visited at-risk relatives of MEN1 patients who were interested in participating but to whom previous procedures were not applicable. This last approach was also used with families with extended genealogies living in a small geographic area. A total of 154 samples, 13 from patients who were MEN1 index cases and 141 from at-risk family members of patients with MEN1, were available for investigation.

Clinical follow-up and genetic counseling were offered to individuals who tested positive for *MEN1* mutation. Furthermore, genetic testing was also offered to their descents. Individuals who did not inherit the mutant allele were excluded from MEN1 clinical screening protocol, and they were informed that their descents would not inherit the familial predisposition to MEN1-related tumors.

Statistics

ANOVA, Kruskall Wallis, and Mann-Whitney tests were used when applicable.

RESULTS

In phase 1 of the study, 13 MEN1 index cases were diagnosed. All index cases reported a familial history of MEN1. During phase 2 of the study, 28 patients with MEN1 were diagnosed. During phase 3, 11 MEN1 cases were discovered. These 11 patients decided to undergo clinical screening only after knowing that they were positive for a *MEN1* mutation.

During the 10-year follow-up of these 13 families involving 52 individual cases of MEN1, the prevalence of MEN1-related pathology was as follows: HPT, 94.2% (49/52); pancreatic/duodenal endocrine tumors (PETs), 63.5% (33/52); and pituitary tumors, 51.9% (27/52). Regarding the 49 cases of HPT, 38 (77.5%) were symptomatic and 11 (22.5%) were asymptomatic. Gastrinomas were the most common type of PET (18/33; 54.5%), and 39.0% (7/18) of them were malignant, as documented by pathology/radiology findings. Insulinomas (8/33; 24.2%) and exclusively nonsecretory tumors (7/33; 21.2%) were also represented within PET cases. Furthermore, prolactinomas were the most frequent pituitary tumor (18/27; 66.7%), whereas nonsecretory pituitary adenomas (8/27; 29.6%) and somatotrophinoma (1/27; 3.7%) were also present (Table 2).

Table 2 - Prevalence of major MEN1-related tumors in patients with MEN1: index cases (group I), clinically diagnosed cases (group II), and genetically diagnosed cases (group III).

	group I (n = 13) (%)	group II (n = 28) (%)	group III (n = 11) (%)	total of patients (n = 52) (%)	literature* (%)
parathyroid (HPT)	100	100	72.7	94.2	73-100
pancreas (PETs)	76.9	67.9	27.3	63.5	30-80
gastrinoma	(60)**	(57.9)**	(33)**	(54.6)**	30-75
insulinoma	(50)**	(15.8)**	(0)**	(24.2)**	10-30
NS	(0)**	(26.3)**	(67)**	(21.2)**	
pituitary adenoma	69.2	46.4	45.5	51.9	20-40
prolactinoma	(77.8)***	(61.6)***	(60)***	(66.7)***	62
NS adenoma	(22.2)***	(38.4)***	(20)***	(29.6)***	15
somatotrophinoma	(0)***	(0)***	(20)***	(3.7)***	9
ACTHoma	0	0	0	0	4
co-secreting	0	0	0	0	10
carcinoids	7.7	10.7	0	7.7	> 10

HPT, hyperparathyroidism; NS, exclusively nonsecreting
*References[1,18,21,28].
** % relative to the cases of pancreatic endocrine tumors (PETs) in this particular group (one case had both gastrinoma and insulinoma).
***% relative to the cases of pituitary adenomas in this particular group

MEN1 patient groups

The mean ages at diagnosis (clinical or genetic) in groups I (39.5 ± 15.7 SD years) and II (42.4 ± 15.0 years) did not significantly differ ($P > 0.05$). However, when both data were compared with group III (27.0 ± 14.0 years), significant differences were noticed ($P = 0.03$; $P = 0.01$, respectively). The occurrence of 2 major secreting MEN1-related tumors in a

single patient tended (P = 0.06) to be more prevalent in groups I and II, (7/13; 53.8% and 9/28; 32.2%, respectively) than in group III (1/11; 9.1%). Also, although 3 coexisting MEN1-related tumors in the same patient were more frequently seen in groups I and II (6/13; 46.2% and 6/28; 21.4%) than in group III (1/11; 9.1%), no significant differences were noticed (P = 0.22). One isolated MEN1-related tumor occurred equally in groups II and III (13/28; 46.4% and 8/11; 72.7%, respectively), as shown in Table 3.

Malignancy and Mortality

Malignancies in MEN1-related tumors were documented by the pathologic findings in 8 cases (data not shown) and/or by the presence of either local or distant metastases in all of them. Malignant tumors were equally represented in groups I (3/13, 23.1%) and II (5/28, 17.9%). Metastases originated from gastrinomas (6/8; 75%) or carcinoid tumors (2/8**;** 25%). No patient in group III exhibited a malignant MEN1-related tumor (Table 3).

All 4 patients with carcinoid tumors were asymptomatic. The first patient had a gastric carcinoid that was identified and removed by endoscopy. The second had a bronchial carcinoid presenting local lymph node metastasis and underwent surgery. The third patient presented with an atypical, multiple metastatic pulmonary carcinoid, and the fourth had a pulmonary carcinoid tumor, but he refused any kind of treatment.

In the 10-year follow-up period (1997-2006), 4 of the 52 patients died of causes related to MEN1 disease as follows: 2 out of the 13 patients in group I (15.4%) and 2 out of the 28 patients in group II (7.1%). Three of them died due to metastatic gastrinoma, and 1 due to secondary complications of a nonsecretory pituitary macroadenoma. No death in group III has occurred (Table 3).

MEN1-related tumor prevalence

Primary hyperparathyroidism was diagnosed in all patients in groups I and II (100%) and in 8 patients (72.7%) in group III. Asymptomatic HPT was present in patients from groups I (7.7%) and II (21.4%), but it prevailed in group III (50%). Conversely, symptomatic HPT (nephrolithiasis) was seen mostly in patients of groups I and II (87.8%) and was less represented in group III (50%).

The prevalence of pancreatic/duodenal endocrine tumors (PETs) in groups I and II (76.9% and 67.9%, respectively) was higher than in group III (27.3%). Within PET cases, gastrinoma was mostly present in groups I (60%) and II (57.9%) and less observed in group III (33%). Most insulinomas were documented in groups I (50%) and II (15.8%) and were absent in group III. Conversely, exclusively nonsecretory PETs were only seen in groups III (67%) and II (26.3%).

Furthermore, in our patients with MEN1, pituitary adenomas were mostly diagnosed in group I (69.2%) and were equally represented in groups II (46.4%) and III (45.5%). In all 3 groups, prolactinoma was highly prevalent (77.9%, 61.6%, and 60%, respectively). Nonsecretory adenomas comprised 22.2%, 38.5%, and 20%, respectively.

Taking into account patients from all 3 groups, most MEN1 cases (73.1%) were recognized after diagnosis of symptomatic HPT, as this condition was highly prevalent in all groups (92.3%, 78.6%, 50%, respectively). Moreover, symptomatic PET cases were significantly more frequent in groups I and II (50%: 76.9%) than in group III (9%; $P < 0.05$). Furthermore, symptomatic pituitary tumors were documented in all 3 groups (61.5%, 28.6%, and 36.4%, respectively).

Furthermore, the annual clinical screening for MEN1 reported here allowed us to diagnose 29 asymptomatic MEN1-related tumors, as follows: 11 patients with HPT; 7 patients with non-secretory pancreatic endocrine tumors, 7 patients with non-secretory pituitary tumors, and 4 patients harboring malignant, asymptomatic carcinoid tumors.

Furthermore, after performing the genetic screening, 11

Table 3 - Clinical manifestations of MEN1 in patients with MEN1: index cases (group I), clinically diagnosed cases (group II), and genetically diagnosed cases (group III).

	probands(index cases) (group I)	patients diagnosed by familial clinical screening(group II)	patients diagnosed by *MEN1* genetic testing(group III)
Mean age at diagnosis (years)	39.5 (18-74)	42.4 (18-61)	27 (14-56) *
0 MEN1-related tumor	-	-	1 (9.1%)
1 MEN1-related tumor	-	13 (46.4%)	8 (72.7%)
2 MEN1-related tumors	7 (53.8%)	9 (32.2%)	1 (9.1%) **
3 MEN1-related tumors	6 (46.2%)	6 (21.4%)	1 (9.1%)
Tumor stage	advanced	early / advanced	asymptomatic / early
Malignancy	3 (23%)	5 (17.9%)	no metastasis
Mortality	2 (15.4%)	2 (7.1%)	no death

*$P < 0.05$; **p = 0.06

Figure 1 - The coding region (exons 2-10) of the *MEN1* gene. *MEN1* mutations identified in 13 MEN1 index cases are indicated. The mutation 360del4 was localized in 2 unrelated patients with MEN1.

MEN1 mutation carriers were identified and included in the annual clinical follow-up.

GENETIC RESULTS

Germline mutations in patients with MEN1 from HC-FMUSP

The following disease-causing mutations where found through *MEN1* mutation analysis: 308del1 (exon 2), proband 1; 375del21 (exon 2), proband 2; I147F (exon 2), proband 3, 360del4 (exon 2), probands 4 and 5; 1059del1 (exon 7), proband 6; 1243del1 (exon 8), proband 7; 1348T>G (exon 9), proband 8; 1351T>C (exon 9), proband 9; 1353C>T (exon 9), proband 10; 1523G>T (exon 10), proband 11; 1689C>T (exon 10), proband 12; and IVS3+1 G>T (exon 2), proband 13 (Figure 1; Toledo RA et al., Clin Endocrinol (Oxf). 2007 Jun 6; [Epub ahead of print] PMID: 1755549, in press). All mutations were confirmed using a second DNA sample from an independently collected blood sample.

No mutational hot spot was found. Mutations of *MEN1* were found at exons 2, 7, 8, 9, and 10; also, 1 splicing mutation was identified at intron 3. All identified *MEN1* mutations were predicted to cause disruption of menin's transcriptional regulation or menin's protein interactions and thus lead to MEN1 tumorigenesis (Toledo RA, data not shown).

Additionally, we performed genetic testing of 141 relatives at-risk for MEN1. Thirty-nine relatives were identified as mutation carriers: 28 had previously undergone clinical exams (symptomatic cases), and 11 had not participated in the previous clinical screening. These 11 relatives after learning that they were positive for a *MEN1* mutation agreed to adhere to the clinical screening. One hundred and one (101/102, 99%) relatives who were negative for a *MEN1* mutation did not present MEN1-related complaints or symptoms, while 1 (1%) developed sporadic primary HPT (MEN1 phenocopy).

DISCUSSION

Multiple endocrine neoplasia type 1 is an inherited disorder with high penetrance, resulting in parathyroid, endocrine-pancreas/duodenum, and pituitary tumors.[1,2] Several other benign and malignant, endocrine and nonendocrine tumors have also been described for patients with MEN1. However, to date, very few data are available on patients with MEN1 from countries outside of North America, Europe, Japan, and Australia. In this report, we present the results of clinical and genetic MEN1 screenings performed in Brazil over a 10-year period. Also, we document important changes in the clinical presentation of patients with MEN1 after the implementation of the *MEN1* genetic diagnosis.

Clinical manifestations in our MEN1 series are in accordance with those in the literature (Table 2). Thus, HPT was the most frequent (94.2%) and usually the first manifestation of MEN1, as reported by Trump et al.[3] The prevalence of PETs in our 52 MEN1 cases was as high as 63.5%, and in groups I (76.9%) and II (67.9%) it was higher than in group III (27.3%). The prevalence of pituitary adenomas in our MEN1 series (51.9%) was slightly higher than previously reported (20%-40%, Table 2); however, our sample is still relatively limited for further conclusions. Finally, carcinoid tumors were equally presented in our patients and data from the literature (Table 2).

In this study, we were able to implement *MEN1* mutation analysis in clinical practice. The genetic screening successfully identified *MEN1* mutations in all 13 MEN1 index cases (Figure 1). Moreover, all 12 different *MEN1* germline disease-causing mutations we identified are predicted to inactivate the tumor suppressor functions of menin and lead to MEN1 tumorigenesis. Although probands 4 and 5 had recurrent *MEN1* mutations, no hot spot was found; mutations were spread throughout the coding and noncoding regions of the gene. This finding confirmed the need for searching the entire *MEN1* coding region as well as its introns, which makes *MEN1* genetic screening a laborious routine procedure. So far, genetic screenings for *MEN1* have been performed in developed countries, whereas no such a program had so far been implemented in South America. Our data should be useful for improving management of the MEN1 syndrome in Brazil.

The familial genetic screening included 141 family members at risk for MEN1 and has identified those who should undergo annual clinical surveillance of MEN1-related tumors and those who should be ruled out from clinical screening. Both positive and negative genetic results were worthwhile and highly informative in the management of individuals at risk for MEN1. The 39 at-risk relatives who inherited the affected *MEN1* allele underwent complete surveillance for MEN1-related tumors. Furthermore, *MEN1* genetic testing ruled out 102 family members who did not harbor a *MEN1* mutation (and had no susceptibility for developing MEN1-associated neoplasias) from unnecessary following-ups, avoiding unnecessary, expensive exams and lifelong surveillance (Figure 2).

Genetic testing was also important for confirming MEN1 disease in previously diagnosed family members. Among such cases, we tested 1 relative presenting a primary HPT, who did not harbor a germline *MEN1* mutation. According to MEN1 clinical criteria, this patient should have been diagnosed as a MEN1-affected case. However, he was indeed a sporadic HPT case. Such rare cases are called MEN1 phenocopies and do not need further surveillance for MEN1.[44,48,49] In the other hand, genetic testing for *MEN1* mutations might disclose inherited cases within "sporadic" patients presenting either HPT at early ages (< 30 yr) or recurrent HPT. The latter patients should be genetically tested, and if a germline mutation is detected, a total parathyroidectomy followed by an autograph to the forearm or subtotal parathyroidectomy should be performed, instead of adenomectomy.[1]

It is largely accepted that the earlier the detection of neoplasias, the better is its management. Patients who are symptomatic for MEN1 usually require more aggressive and risky surgeries and a higher number of exams (Table 4). Also, the chance of cure could be reduced in late-diagnosed cases.[16,17,25] Despite that, the identification of *MEN1* mutations has been reported to have a limited influence in guiding early therapeutic surgery[1,13]; however, our data have shown a relevant impact of *MEN1* genetic screening in the management of at-risk patients. Importantly, we noticed a shift towards milder clinical presentations in our 11 genetically diagnosed MEN1 patients. These patients (group III) presented different phenotypes from the clinically diagnosed patients (groups I-II) as follows, a) they were significantly (11-15 years) younger; b) they were usually asymptomatic; c) they mostly presented only 1 or no MEN1 tumor; d) they tended to present MEN1 tumors at their early stages; e) they had no MEN1-related malignancy; and f)

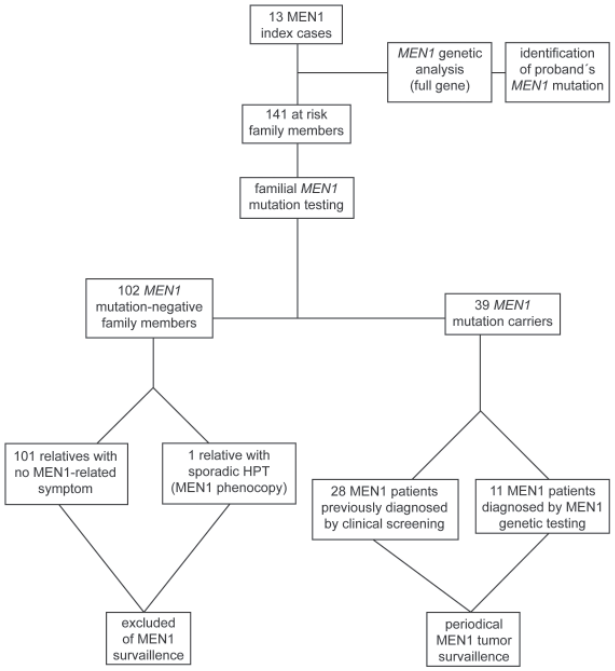

Figure 2 - MEN1 screening program.

Table 4 - Usual major clinical manifestations of MEN1 at its early and late stages. Appropriate treatments for early- and late-diagnosed MEN1 patients are listed.

	Early recognition of MEN1		Late recognition of MEN1	
Major MEN1 manifestations	**Clinical features**	**Treatment**	**Clinical complications**	**Treatment**
Hyperparathyroidism	↑PTH ↑Ca^{++}	total parathyroidectomy*	renal complications osteoporosis, renal calculi, renal insufficiency	renal dialysis or transplant
Insulinoma	↑insulin ↓glycemia, hypoglycemic symptoms	surgery	hypoglycemic shock, neuro-psychiatric disorders, metastasis (10%)	surgery
Gastrinoma	↑gastrin, gastritis, ulcer, gastric acid hypersecretion	drug therapy surgery	esophageal stenosis, gastroduodenal ulcers, metastasis (60%)	surgery chemotherapy interpheron somatostatin analogues
Prolactinoma	microadenoma	drug therapy	infertility, osteoporosis, hypogonadism, macroadenoma,visual defects,	drug therapy radiotherapy surgery
Thymic carcinoid	-	preventive thymectomy	metastasis	surgery chemotherapy radiotherapy

*with autograph to the forearm

they all remained alive during the follow-up period (Tables 2-3). Our present findings corroborate a previous prospective study documenting that genetically diagnosed MEN1 patients have biochemical evidence 10 years preceding the signs and symptoms of the disease.[50]

Therefore, the implementation of a genetic screening program for MEN1 in a reference health center, such as our hospital, would be an important step for improving the management of this complex disease in Brazil. This program is a new effort among many others in the study of cancer and cancer prevention in Brazil.[51–56] Also, such a program would save significant amounts of money spent on surveillance of patients that are negative for a *MEN1* mutation.

In conclusion, we present the first clinical and genetic MEN1 screening trial performed in South America. Our study shows that *MEN1* genetic testing greatly contributes to a more adequate clinical management of this complex syndrome and may benefit patient care.

RESUMO

Lourenco Jr. DM, Toledo RA, Coutinho FL, Margarido LC, Siqueira SAC, Cortina MA, Montenegro FL, Machado MC, Toledo SP. Impacto do rastreamento clínico e genético para Neoplasia Endócrina Múltipla tipo 1. CLINICS. 2007;62(4):465-76.

OBJETIVOS: Realizar rastreamentos clínico e gênico para Neoplasia Endócrina Múltipla tipo 1 (NEM1) e analisar seu impacto no seguimento clínico desses pacientes no Hospital das Clínicas, SP.

MÉTODOS: O diagnóstico clínico de NEM1 foi realizado de acordo com o Consenso sobre neoplasias endócrinas múltiplas. A análise genética para identificação de mutações foi realizada por sequenciamento automático de todas as regiões codificadoras e fronteiras exon/intron do gene *MEN1*. Os casos afetados foram sub-divididos em 3

grupos e analisados separadamente: casos-índices (grupo I), familiares diagnosticados clinicamente (grupo II) e genicamente (grupo III).

RESULTADOS: Um total de 154 casos participou desse estudo, sendo 52 diagnosticados com NEM1: 13 do grupo I, 28 do grupo II e 11 do grupo III. A idade média ao diagnóstico no grupo III (27 anos) foi significativamente menor que a dos grupos I (39,5 anos; $p = 0,03$) e II (42,4 anos; $p = 0,01$). A maioria dos pacientes dos grupos I e II apresentou 2 ou 3 tumores, enquanto que 81,8% dos casos do grupo III apresentavam 1 ou nenhum tumor relacionado à NEM1. Além disto, 45,4% dos casos do grupo III eram assintomáticos, não sendo observados nenhuma metástase ou óbito. Os demais 102 familiares sob-risco estudados não herdaram mutação *MEN1* e foram excluídos do rastreamento clínico. Um caso de fenocópia NEM1 foi também localizado.

DISCUSSÃO: Nossos dados demonstraram importantes benefícios no seguimento dos pacientes NEM1, obtidos pela implementação dos rastreamentos clínico e gênico para essa doença.

UNITERMOS: Neoplasia endócrina múltipla. NEM1. Gene *MEN1*. Rastreamento. Diagnóstico gênico.

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