Società Medico Chirurgica di Ferrara

dal 1846

AGGIORNAMENTI NELL'APPROCCIO DIAGNOSTICO-TERAPEUTICO AI TUMORI NEUROENDOCRINI GASTRO-ENTERO-PANCREATICI


REMDICONTO
DELLE ADUNANZE
dell' Accademia
MEDICO-CHIRURGICA
DI FERRARA
DEGLI ANNI
1846 -- 1847 -- 1848 -- 1849

LE FORME FAMILIARI GEP-NET: quando pensare alla MEN1?

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Quando pensare alla MEN1?

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Multiple Endocrine Neoplasia

Several distinct syndromes featuring benign or malignant tumors of endocrine glands, each with its own characteristic pattern.

Benign or malignant tumors of nonendocrine tissues may occur

Quando pensare alla MEN1?

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Multiple Endocrine Neoplasia

classically	...but now
MEN1	several genetic syndromes
MEN2	

Quando pensare alla MEN1?

…when should we suspect MEN1…?

parathyroid adenomas
entero-pancreatic endocrine tumors
pituitary tumors

…but MEN1 causes
combinations of
over 20 different endocrine
and nonendocrine tumors

Quando pensare alla MEN1?

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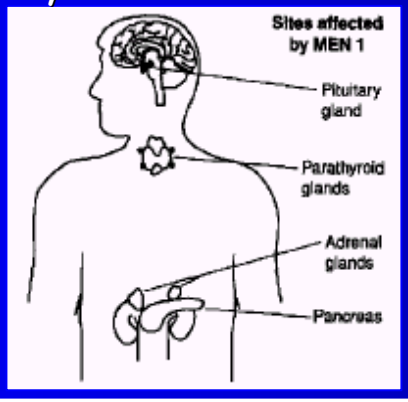
MEN 1
Definition

MEN 1 is an autosomal dominant disorder characterized by

- parathyroid adenomas
- entero-pancreatic endocrine tumors
- pituitary tumors

various combinations of more than 20 endocrine and nonendocrine tumours

- foregut, bronchial and thymic carcinoids
- lipomas
- skin tumours



Expression of MEN1 with estimated penetrance at age 40 yr

Endocrine features	Nonendocrine features
Parathyroid adenoma (90%)	Lipomas (30%)
Entero-pancreatic tumor	Facial angiofibromas (85%)
Gastrinoma (40%)@	Collagenomas (70%)
Insulinoma (10%)	
NF[a] including **pancreatic polypeptide** (20%[b])	Rare, maybe innate, endocrine or nonendocrine features
Other: **glucagonoma**, **VIPoma**, **somatostatinoma**, *etc.* (2%)	
Foregut carcinoid	
Thymic carcinoid NF (2%)	Pheochromocytoma (<1%)
Bronchial carcinoid NF (2%)	Ependymoma (1%)
Gastric enterochromaffin-like tumor NF (10%)	
Anterior pituitary tumor	
Prolactinoma (20%)	
Other: GH + PRL, GH, NF (each 5%)	
ACTH (2%), TSH (rare)	
Adrenal cortex NF (25%)	

Brandi ML et al. J Clin Endocrinol Metab 2001; 86:5658

Quando pensare alla MEN1?

MEN 1
Definition

a case with 2 of the 3 main MEN 1-related endocrine tumors

Familial MEN1 is defined as
at least 1 MEN1 case plus at least 1 first degree relative with 1 characteristic tumor

Brandi ML et al. J Clin Endocrinol Metab 2001; 86:5658

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MEN 1

Epidemiology

- MEN-1 is rare ~ 1 : 30,000
- 0.25% in autoptic study
- 1-18% patients with primary HPT
- 16-38% patients with gastrinoma
- <3% patients with pituitary adenomas

Observed penetrance and spectrum of manifestations in MEN-1

	Present as initial feature *n* (%)	Age-specific penetrance at 50 yr (%)	Proportion female (range)	Mean age at diagnosis	Prevalence (%)
HPTH	23 (65%)	73%	16/28	36 (21–73)	82%
Pituitary Tumor	8 (24)	48	15/22	40 (18–73)	65
Islet Tumor	13 (38)	49	13/24	46 (23–84)	74
Carcinoid	1 (3)	50	1/2	43 (22–63)	6

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MEN 1
Clinical presentation

Primary HPT

most common endocrinopathy in MEN1
(~ 100% penetrance by 50 yr)

age of onset: 20–25 yr → low bone mass

hypercalcemia → hypergastrinemia → ZES

Biochemical screening (Ca^{2+}, PTH) should be started at 8 yr of age in MEN1 gene mutation carriers

Pieterman et al. Familial Cancer 2011; 10:157–171

Quando pensare alla MEN1?

MEN 1
Clinical presentation

neuroendocrine tumors

Duodenopancreatic NETs (35-75%)

Gastric NETs (21-37%)

Thymic NETs (2-8%)

Bronchopulmonary NETs (1.4-9.5%)

Pieterman et al. Familial Cancer 2011; 10:157–171

Quando pensare alla MEN1?

MEN 1
Clinical presentation

entero-pancreatic islet tumors

prevalence = 30–75% in clinical series
~ 80% in necropsy series

age of onset = 40 yr

- multicentric
- microadenomas – macroadenomas
- islet cell hyperplasia (rare)
- invasive
- metastatic carcinomas

gastrinoma
is the most frequent tumor

Vasen et al. 1989 Arch Intern Med 149:2717–2722
Skogseid et al. 1991 J Clin Endocrinol Metab 73:281–287

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MEN 1

Clinical presentation

pituitary adenomas

Prevalence : 20-65%

first clinical manifestation in up to 25% of sporadic cases

Carty et al. 1998 Surgery 124:1106–1114

age of onset = 40 yr

60% are microadenomas

Every type of anterior pituitary adenoma, except the true gonadotropinoma, has been reported in MEN1

Anterior pituitary tumor
- Prolactinoma (20%)
- Other: GH + PRL, GH, NF (each 5%)
- ACTH (2%), TSH (rare)

Prolactinoma is the most frequent tumor (40-71%)

Pieterman et al. Familial Cancer 2011; 10:157–171

Vasen et al. 1989 Arch Intern Med 149:2717–2722
Skogseid et al. 1991 J Clin Endocrinol Metab 73:281–287

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MEN 1

Clinical and biochemical assessment

parathyroid adenomas

pituitary tumors

neurodocrine tumors

does not differ from that of sporadic forms

but

starts early in life
in gene mutation carriers

and

continues after first surgery
due to the high likelyhood of recurrence

Norton et al. Ann Surg 247(3):501–510

Quando pensare alla MEN1?

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MEN 1

Clinical and biochemical screening

TABLE 2. A representative program of tests and test schedules to screen for tumor expression in a highly likely carrier of *MENI* mutation (identified from *MENl* mutation or other criteria)

Tumor	Age to begin (yr)	Biochemical tests annually	Imaging tests every 3 yr
Parathyroid adenoma	8	Calcium (especially Ca++), PTH	None
Gastrinoma	20	Gastrin, gastric acid output[a]; secretin-stimulated gastrin[a]	None
Insulinoma	5	Fasting glucose; insulin	
Other enteropancreatic	20	Chromogranin-A; glucagon; proinsulin	^{111}In-DTPA octreotide scan; CAT or MRI
Anterior pituitary	5	PRL; IGF-I	MRI
Foregut carcinoid[b]	20	None	CAT

[a] Gastric acid output measured if gastrin is high; secretin-stimulated gastrin measured if gastrin is high or if gastric acid output is high (Footnote 4).
[b] Stomach best evaluated for carcinoids ("ECLomas") incidental to gastric endoscopy. Thymus removed partially at parathyroidectomy in MENl.

Brandi ML et al. J Clin Endocrinol Metab 2001; 86:5658

decreased morbidity and mortality at follow-up

Lourenco et al. 2007 Clinics 62;465–476
Pieterman et al. 2009 Clin Endocrinol (Oxf) 70;575–581

but high cost!

Waldmann et al. 2009 World J Surg 33:1208–1218

Quando pensare alla MEN1?

MEN 1

Clinical and biochemical screening

Alternative schedule

Table 1 Protocol for periodic 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 years	MRI[a] of upper abdomen
		Every 2–3 years	MRI[a] of pituitary (intravenous contrast with gadolinium)
		Every 3–5 years	CT[b] of thorax

Pieterman et al. Familial Cancer 2011; 10:157–171

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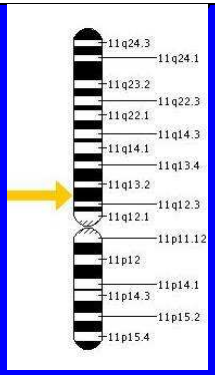
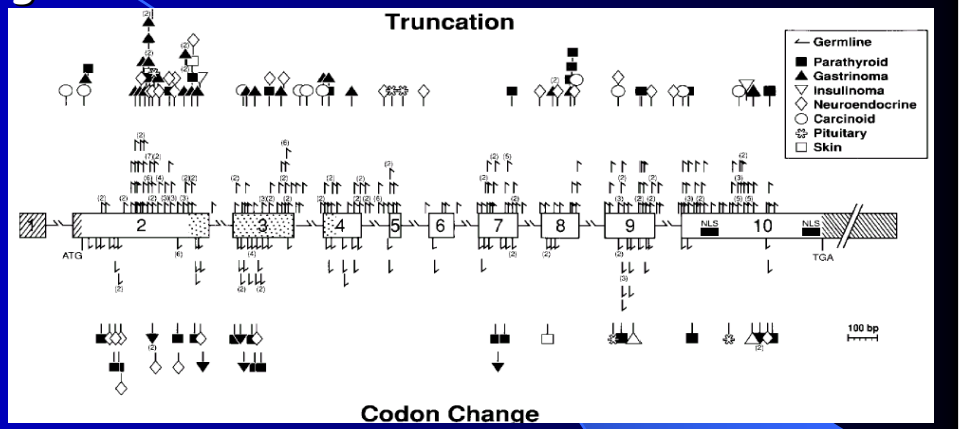
Etiology

700 different somatic and germline mutations



The gene responsible for MEN1 maps at 11q12-13



Agarwal SK et al. Ann NY Acad Sci 2004; 1014: 189–198

Most MEN1 mutations are inactivating:

60% frameshift
25% non-sense
→ protein truncation

5-20% mis-sense mutations → alter interaction with menin partners and/or favour rapid degradation

1% small indels, deletions or insertions → protein truncation and/or alter interaction with menin partners and/or favour rapid degradation

absence of genotype/phenotype relation

Falchetti A et al . Best Pract & Res Clin Rheumatol 2008; 22:149

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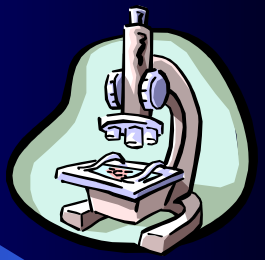
MEN 1 Pathology



the MEN-1 endocrine tumors do not differ from their nonhereditary counterparts

- high degree of differentiation
- ultrastructural and immunohistochemical features close to those of the corresponding normal endocrine cells

Brandi ML 2000. Reviews Endocr Metab Dis 1:275-282

- multicentricity
- bilaterality in paired organs
- cell hyperplasia → adenomatous lesions

progression

Agarwal SK et al 2004 Ann NY Acad Sci 1014: 189–198

Quando pensare alla MEN1?

MEN 1

Treatment

parathyroid adenomas

Surgery performed by an experienced endocrine surgeon is the treatment of choice, although the optimum timing has not been defined.

Conventional open bilateral exploration with subtotal parathyroidectomy(at least 3.5 glands) or total parathyroidectomy is recommended .

Total para- thyroidectomy with autotransplantation may be considered

Thakker RV et al; Endocrine Society. J Clin Endocrinol Metab. 2012

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MEN 1

Treatment

neurodocrine tumors

The aim of treatment for individuals with symptomatic functioning pancreatic NET including insulinoma is to achieve cure, if possible, by surgery

The optimal therapy of gastrinoma remains controversial. Medical management using proton-pump inhibitors for the majority of patients.

Treatment of nonresectable tumor mass includes somatostatin analogs, biotherapy, targeted radionuclide therapy, locoregional treatments, and chemotherapy

Surgery for tumors that are more than 1cm in size and/or demonstrate significant growth over 6–12 months.

Thakker RV et al; Endocrine Society. J Clin Endocrinol Metab. 2012

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MEN 1

Treatment

pituitary tumors

Treatment of MEN1-associated pituitary tumors is similar to that for non-MEN1 pituitary tumors and con sists of appropriate medical therapy

Selective transsphenoidal surgical hypophysectomy, with radiotherapy reserved for residual unresectable tumor tissue

Thakker RV et al; Endocrine Society. J Clin Endocrinol Metab. 2012

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How is genetic analysis perfomed?

1. Patient referral for MEN or family history of MEN
2. History – evaluate family history
3. Clinical examination
4. Informed consent signature
5. Blood withdrawal (no fasting needed)
6. Sample sent to the Lab

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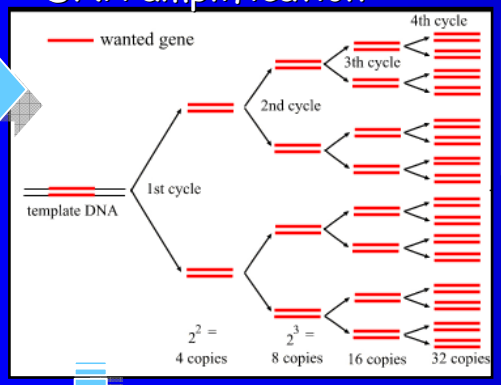
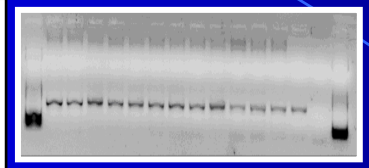
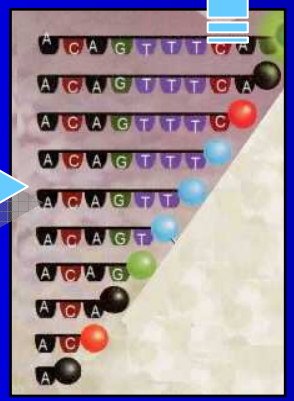
DNA isolation

DNA amplification





purification



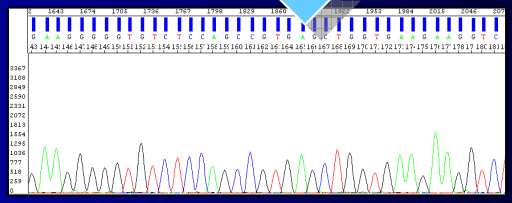
Sequencing reaction

purification

denaturation

GENOMIC DNA DIRECT SEQUENCING

Capillary electrophoresis



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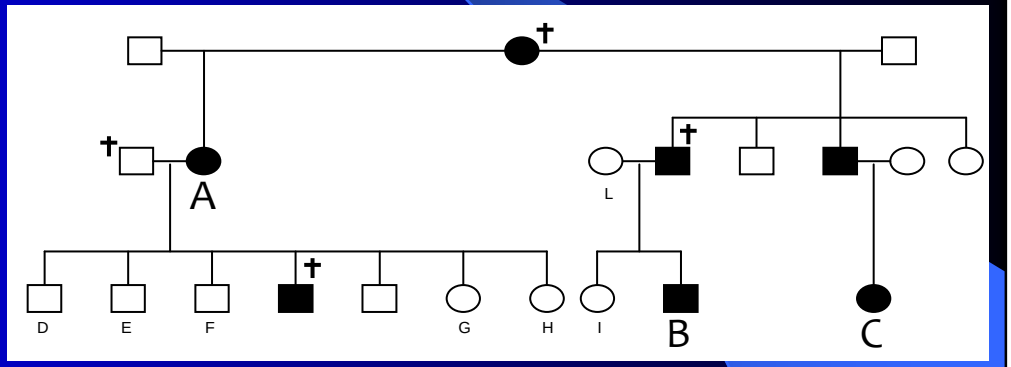
PATIENTS

A (F, 74 ys)	
1989	left parathyroid adenoma
1991	right parathyroid adenoma PRL-secreting pituitary macroadenoma
1995	left adrenal gland macronodular hyperplasia
1998	pancreatic glucagonoma
B (M, 48 ys)	
1998	ACTH-secreting pituitary adenoma
2002	multiple parathyroid adenomas
2008	pancreatic neuroendocrine carcinoma with lymphnode metastases Bilateral diffuse adrenal gland macronodular hyperplasia
C (F, 23 ys)	
2000	left parathyroid adenoma PRL-secreting pituitary microadenoma
2008	left adrenal gland macronodular hyperplasia pancreatic insulinoma



21 family members
7 affected

No SNP mutations

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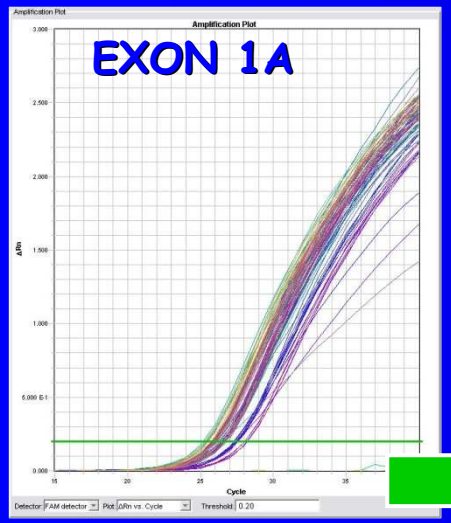

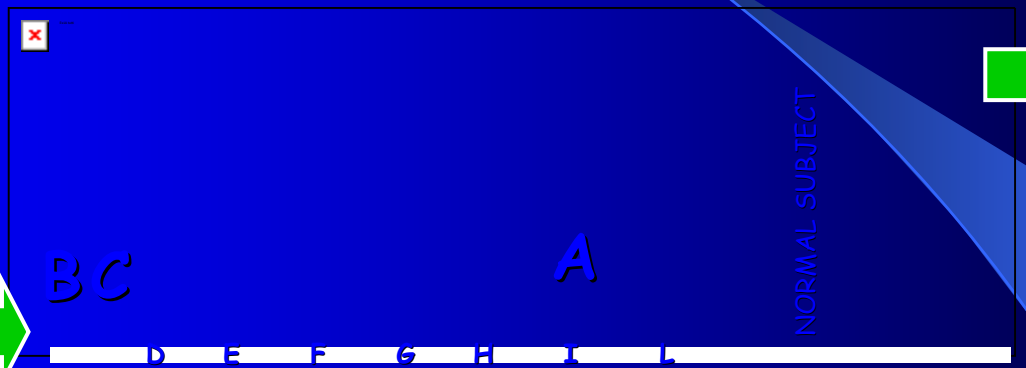

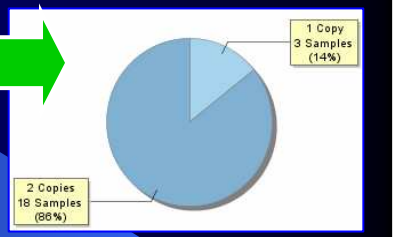

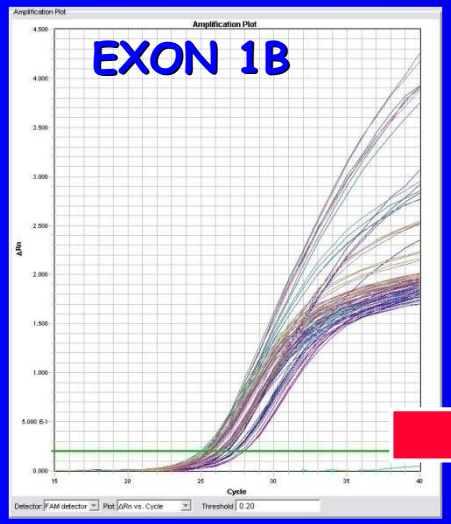

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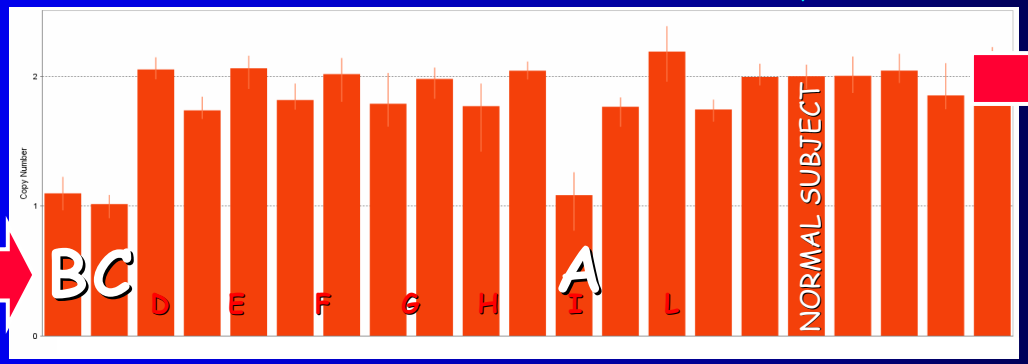

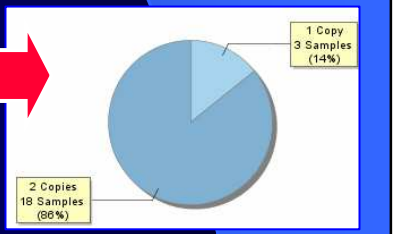

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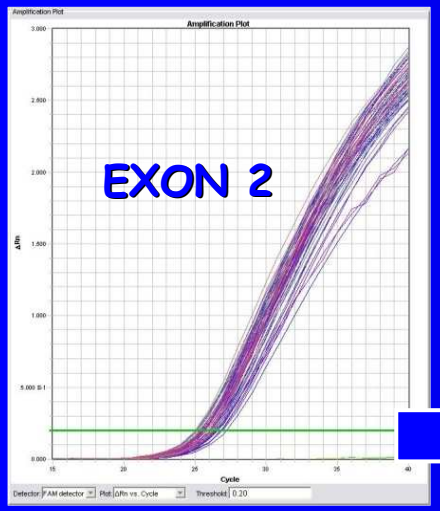

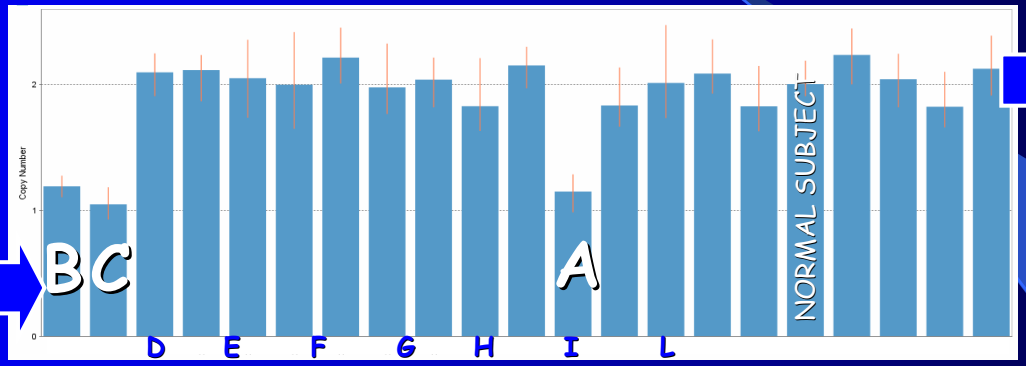

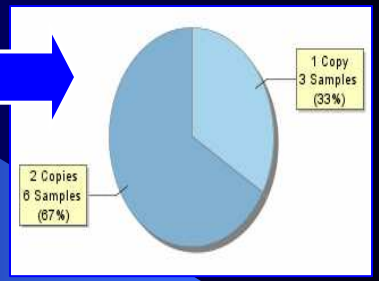

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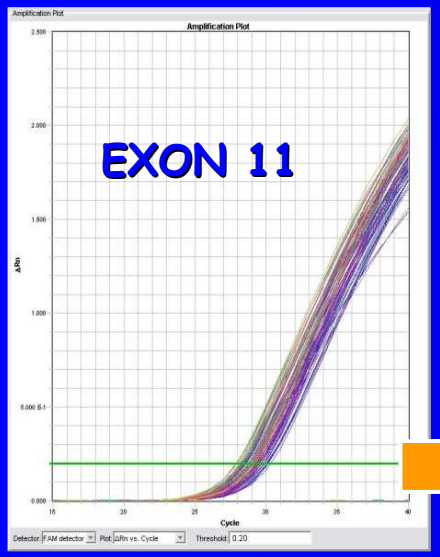

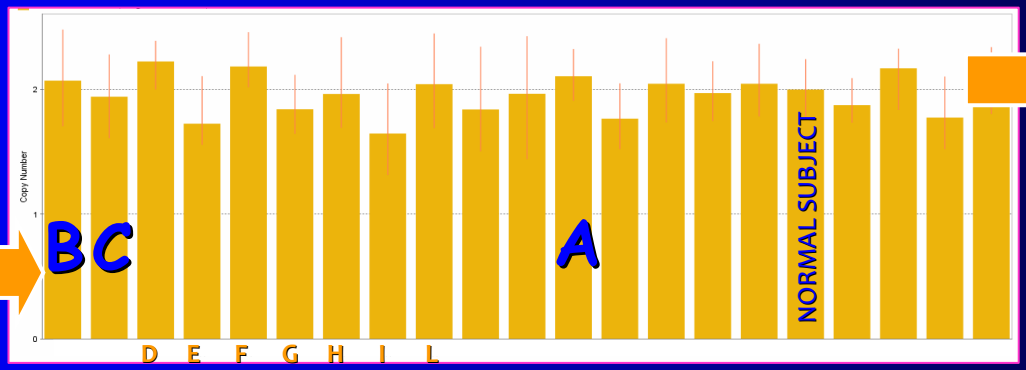

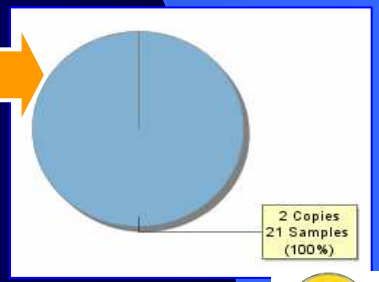

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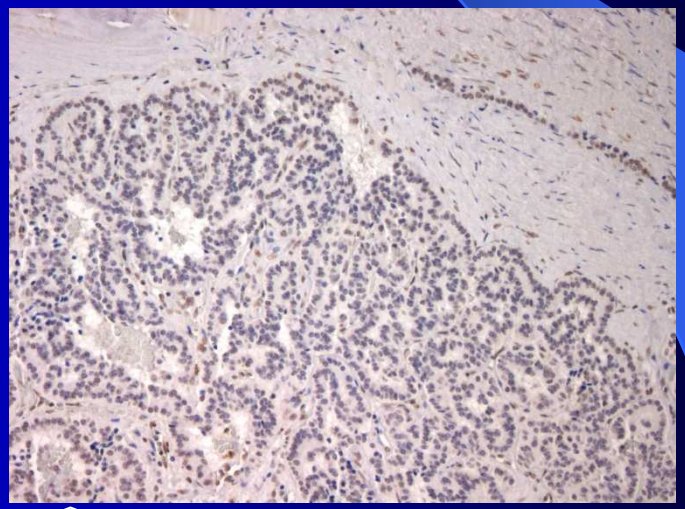
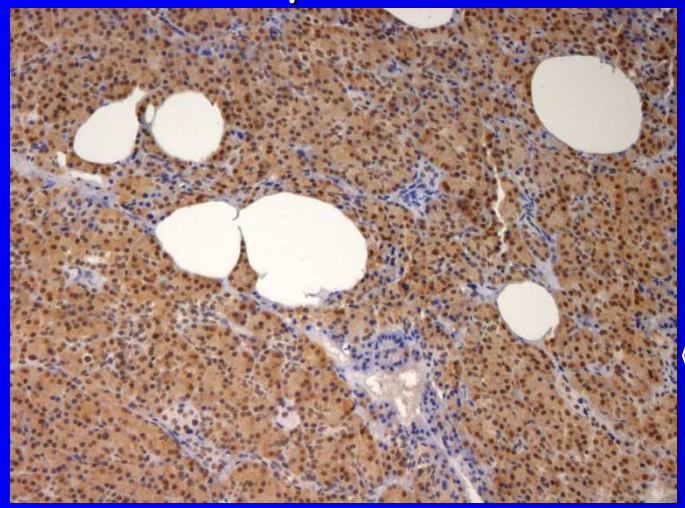
Quando pensare alla MEN1?

A (F, 74 ys)	
1989	left parathyroid adenoma
1991	right parathyroid adenoma PRL-secreting pituitary macroadenoma
1995	left adrenal gland macronodular hyperplasia
1998	pancreatic glucagonoma

pancreatic glucagonoma



normal pancreas



immunostaining for menin

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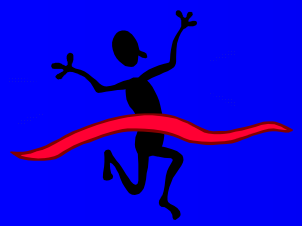
Deletion encompassing exon 1 and 2 of the MEN1 gene in the affected patients, but not in the unaffected family members and in the control group

This new approach allowed us to correctly diagnose 3 MEN1 patients that were considered MEN1 phenocopies

We excluded the presence of any MEN1 genetic alteration in the unaffected family members



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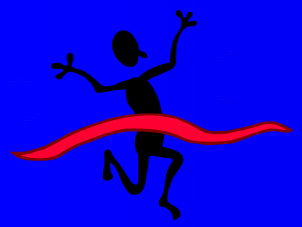
MEN 1

direct sequencing

is not sufficient for a complete genetic analysis in patients with MEN1 phenotype

New biotechnology approaches
Real-Time PCR
MLPA

help us to achieve a correct diagnosis



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MEN1 phenocopies may be caused by other germ-line mutations

AIP (aryl hydrocarbon receptor-interacting protein)

CDKN1B (p27, Kip1),
CDKN1A (p21, Cip1, Waf1)
CDKN2B (p15, CDK4I) and
CDKN2C (p18, INK4C

} cyclin-dependent kinase inhibitor genes

New MEN syndromes

Vierimaa et al. 2006 Science 312:1228–1230
Pellegata et al. 2006 Proc Natl Acad Sci USA 103;:15558–15563
Georgitsi et al. 2007 J Clin Endocrinol Metab 92(8):3321–3325
Georgitsi et al. 2007 Proc Natl Acad Sci USA 104:4101–4105
Agarwal et al. 2009 J Clin Endocrinol Metab 94:1826–1834

Quando pensare alla MEN1?

MEN 4

Table 1. Clinical and molecular characteristics of the identified *CDKN1B*/p27 variants

CDKN1B mutation	Clinical phenotype of proband	Relative affected	Mutation description	CDKN1B status in the tumor	Localization of p27 mutant	Reference
W76X	1°HPT, GH-pituitary tumor	2	truncated protein	no LOH	cytoplasm	[10]
K25fs	1°HPT, ACTH-pituitary tumor, carcinoid tumor of uterine cervix	0	frameshift longer protein	LOH		[11]
ATG–7G>C	1°HPT (1 parathyroid tumor) bilateral adrenal mass nonfunctioning	0	reduction in protein expression *in vitro*	no LOH		[12]
P95S	1°HPT (2 parathyroid tumors), ZES	0	reduced binding of the mutant protein with Grb2	ND		[12]
Stop>Q	1°HPT (3 parathyroid tumors)	3	longer protein, very unstable	ND		[12]
P69L	1°HPT, bronchial carcinoids, papillary thyroid carcinoma, pituitary macroadenoma and bilateral multiple lung metastasis	ND	unstable protein, impared CDK2 binding	ND	nuclear/ cytoplasmic	[13]

1°HPT = Primary hyperparathyroidism; ZES = Zollinger-Ellison syndrome; LOH = loss of heterozygosity; ND = not determined.

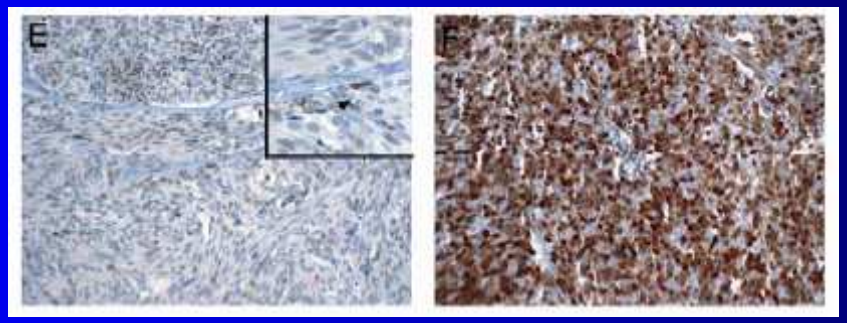
Marinoni et al. Neuroendocrinology 2011;93:19–28

Quando pensare alla MEN1?

MEN 4

P69L	1°HPT, bronchial carcinoids, papillary thyroid carcinoma, pituitary macroadenoma and bilateral multiple lung metastasis	ND	unstable protein, impared CDK2 binding	ND	nuclear/ cytoplasmic	[13]

bronchial carcinoid



P69L mutation-positive patient sporadic patient (wild-type p27)

Loss of p27 protein in tumors of affected patients

Molatore et al. Human Mutation 2010 Mutation in Brief 31: E1825-E1835

Quando pensare alla MEN1?

MEN 4

p27 is a new tumor susceptibility gene for multiple neuroendocrine tumors

Marinoni et al. Neuroendocrinology 2011;93:19–28

Quando pensare alla MEN1?

Società Medico Chirurgica di Ferrara

Multiple Endocrine Neoplasia

Several distinct syndromes featuring benign or malignant tumors of endocrine glands, each with its own characteristic pattern.

Genetic screening may greatly help in identifying yet unaffected family members

Diagnostica delle neoplasie endocrine multiple

Mutazioni germinali

MEN1, MEN2, AIP, p27

Mutazioni somatiche

BRAF, H-RAS, N-RAS, K-RAS

RET/PTC1, RET/PTC3

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THANK YOU

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