12° AME Italian Meeting
6° Joint Meeting with AACE

Bari,
7-10 novembre 2013

Carcinoma midollare tiroideo familiare

Profilo genetico e stratificazione del rischio

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Familial MTC: genetics and risk stratification

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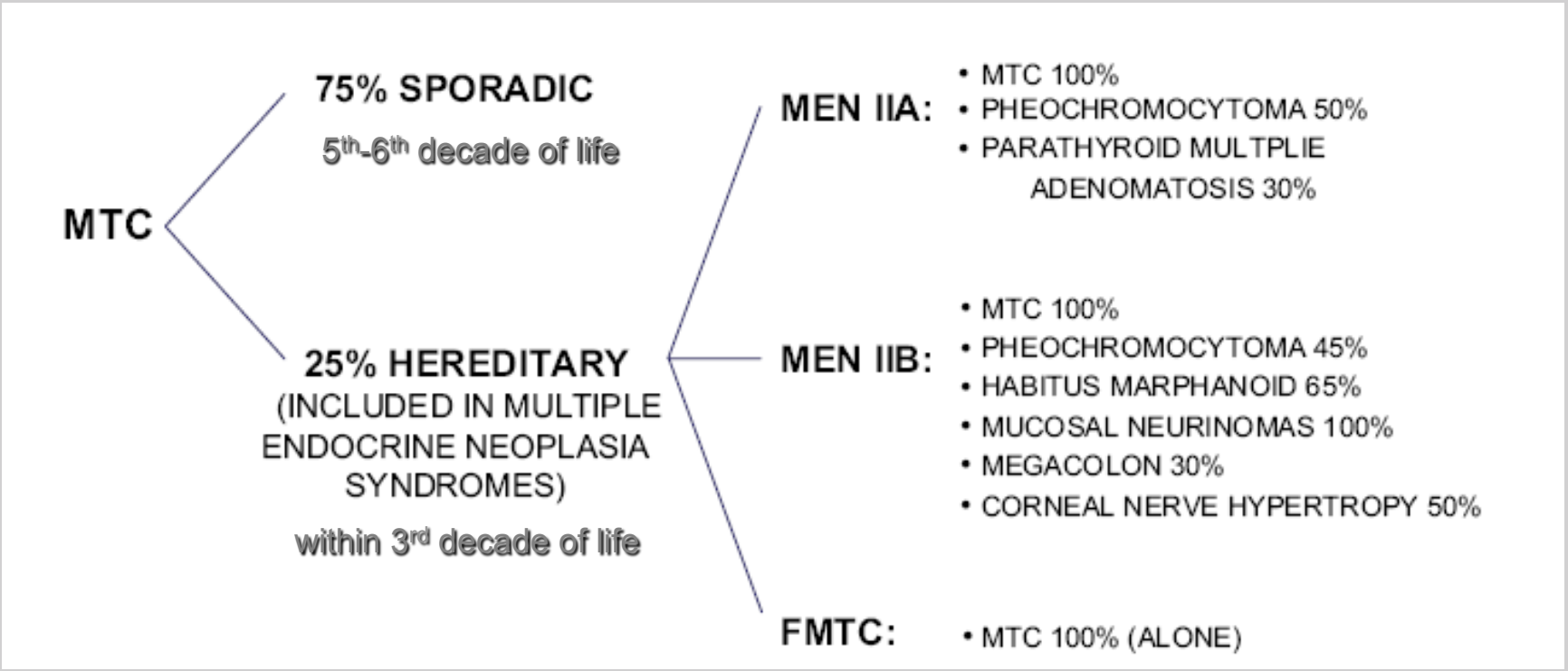
Different forms of medullary thyroid cancer



Elisei R 2008 Best Pract Res Clin Endocrinol Metab 22: 941–953

Familial MTC: genetics and risk stratification

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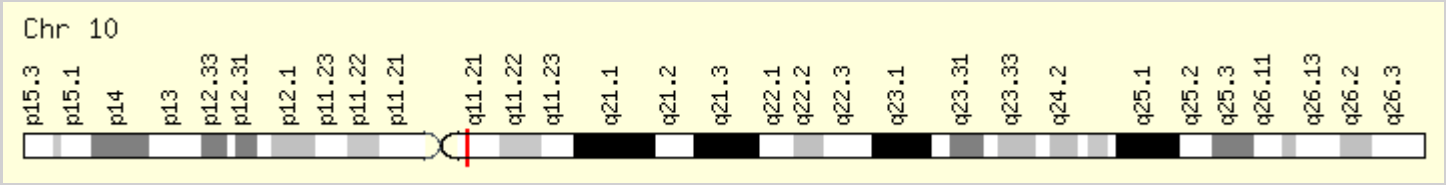
RET (REarranged during Transfection) protooncogene



Long arm of chromosome 10
(10q11.2)

susceptibility gene for

- familial medullary thyroid cancer (FMTC)
- pheochromocytoma
- parathyroid hyperplasia/adenomas

multiple endocrine neoplasia type 2 (MEN 2)

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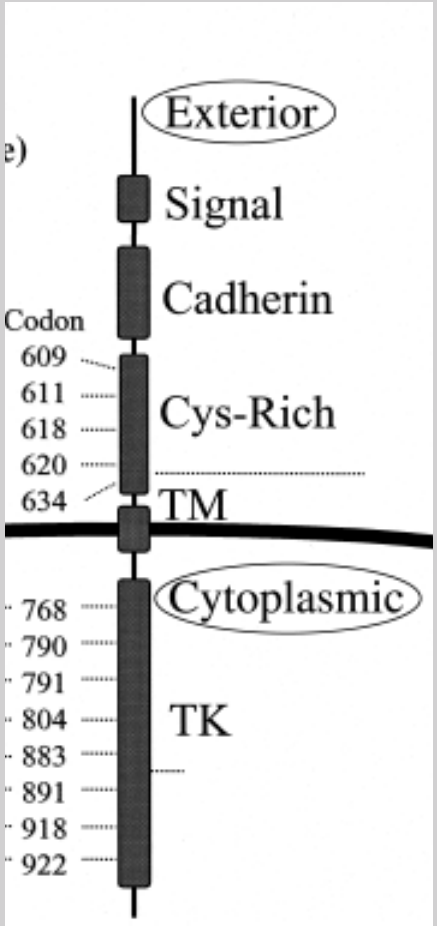
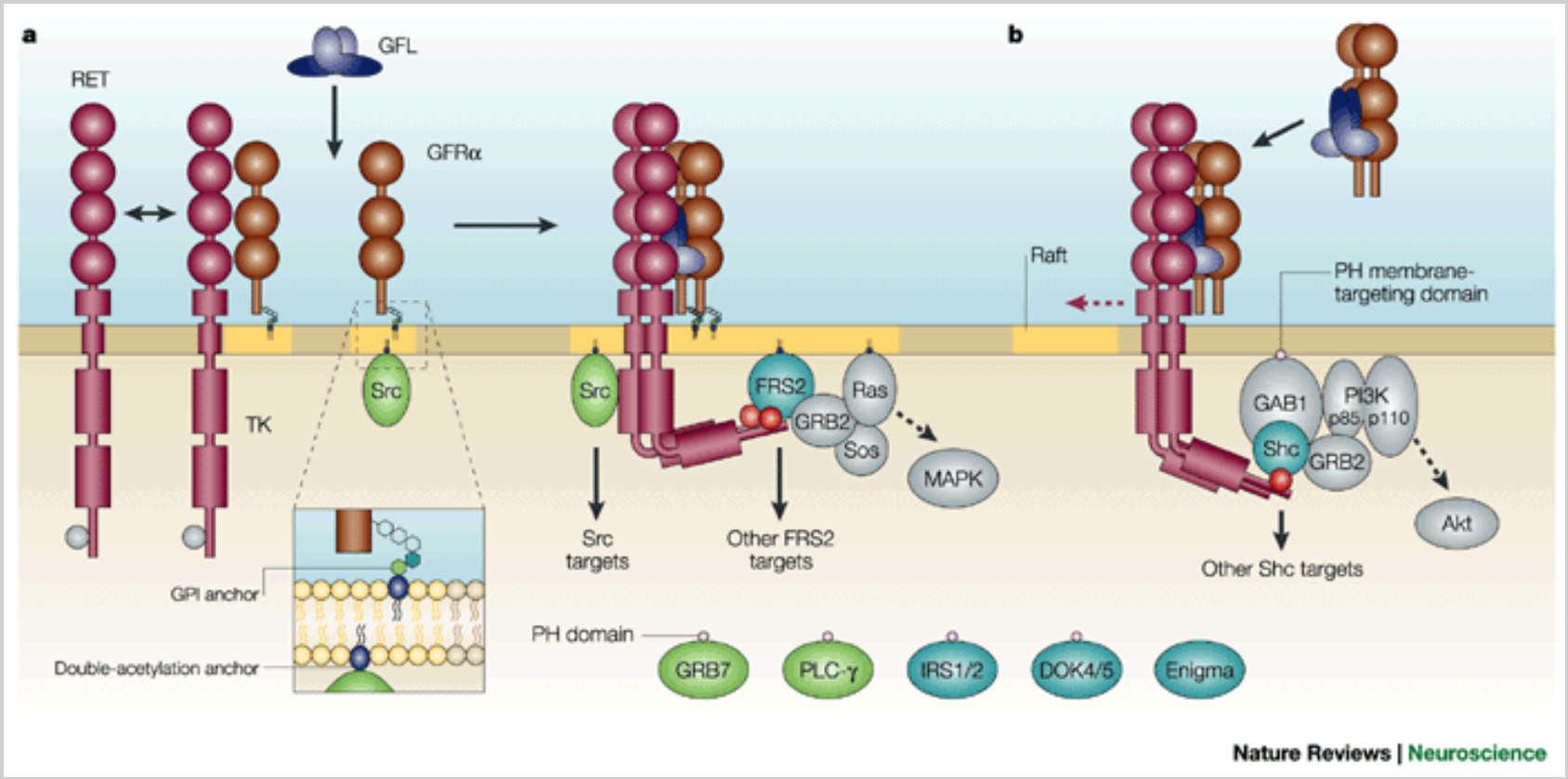
RET ➔ transmembrane receptor

- extracellular domain
- transmembrane domain
- tyrosine kinase domain



Familial MTC: genetics and risk stratification



In the presence of GDNF the receptor complex is activated

- autofosforilation
  - signal transduction pathway activation

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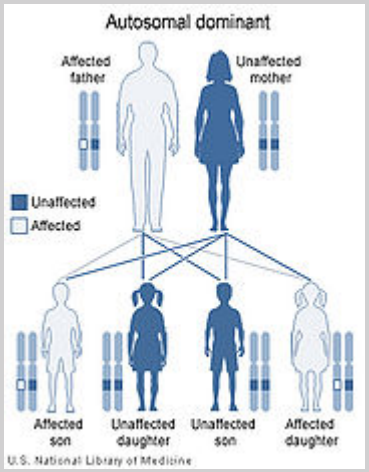

RET mutations

constitutive supraphysiological activation of the RET receptor tyrosine kinase

↓

cell hyperstimulation

↓

thyroid C cells

↓

adrenal medullary cells

↓

parathyroid chief cells

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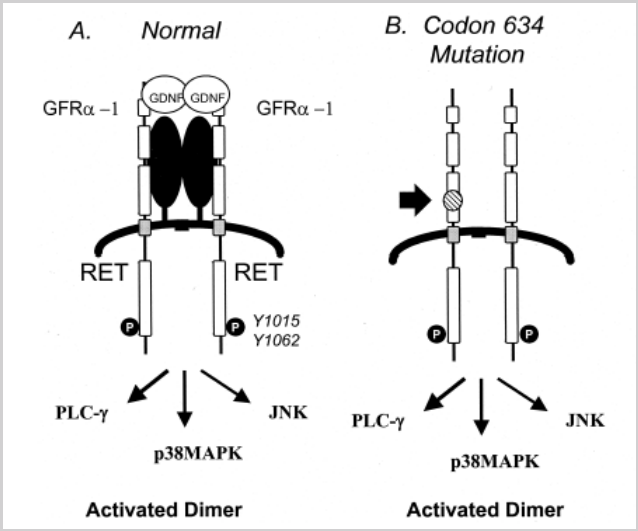
'Gain-of-function' mutations

cysteine-rich extracellular domains

High transforming ability

ligand-independent dimerization and cross-phosphorylation of mutant RET receptor proteins



addition of one more cysteine residue in codons 533, 606 or 631

loss of a cysteine residue irrespective of the amino acid substituting for cysteine

Machens et al. 2009 J Intern Med 266: 114

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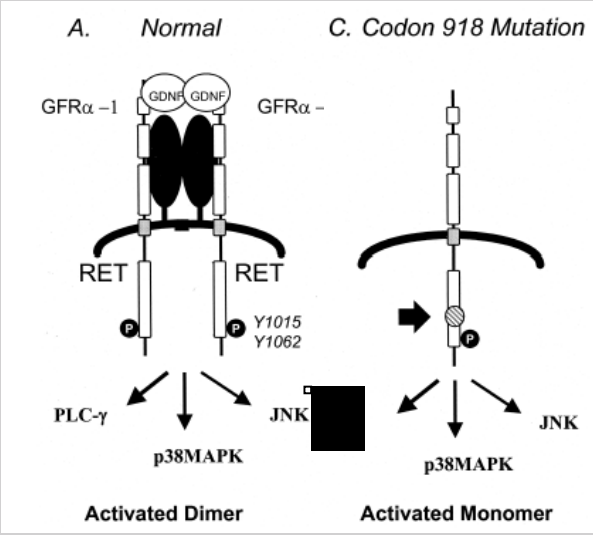
'Gain-of-function' mutations

intracellular tyrosine kinase domain codons 768, 790, 791, 804 and 891

- facilitate the access of ATP to its binding site
- preferential binding of intracellular substrate

intracellular catalytic core codon 918

Very high transforming ability



Mutations in the intracellular kinase domain cause <u>RET autophosphorylation</u>, independently of dimerization and of the ligand

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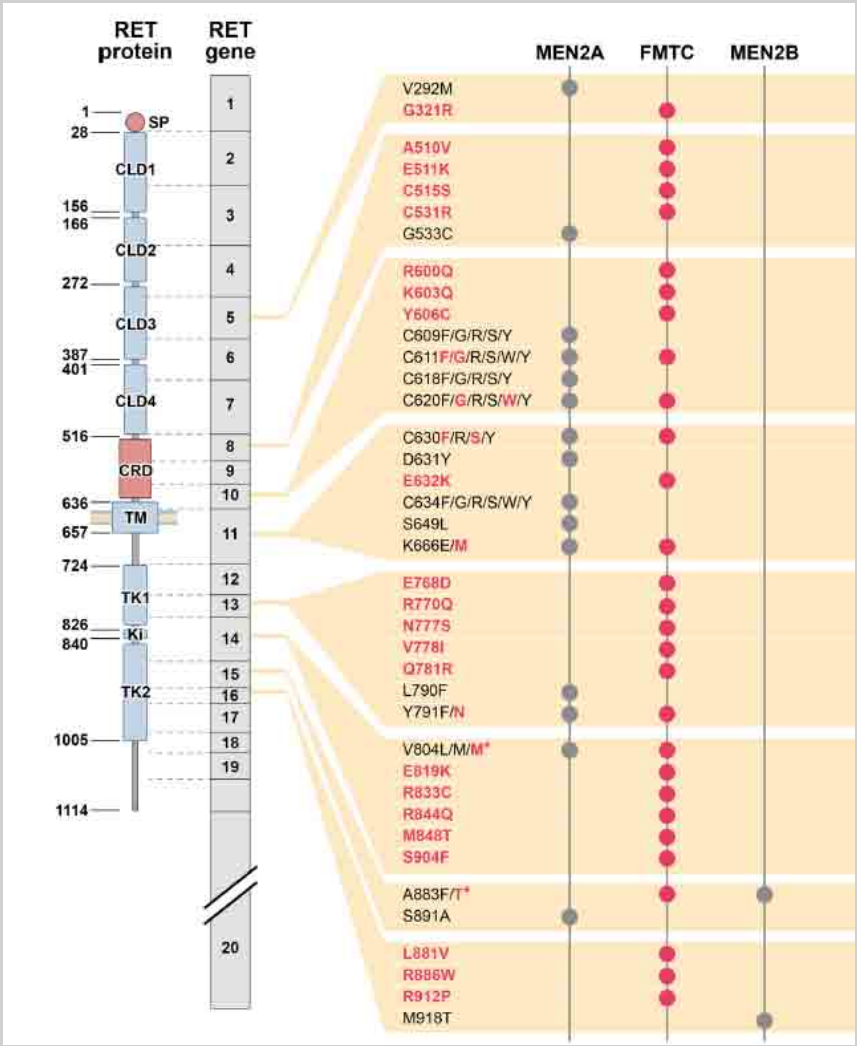
GENOTYPE-PHENOTYPE CORRELATION



Mutations in codons 609, 611, 618, 620 (exon 10) co-segregate with Hirshprung Disease and MEN2 syndromes

Mulligan et al. 1994 Hum Mol Genetics 3:2163
Mulligan et al. 1994 Nat Genetics 6: 70
Romeo et al. 1998 J Int Med 243: 515
Sijmons et al. 1998 Gut 43: 542
Nishikawa et al. 2003 Eur J Hum Genetics 11:364
Pasini et al. 2002 Surgery 131: 373

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RET mutations

Table 2. Germline mutations of the *RET* proto-oncogene in MEN-2A (24)

Affected codon	Exon	Clinical syndrome	Percentage of all MEN-2 mutations
609	10	MEN-2A/FMTC	0-1
611	10	MEN-2A/FMTC	2-3
618	10	MEN-2A/FMTC	3-5
620	10	MEN-2A/FMTC	6-8
630	11	FMTC	<0.1
634	11	MEN-2A	80-90
768	13	FMTC	0-1
790	13	MEN-2A/ FMTC	<0.1
791	13	FMTC	<0.1
804	14	FMTC (age of onset variable)	0-1
883	15	MEN-2B	
891	15	FMTC	0-1
918	16	MEN-2B	10-20
920			
922		Sporadic/MEN-2B	

MEN-2A: multiple endocrine neoplasia type 2, FMTC: familial medullary thyroid carcinoma

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Table 3 Distribution of *RET* missense germline mutations in Continental Europe (500 RET families)[a]. Owing to rounding, not all numbers add up.

ATA class	*RET* mutation	Germany, Halle 1994–2012[b] *n* (%)	Italy, multicenter (18) *n* (%)	France, multicenter (19, 20) *n* (%)	Total *n* (%)
D	M918T	32 (16)	17 (9)	3 (3)	52 (10.4)
D	A883F	0	0	0	0
C	C634R/G/F/S/W/Y	73 (36)	52 (26)	46 (47)	171 (34.2)
B	C630R/F/S/Y	1 (0.5)	4 (2)	0	5 (1.0)
B	C620R/G/F/S/W/Y	14 (7)	9 (5)	12 (12)	35 (7.0)
B	C618R/G/F/S/Y	11 (5)	15 (8)	6 (6)	32 (6.4)
B	C611R/G/F/S/W/Y	6 (3)	1 (1)	1 (1)	8 (1.6)
B	C609R/F/S/Y	1 (0.5)	6 (3)	1 (1)	8 (1.6)
A	G533C	0	0	0	0
A	E768D	2 (1)	9 (5)	2 (2)	13 (2.6)
A	L790F	26 (13)	8 (4)	4 (4)	38 (7.6)
A	Y791F	14 (7)	2 (1)	0	16 (3.2)
A	V804L/M	19 (9)	52 (26)	15 (15)	86 (17.2)
A	S891A	6 (3)	23 (12)	7 (7)	36 (7.2)
Total	Any	205 (100)	198 (100)	97 (100)	500 (100)

ATA, American Thyroid Association; RET, rearranged during transfection.
[a]Considering series with a minimum of 30 European RET families only that specified familial RET prevalence.
[b]Updated from reference (17) (141 RET families).

Machens et al Eur J Endocrinol 2013;168: 307–314

Risk associated to RET mutations

Table 3. American Thyroid Association (ATA) risk level and timing of prophylactic thyroidectomy in multiple endocrine neoplasia type 2 (MEN-2A)

ATA risk level	Age of prophylactic surgery
Level A (codons 768, 790, 791, 804, and 891)	Consider operative resection before age 5 years May delay operative resection if: ▪ Normal annual serum calcitonin and ▪ Normal annual neck ultrasound (no lesions >5 mm and no concerning adenopathy) and ▪ Less aggressive family history and ▪ Family preference
Level B (codons 609, 611, 618, 620, and 630)	Consider operative resection before age 5 years May delay operative resection if: ▪ Normal annual serum calcitonin and ▪ Normal annual neck ultrasound (no lesions >5 mm and no concerning adenopathy) and ▪ Less aggressive family history and ▪ Family preference
Level C (codon 634)	Before 5 years of age
Level D (codon 883, 918) Tandem mutation (804-805, 804-806, 804-904)	First month of life

J Clin Res Pediatr En docrinol 2013;5(Suppl 1):70-78

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DNA-based screening

Following the discovery of RET mutations in the germline of patients with MEN type 2, DNA-based analysis of the RET proto-oncogene was rapidly integrated into the routine clinical armamentarium.

Machens et al. 2009 J Intern Med 266: 114

Cost-effective identification of affected family members

Gilchrist et al. Clin Genet 2004; 66: 349–53

legal and ethical importance indicating the need for prophylactic thyroidectomy

gold standard of care

Rosenthal et al Thyroid 2005; 15: 140–5

Familial MTC: genetics and risk stratification

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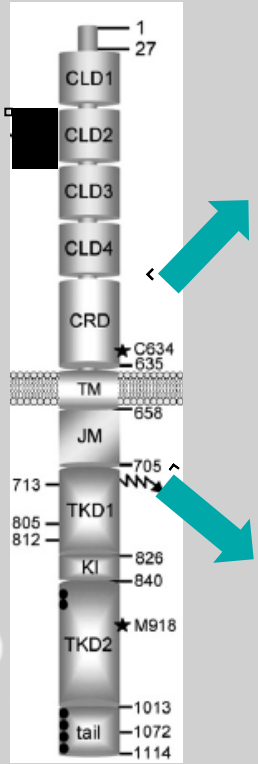
Hereditary MTC

- Germline RET mutations
  - 95% of MEN2A kindreds
    - Hirshprung disease
    - Lichen amyloidosis
  - 88% of FMTC kindreds
  - >95% of MEN 2B kindreds (codon 918)



- Prospective family screening

MUTATED CODONS	EXONS	PHENOTYPE	NC
609	10	FMTC	
611		FMTC	
618		FMTC-MEN 2A	
620		FMTC	
630	11	FMTC	
634		FMTC-MEN 2A	
768	13	FMTC	
790	13	FMTC	
804	14	FMTC	
848	14	FMTC	
883	15	FMTC	
891	15	FMTC	
904	15	FMTC	
918	16	MEN 2B	

Elisei et al. 2007 J Clin Endocrinol Metab 92:4725-9

Castellone et al. 2008 Endocrinol Metab Clin North Am 37:363-74, viii

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Hereditary MTC

- 6-7% of "sporadic" MTC carry a germline RET mutation

Elisei et al. 2007 J Clin Endocrinol Metab 92:4725-9

Wohllk et al. 1996 J Clin Endocrinol Metab 81: 3740-3745

RET genetic testing should be encouraged
in all newly diagnosed MTC patients

The National Comprehensive Cancer Network
Clinical Practice Guidelines in Oncology
2009

biochemical screening
for MEN2

hyperparathyroidims

pheochromocytoma

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How is genetic analysis performed?

1. Patient referral for MTC or family history of MTC
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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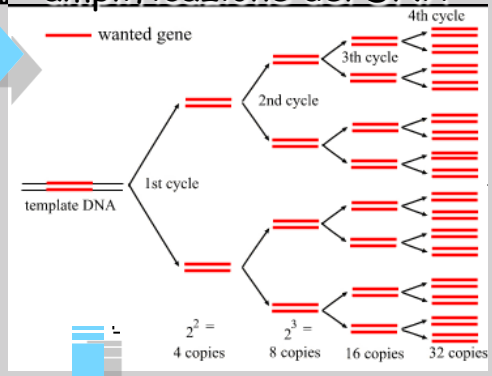
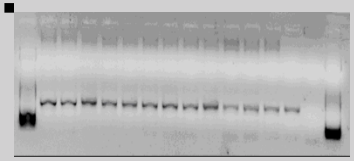
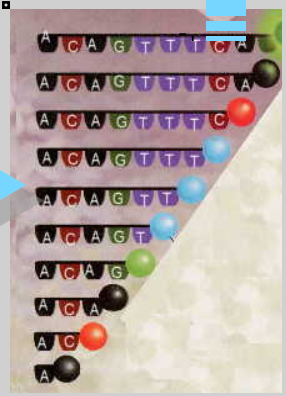
DNA isolation

amplificazione del DNA





purification



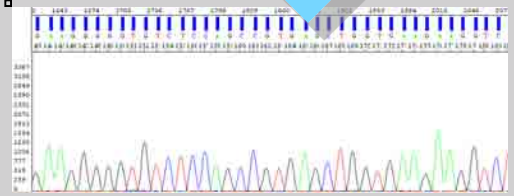
Sequencing reaction

purification

denaturation

GENOMIC DNA DIRECT SEQUENCING

Capillary electrophoresis



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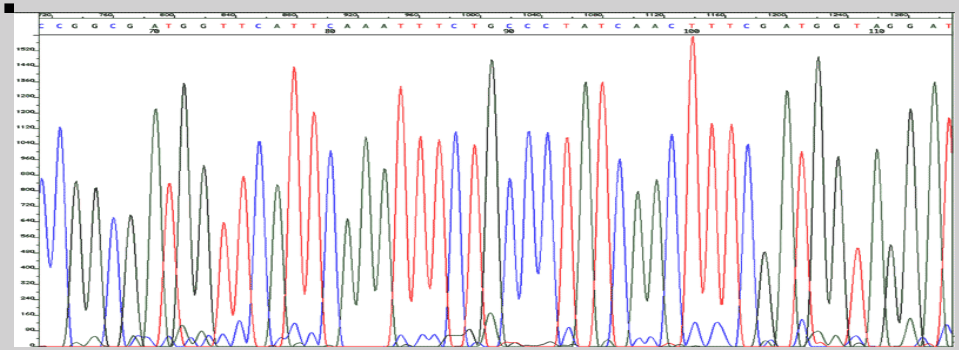

Electropherogram analysis
by a Technician
by the Physician in charge

Comparison with the normal sequence
➔ any SNP?

DIAGNOSIS

Familial MTC: genetics and risk stratification

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Genetic screening in proband and in first degree relatives is fundamental

High likelihood of developing MTC during lifespan

consider prophylactic thyroidectomy

follow up

Familial MTC: genetics and risk stratification

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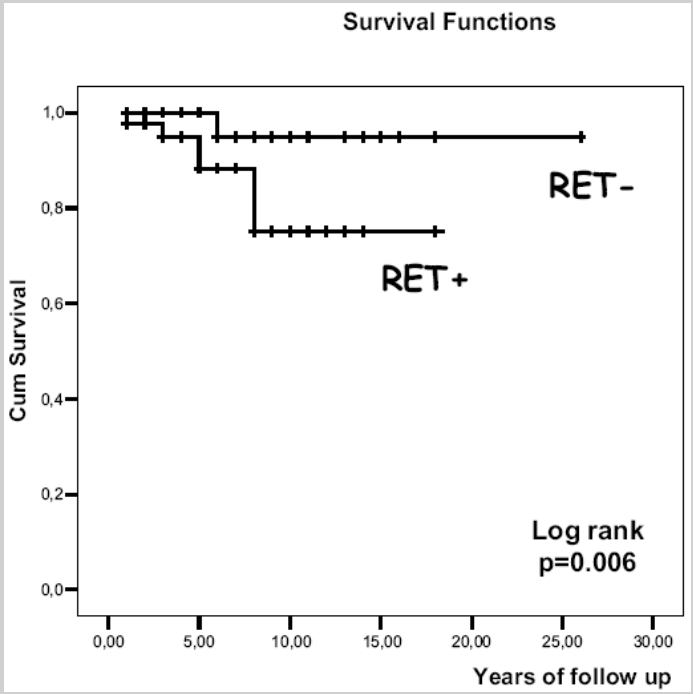
Sporadic MTC

40-50% of sporadic MTC display somatic RET mutations

Romei et al. 1996 J Clin Endocrinol Metab 81:1619–1622
Schilling et al. 2001 Int J Cancer 95:62–66

Zedenius et al. 1995 J Clin Endocrinol Metab 80:3088–3090
Zedenius et al. 1998 Cancer Detect Prev 22:544–548



Somatic RET mutations correlate with

- presence of lymph node metastases at diagnosis
- worse outcome
- disease persistence after surgery
- lower survival rate

bad prognostic factor

Elisei et al. 2008 J Clin Endocrinol Metab 93:682-7

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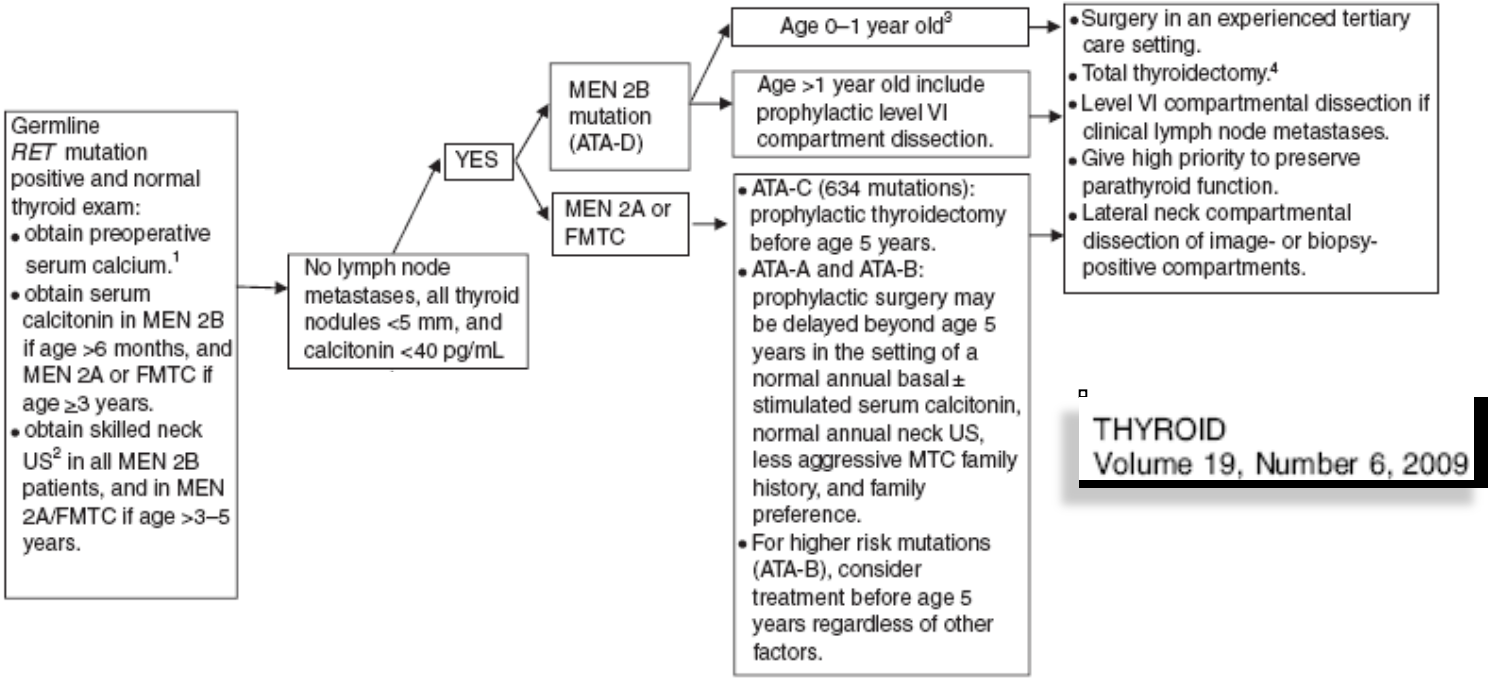
Medullary Thyroid Cancer: Management Guidelines of the American Thyroid Association



THYROID
Volume 19, Number 6, 2009

[1]Treat hyperparathyroidism with 4 gland resection and autograft to heterotopic site, or subtotal parathyroidectomy. Consider cryopreservation. PHEO preoperative screening should begin by age 8 years for MEN 2B and mutated *RET* codons 634 and 630; otherwise by age 20 years for other *RET* mutations.

[2]Neck US to include the superior mediastinum and central and lateral neck compartments.

[3]Insufficient data to recommend routine prophylactic level VI compartment dissection.

[4]Parathyroid glands resected or devascularized should be autografted in the neck in *RET*-negative, MEN 2B, and FMTC patients, while MEN 2A glands should be auto graphed to a heterotopic site.

FIG. 1. Initial diagnosis and therapy of pre-clinical disease.

RET mutation analysis is a fundamental step in the diagnostic work-up in medullary thyroid carcinoma patients

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THANK YOU!

University of Ferrara
Department of Medical Sciences
Section of Endocrinology

Ettore degli Uberti