A KINDRED WITH a *RET* CODON Y791F MUTATION PRESENTING WITH HIRSCHSPRUNG'S DISEASE.

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הדסה עין כרם, ירושלים

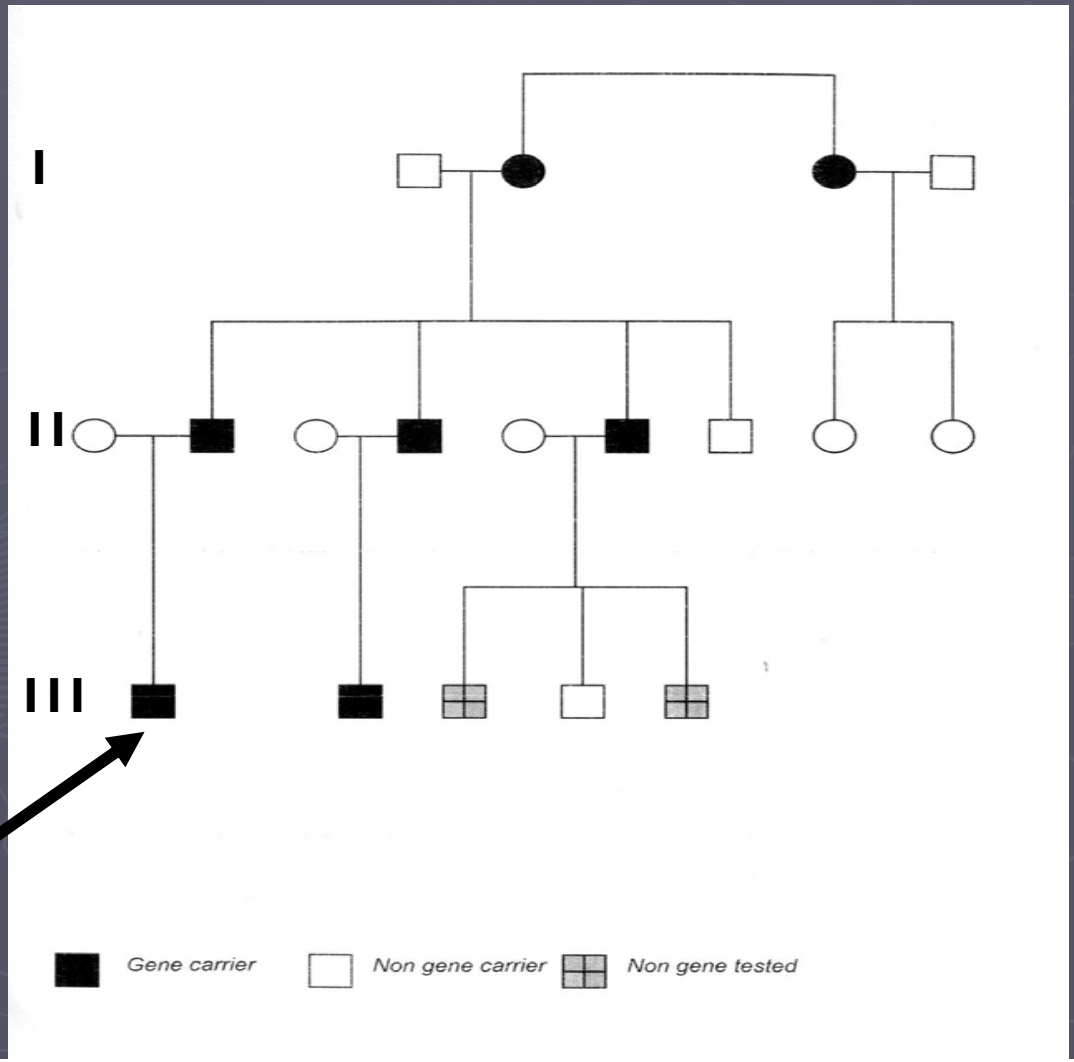
Case Report

- 36 year old male married+1
- Hirschesprung's disease (HD) diagnosed at age 3 months in his first son.

RET Gene Sequencing

- Sequence analysis of exons 5, 8-11, 13-16 of the RET gene.
- Heterozygous for **exon 13 Y791F** Tyr791Phenylalanine (TAT→TTT)
- No previous family Hx of HD, MEN2, MTC or pheochromocytoma.
- PE: no nodules in thyroid. Normotensive.

6 other family members are Y791F carriers (ages1-75 years)



Neck US in Y791F carries

- None had palpable thyroid nodules.
- n=1 had MNG
- n=1 has a 7 mm nodule with gross calcifications-FNA: colloid nodule (Ca infusion→ CCH).
- n=2 had several <1 cm nodules which did not require an FNA.

Calcitonin Levels and Calcium Stimulation Test in Y791F Carries

- Basal calcitonin levels were normal in all carriers.
- Calcium infusion tests were preformed in 5/6 mutation carriers
- n=2 had calcitonin levels consistent with C cell hyperplasia (peak calcitonin 85 pg/mL)
- n=3 had a normal response;
- 4/6 had urinary catecholamines performed with normal results.

Inactivating Mutations in *RET* can cause HD

- HD: absence of ganglion cells in the submucosal and myenteric plexus along the distal GIT.
- Inactivating mutations in the *RET* gene may be found in 35-75% of HD.
- These mutations may be distributed throughout he coding region of *RET*.

Frequency of RET Mutations in Long- and Short-Segment Hirschsprung Disease

Seri et al., Human Mutation 1997

TABLE 1. *RET* Mutations Identified By Our Group in HSCR Patients. Mutations are Designated According to the Nomenclature Recommended by Beaudet and Tsui (1993)

Exons	Mutation	Nucleotide Change	Alteration Coding Sequence	Occurence	Length of Aganglionosis	References
2	L40P	CTC→CCG	Missense	de novo	Long	Yin et al. (1994)
3	R180P	CGA→CCA	Missense	Sporadic[2]	Long	present work
5	R313Q	CGG→CAG	Missense	Sporadic[2]	Long[3]	present work
5	1063+9 G→A*	G→A	Splicing alteration	Sporadic[3]	Short	Yin et al. (1994)
6	S365X	TCG→TAG	Missense	Sporadic[2]	Long	Yin et al. (1994)
6	1120delG*	del 1 bp	Frame shift	Sporadic[2]	Long	Romeo et al. (1994a)
6	P399L	CCG→CTG	Missense	Sporadic[2]	Short	Yin et al. (1994)
8	C541X	TGT→TGA	Nonsense	Familial	Short	Yin et al. (1994)
10	C620R	TGC→CGC	Missense	Sporadic[1]	Long	present work
10	1879+1 G→A*	G→A	Splicing alteration	Sporadic[1]	Short	present work
12	E762Q	GAG→CAG	Missense/Splicing alt.	Sporadic[1]	Long	Yin et al. (1994)
12	2284+13 C→T*	C→T	Splicing alteration	Familial	—	present work
12	2284+19 C→T*	C→T	Splicing alteration	de novo	Long	Yin et al. (1994)
13	S765P	TCC→CCC	Missense	de novo	Long	Romero et al. (1994a)
13	Y791F	TAT→TTT	Missense	de novo	—	present work
15	R897Q	CGA→CAA	Missense	Sporadic[1]	Long	Romero et al. (1994a)
17	R972G	AGG→GGG	Missense	Familial	Long	Romero et al. (1994a)
17	P973L	CCA→CTA	Missense	Familial	Long	Yin et al. (1994)
19	3118del4*	del 4 bp	Frame shift	de novo	Long	present work
Deletions in Hirschsprung Patients						
	del 10q11.2–q21.2		del RET	de novo	Long[3]	Fewtrell et al. (1994)
	del 10q11.2–2q21.2		delRET	Sporadic[1]	Long[3]	Martucciello et al. (1992)
	micro del 10q11.2		delRET	Familial	Long	Yin et al. (1993)
	micro del 10q11.2		delRET	Familial	Long	Yin et al. (1994)

Activating Mutations in *RET* cause MEN2/FMTC

- Germline activating mutations in *RET* → autosomal dominant inheritance of MTC.
- Genotype/phenotype correlation between mutations in the *RET* gene and severity and age of onset of MTC.
- Most activating mutations in *RET* are in exons 10 & 11.

***RET* mutations in MEN2/FMTC- active RET dimmers in the absence of ligand**

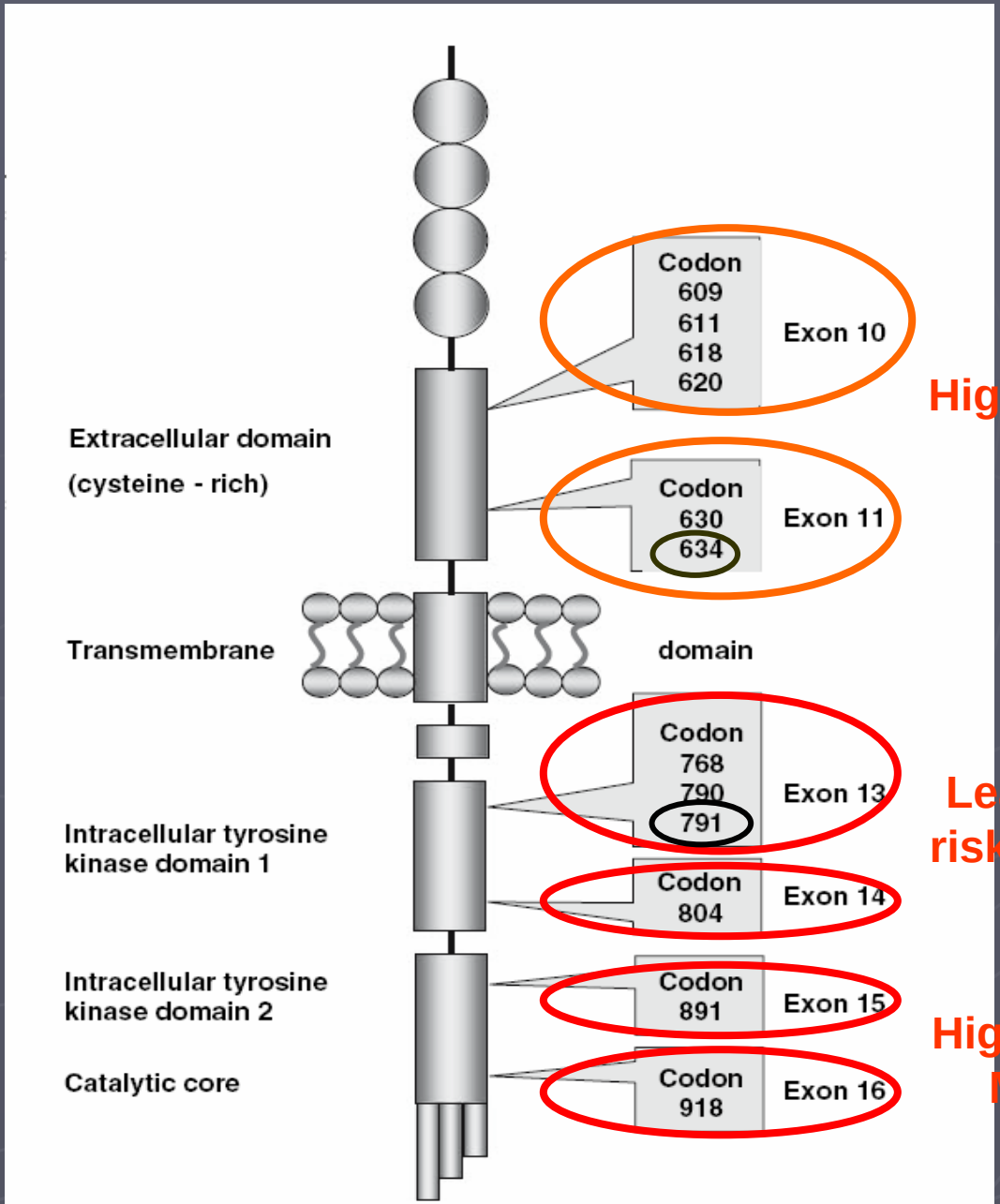


Figure 1. The RET tyrosine kinase receptor.

HD & MTC

The risk of MTC in patients with HD, Skaba et al., Pediat Surg, Int 2006

- Co-segregation of HD with MTC is rare.
- Most commonly associated with mutations in exon 10 codons 609, 611,618 & 620.
- Paradox: same mutation causes loss of function in the myenteric neurons and gain of function in the thyroid.

Medullary Thyroid Cancer in a Patient with Hirschsprung Disease with a C609Y Germline *RET*-mutation

TABLE 1. *Overview of all published HSCR cases in whom a MEN2A/FMTC associated RET germline mutation was identified*

Mutation	Phenotype	Author (Reference)
C609W	HSCR (familial)	Mulligan *et al.* (6)
C609Y	HSCR (familial)	Angrist *et al.* (3)
C609Y	HSCR/MEN2A	Decker *et al.* (7)
C609Y	HSCR/MEN2A	Decker *et al.* (7)
C609Y	HSCR (sporadic)	Sijmons *et al.* (4)
C611S	HSCR/FMTC	Nishikawa *et al.* (8)
C618R	HSCR/MEN2A	Caron *et al.* (9)
C618R	HSCR/MEN2A	Mulligan *et al.* (6)
C618R	HSCR/FMTC	Peretz *et al.* (10)
C618R	HSCR/FMTC	Peretz *et al.* (10)
C618R	HSCR/MEN2A	Pasini *et al.* (11)
C618S	HSCR/MEN2A	Decker *et al.* (7), Borst *et al.* (12)
C618S	HSCR/MEN2A	Decker *et al.* (7,13), Borst *et al.* (12)
C618S	HSCR/MEN2A	Decker *et al.* (7)
C620W	HSCR/MEN2A	Decker *et al.* (7)
C620R	HSCR/MEN2A	Decker *et al.* (7)
C620R	HSCR/MEN2A	Mulligan *et al.* (6)
C620R	HSCR/MEN2A	Mulligan *et al.* (6)
C620R	HSCR/FMTC	Romeo *et al.* (14)
C620R	HSCR/MEN2A	Blank *et al.* (15)
C620R	HSCR/MEN2A	Sijmons *et al.* (4)
C620R	HSCR (sporadic)	Angrist *et al.* (3)
C620R	HSCR (sporadic)	Seri *et al.* (16)
C620R	HSCR (sporadic)	Sijmons *et al.* (4)
C620S	HSCR/FMTC	Romeo *et al.* (14)
C620S	HSCR/MEN2A	Borrego *et al.* (17)
C620S	HSCR/MEN2A	Inoue *et al.* (18)

Jan Willem B. de Groot et al, 2005

- The precise risks of developing a MEN2A-tumor in a HD patient with a gain of function mutation is unknown.
- These risks might very well be similar to the risks MEN2A and FMTC patients with the same *RET*-mutations.
- HD patients with such mutations should be treated similarly to MEN2A and FMTC patients with such mutations.

0021-972X/98/$03.00/0
Journal of Clinical Endocrinology and Metabolism
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Vol. 83, No. 3
Printed in U.S.A.

A New Hot Spot for Mutations in the *ret* Protooncogene Causing Familial Medullary Thyroid Carcinoma and Multiple Endocrine Neoplasia Type 2A*

I Berndt et al., JCEM 1998

- 181 families with MEN-2A or FMTC from Germany
- n=8 families no mutation in exons 10 and 11.
- n=5 mutations in exon 13, codons 790 & **791**, (4/5 MTC only; 1/5 MTC & pheochromocytoma).

0021-972X/98/$03.00/0
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A New Hot Spot for Mutations in the *ret* Protooncogene Causing Familial Medullary Thyroid Carcinoma and Multiple Endocrine Neoplasia Type 2A*

- Age of onset of symptoms 21-64; 50% 30-50.

- Exon 13 mutations probably represent:
  - 30% of *RET* mutations in “sporadic” MTC
  - 10% of the mutations in MEN2A/FMTC.

- Penetrance for MTC is m/p low.

- Penetrance is very low for Pheochromocytoma.

RET proto-oncogene mutations affecting codon 790/791: A mild form of multiple endocrine neoplasia type 2A syndrome?

Gimm et al, Dralle; Germany, Surgery 2002

- N=40 RET exon 13 codon 790/791 mutations (n=23 & 17)
- Operated in 4 specialized centers.
- 13 patients were index patients (57.7 ± 11.3 years)
- 27 patients were screening patients (24.4 ± 16.5 years).
- Youngest patient with MTC 13.8 years,
- Youngest patient with LN metastases 46.4 years.

Index group n=13

- L790F n = 7, all had MTC;
- Y791F, n = 6, 4/6 had MTC
- Mean age did not differ between the 2 codons:
  - L790F 58.0 ± 11.9
  - Y791F, 57.4 ± 11.7

Index group

- 2/13 had Pheo diagnosed before thyroid surgery, both Y791F carriers.

- 5/13 LN metastasis
  - L790F n=4 (4/23; 26%)
  - Y791F n=1 (1/17; 6%)

- Distant metastasis 1/13 L790F

Screening group n=27

- L790F n = 16, 8/16 had MTC; 2/16 had CCH
- Y791F, n = 11, **0**/11 had MTC; 8/11 had CCH
- LN metastases n=2 (2/16 of L790F).

Conclusions

- 790/791 mutations seemed to have a less aggressive clinical course (790>791).

- Biochemical cure rate: screening patients>index patients.

- Risk of MTC
  - very low < 10 years
  - Low if stimulated calcitonin<90pg/ml.

- Risk of LNM is low if basal calcitonin<81pg/ml.

Various Penetrance of Familial Medullary Thyroid Carcinoma in Patients With *RET* Protooncogene Codon 790/791 Germline Mutations

Fitze G et al. Germany, Annals of Surgery 2002

- 45 patients with "sporadic MTC"
- *RET* genotyping in all family members of the index patients.
- All mutation carriers underwent subsequent prophylactic TT.

- 5/45 index patients had *RET* mutations (11%).
- 4/5 patients with sporadic MTC who carried *RET* mutations had exon 13 codons 790 (n=3), 791(n=1) mutations.
- The one index patient with Y791F mutation had a somatic M918T mutation in his tumor, this might explain the more aggressive behavior of the low risk mutation in this patient.
- Age of clinical MTC presentation 34-62 years.
- None of the mutation carriers had biochemical evidence of Pheo or PHP.

- 4/4 asymptomatic carrieres of the L790F mutation had MTC or CCH.
- 1 Y791F mutation carrier did not have MTC or CCH.
- Prophylactic thyroidectomy in childhood in carriers of the **Y791F** mutation is not recommended.
- Thyroidectomy should be preformed at first biochemical sign of MTC or by the age of 20.
- Carriers of **codon 790** mutations should be treated like other higher risk mutations.

World Journal of Surgery

Published Online: 1 February 2007

World J Surg (2007) 31: 996–1001
DOI: 10.1007/s00268-006-0446-1

ORIGINAL SCIENTIFIC REPORTS

Codon Y791F Mutations in a Large Kindred: Is Prophylactic Thyroidectomy Always Indicated?

Peter Vestergaard, MD, PhD, DrMedSc,[1] Else Marie Vestergaard, MD, PhD,[2]
Helle Brockstedt, MD, PhD,[3] Peer Christiansen, MD, DrMedSc[4]

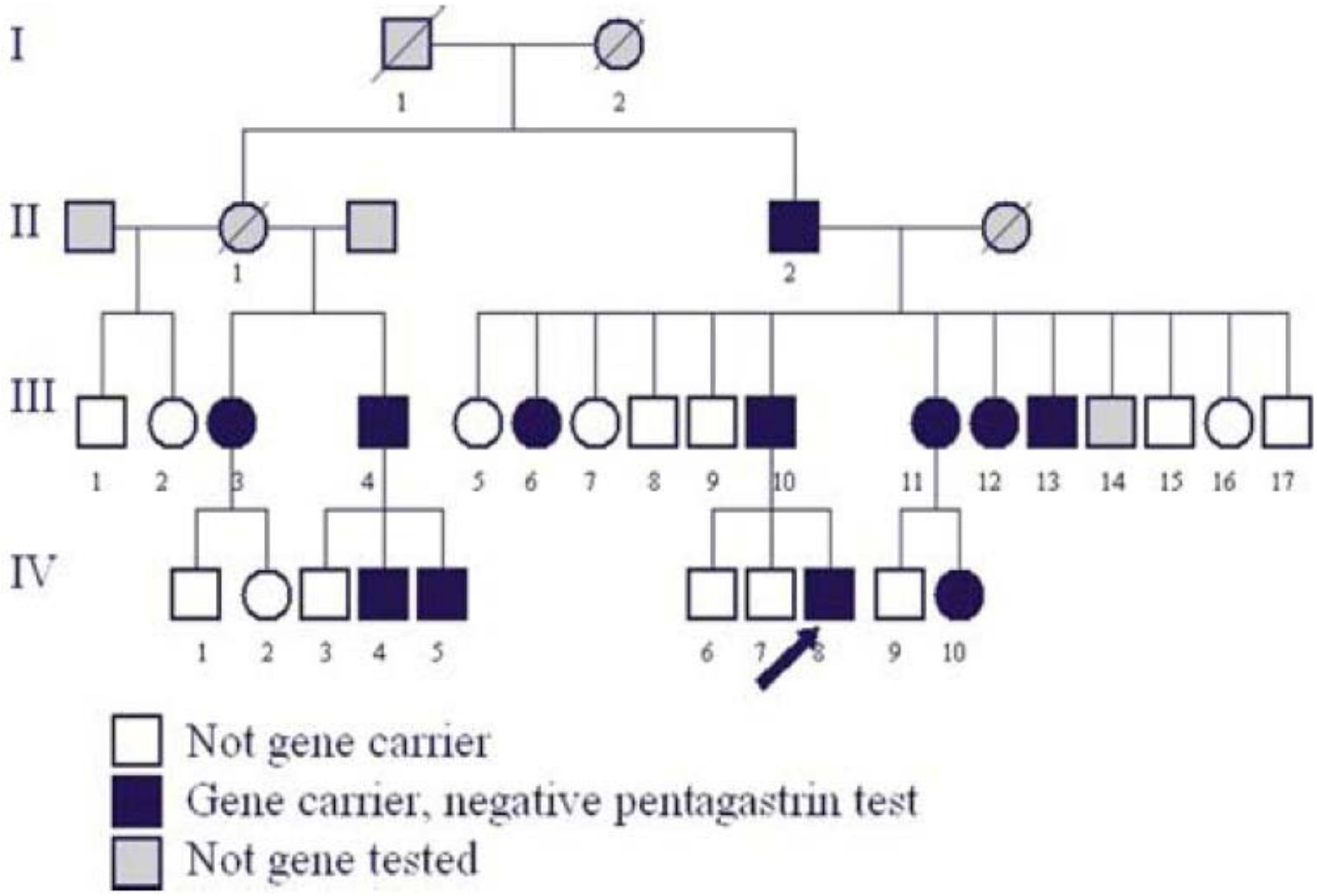

I
1
2
II
1
2
III
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
IV
1 2 3 4 5 6 7 8 9 10
Not gene carrier
Gene carrier, negative pentagastrin test
Not gene tested

Table 1.
Characteristics of the family members

Case	Gender	Age	Mutation	Comments	Basal calcitonin (<3.8 pmol/l)	Pentagastrin test (serum calcitonin in pmol/L before and after)			
						3 minutes before (<3.8 pmol/l)	1 minutes before (<3.8 pmol/l)	2 minutes after (<29 pmol/l)	5 minutes after (<29 pmol/l)
I-1	Male	N/A	N/A	Deceased	–	–	–	–	–
I-2	Female	N/A	N/A	Deceased	–	–	–	–	–
II-1	Female	N/A	(+)	Deceased, obligate gene carrier	–	–	–	–	–
II-2	Male	84	+	–	< 1.46	< 1.46	< 1.46	4	4
III-1	Male	64	–	–	–	–	–	–	–
III-2	Female	62	–	–	–	–	–	–	–
III-3	Female	59	+	Not tested in our centre	–	–	–	–	–
III-4	Male	50	+	–	0	< 1.46	< 1.46	3	2
III-5	Female	60	–	–	< 1.46	–	–	–	–
III-6	Female	57	+	–	< 1.46	< 1.46	< 1.46	4	3
III-7	Female	56	–	Goitre; surgery; no malignancy	–	–	–	–	–
III-8	Male	54	–	–	< 1.46	–	–	–	–
III-9	Male	52	–	–	1.74	–	–	–	–
III-10	Male	51	+	Goitre; FNAB; no malignancy	–	1.77	1.96	7.54	5.47
III-11	Female	50	+	–	< 1.46	< 1.46	< 1.46	< 1	< 1
III-12	Female	48	+	–	< 1.46	< 1.46	< 1.46	< 1	< 1
III-13	Male	47	+	–	< 1.46	< 1.46	< 1.46	4	4
III-14	Male	46	N/A	–	–	–	–	–	–
III-15	Male	44	–	–	–	–	–	–	–
III-16	Female	43	–	Goitre; surgery; no malignancy	< 1.46	–	–	–	–
III-17	Male	40	–	–	–	–	–	–	–
IV-1	Male	40	–	–	–	–	–	–	–
IV-2	Female	32	–	–	–	–	–	–	–
IV-3	Male	23	–	–	–	–	–	–	–
IV-4	Male	20	+	–	–	0	0	3	3
IV-5	Male	13	+	–	–	0	0	2	2
IV-6	Male	24	–	–	–	–	–	–	–
IV-7	Male	15	–	Goitre	–	< 1.46	< 1.46	4.82	4.58
IV-8	Male	13	+	Goitre; FNAB; no malignancy	–	< 1.46	< 1.46	4.71	3.29
IV-9	Male	33	–	–	1.57	–	–	–	–
IV-10	Female	26	+	–	< 1.46	< 1.46	< 1.46	< 1	< 1

N/A: not available; +: presence of mutation or goitre; –: absence of mutation or goitre; FNAB: fine-needle aspiration biopsy from the thyroid. Pentagastrin test: serum calcitonin values before and after stimulation with pentagastrin, the values in parentheses are the normal range.

Summary of previous cases

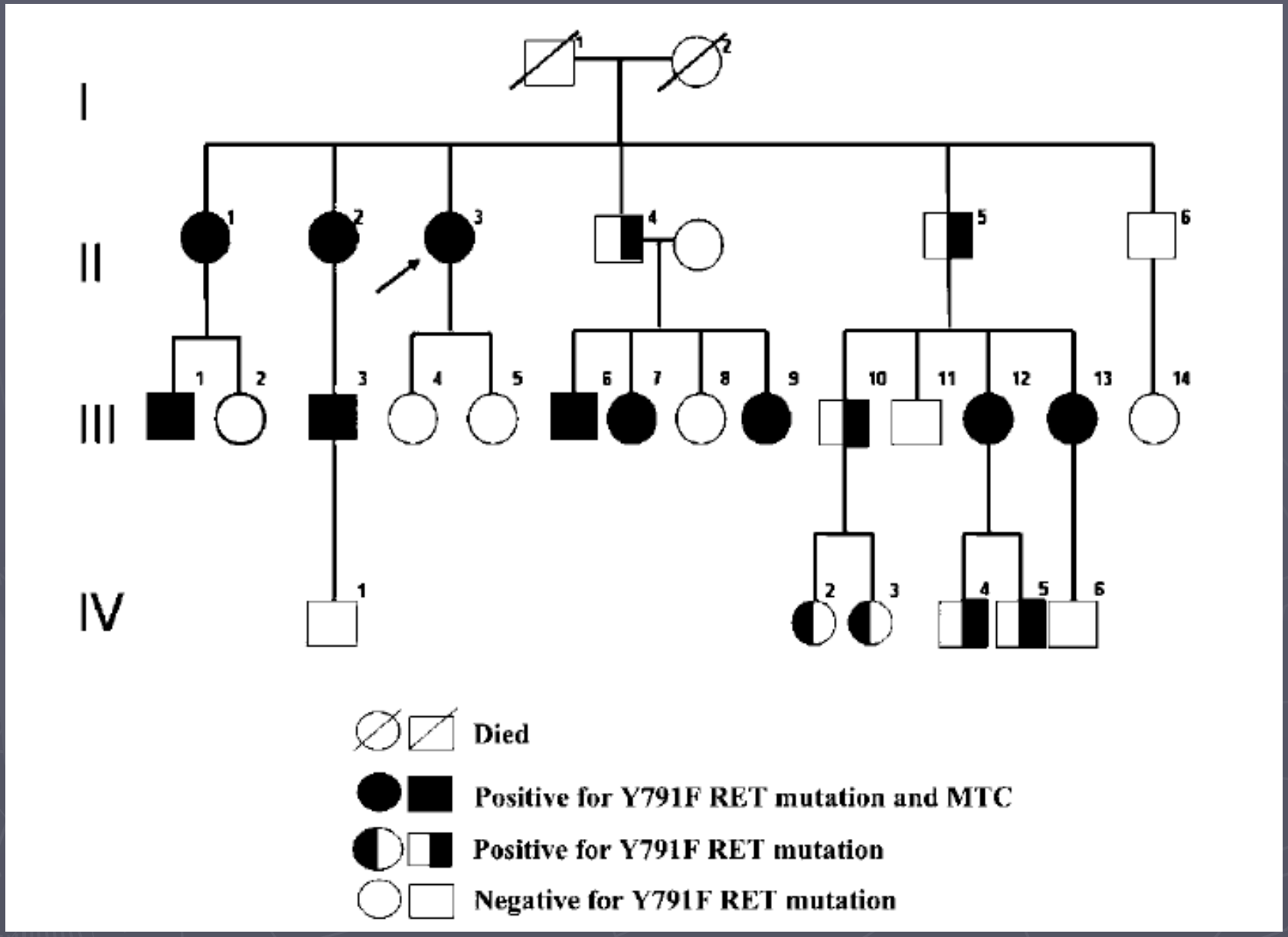
- 62/80 described in detail.
- n=15 (24.2%) had MTC
- n=30 had CCH (48.4%)
- n=2 had PTC (3.2%)
- n=2 had a phaeochromocytoma (3.2%)
- n=14 did not have MTC or other abnormalities (22.6%).
- No deaths have been reported in patients with the Y791F mutation.

Conclusions

- Current series: 0% had abnormal pentagastrin tests
- Previously recorded cases-all clustered around index cases of MTC.
- May lead to a biased estimate of the likelihood of developing MTC.
- Questions the penetrance of the Y791F mutation in the development of a MTC.
- Genotype–phenotype variations ?

Y791F *RET* mutation and early onset of medullary thyroid carcinoma in a Brazilian kindred: evaluation of phenotype-modifying effect of germline variants

 Clinical Endocrinology, 67, 805–808



No biochemical evidence of hyperparathyroidism and phaeochromocytoma was found.

Y791 kindred from Brazil

- N=2 <20 years
- Stimulated CT is ↑ in a 5 and 8 -year-old
- Both refused surgery.

Y791F and MTC-Other modifying genes?

- It is not clear why patients with the same *RET* mutations have such a variable course.
- SNPs?
- Affected and nonaffected family members were screened for five known *RET* SNPs
- SNPs: association with clinical course of MTC, in particular promoting an early onset of disease.

Results

- 100 unrelated healthy normal controls.
- Co- occurrence of Y791F and the L769L
- The L769L HZ in 15 carriers; HM in 2.
- Median age at Dx
  - HZ- 28·5;
  - HZ 18 years.
- n=2 HM for L769L MTC < 20 years; bilateral MTC and LN involvement at Dx

Conclusions

- Y791F mutation may be considered at risk in an age earlier than 20
- L769L variant of *RET* may be related to the earlier age of onset in these patients.
- SNP's may help optimize an individualized approach in the timing and extent of prophylactic thyroidectomy.

CONSENSUS

Brandi et al., JCEM 2001

Guidelines for Diagnosis and Therapy of MEN Type 1 and Type 2

Mutation	Risk level	Age of TT	LN dissection
883, 918, 922	Level 3- highest risk	1-6 months	Central compartment
611, 618, 620, 634	Level 2- high risk	<5 years	Central compartment? After age 5
609,768,**790**, **791**,804,891	Level 1- least high risk	Age 5? 10? Biochemical follow up?	Maybe after age 20.

ORIGINAL ARTICLE

Early Malignant Progression of Hereditary Medullary Thyroid Cancer

Andreas Machens, M.D., Patricia Niccoli-Sire, M.D., Josef Hoegel, Ph.D.,
Karin Frank-Raue, M.D., Theo J. van Vroonhoven, M.D.,
Hans-Dietrich Roeher, M.D., Robert A. Wahl, M.D., Peter Lamesch, M.D.,
Friedhelm Raue, M.D., Bernard Conte-Devolx, M.D., and Henning Dralle, M.D.,
for the European Multiple Endocrine Neoplasia (EUROMEN) Study Group

2003

Table 4. Genotypic Testing for *RET* Mutations and Clinical Implications.

Affected Codon	No. of Patients	Earliest Age at Presentation of Medullary Thyroid Cancer		Positive for Familial MEN Phenotype*
		Current Study	Other Studies	
				%
918	4	9 mo	13 mo[19]	100†
634	130	15 mo	17 mo[20]	95
618	19	7 yr	7 yr[11,21]	80
611	4	7 yr	20 yr[22]	50
620	14	11 yr	12 yr[23]	40
790	14	12 yr	12 yr[11]	14
891	6	13 yr	48 yr[24]	0
630	1	15 yr	34 yr[25]	100
804	4	20 yr	6 yr[26,27]	0
609	4	>20 yr	5 yr[28]	100
791	5	>20 yr	21 yr[29]	67
768	2	>20 yr	22 yr[30-32]	0

Genotype-Phenotype Based Surgical Concept of Hereditary Medullary Thyroid Carcinoma

Machens A and Dralle H, World J Surgery 2007.

Meta-analysis

"The genotype/phenotype correlation with some of the more rare *RET* mutations: 609, 611, 630, **790, 791**, 891, 918 needs to be verified."

RET carriers in hereditary MTC

Mutation	Risk level	Youngest age of MTC	Youngest age of LN Mets	Youngest age of Distant Mets
883, 918, 922	Level 3- highest risk	9 months	2.7 years	5 years
611, 618, 620, 634	Level 2- high risk	1-5 years	1st-2nd decade	3rd decade
609,768,**790, 791,**804,891	Level 1- least high risk	2nd-3rd decade	2nd -4th decade	4th decade

Schaffer family-What now??

- One 36 year old male with solitary 7 mm colloid nodule and Ca infusion compatible with CCH.
- One 75 year old carrier with several small thyroid nodules and normal basal and stimulated calcitonin.
- 1 year old boy with HD.