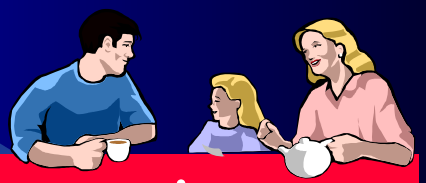
FORME FAMILIARI DI ADENOMI IPOFISARI

FAMILIAL FORMS of PITUITARY ADENOMA



Familial clustering of pituitary adenomas is rare

may present as a component of

- Carney Complex
- Wermer syndrome (Multiple Endocrine Neoplasia type 1, MEN-1)
- Familial acromegaly (FA)
- Familial prolactinomas
- Familial Cushing's disease

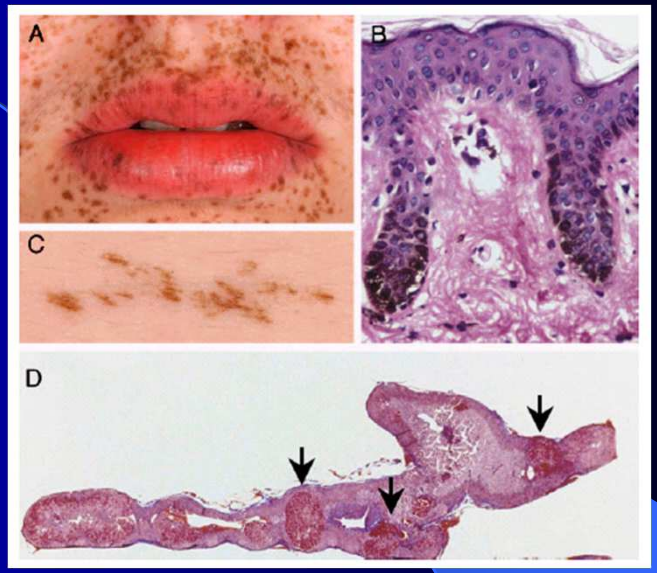
FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

Carney Complex,
the complex of spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas, is a form of multiple endocrine neoplasia



Patients often have tumors of two or more endocrine glands, including primary pigmented nodular adrenocortical disease (PPNAD), GH- and PRL-producing pituitary adenoma, thyroid adenoma or carcinoma, testicular neoplasms, and ovarian cysts.

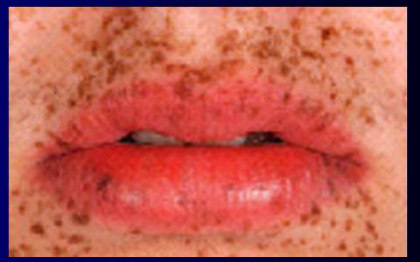
Bossis I et al. 2004 Endocrinology 145(12):5452–5458

Nonendocrine tumors that occur frequently are myxomas and ear canal trichofolliculo-epitheliomas. Additional, but rare, manifestations include psammomatous melanotic schwannoma, breast ductal adenoma, and osteochondromyxoma

Stratakis CA 2000 Front Biosci 5:353–366

FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX



Diagnosis

- two of the disease manifestations or
- one disease manifestations and an affected first-degree relative or an inactivating mutation of the PRKAR1A gene

Sandrini F et al. 2003 Molecular Genetics and Metabolism 78: 83–92

Manifestations of disease

1. Spotty skin pigmentation with a typical distribution (lips, conjunctiva and inner or outer canthi, vaginal and penile mucosa)
2. Myxoma (cutaneous and mucosal)*
3. Cardiac myxoma*
4. Breast myxomatosis*
5. PPNAD* *or* paradoxical positive response of urinary glucocorticosteroids to dexamethasone administration during Liddle's test
6. Acromegaly due to GH-producing adenoma*
7. LCCSCT* *or* characteristic calcification on testicular ultrasonography
8. Thyroid carcinoma (at any age)* *or* multiple, hypoechoic nodules on thyroid ultrasonography in a prepubertal child
9. Psammomatous melanotic schwannoma*
10. Blue nevus, epithelioid blue nevus (multiple)*
11. Breast ductal adenoma (multiple)*
12. Osteochondromyxoma*

Supplemental criteria

1. Affected first-degree relative
2. Inactivating mutation of the *PRKAR1A* gene

*With histological confirmation.

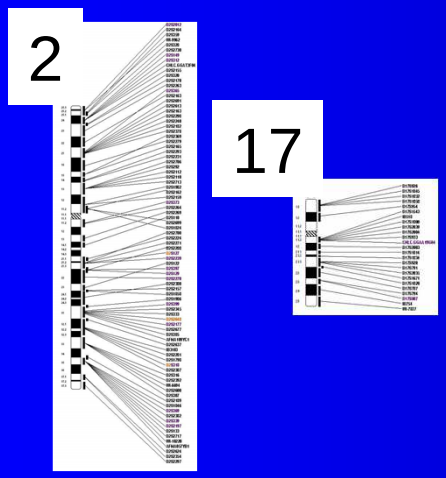
Bossis I et al. 2004 Endocrine-Related Cancer 11:265–280

FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

Etiology

Chromosomal mapping of mutations on the PRKAR1A gene and its characteristics observed in familial or sporadic cases of CNC genetically evaluated at National Institutes of Health



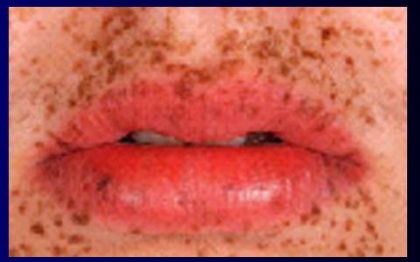
Subject	Chromosomal mapping[a]	Mutation[b]	Characteristic of mutation[c]
Familial cases			
CAR01	17	578delGT	Frameshift/stop
CAR03	2		
CAR04		NO	
CAR05		NO	
CAR06		NO	
CAR07		NO	
CAR08		169C > T	Nonsense
CAR09		NO	
CAR10		NO	
CAR11		NO	
CAR12		NO	
CAR13		873GG > CT	Missense/nonsense
CAR15	2	NO	
CAR16		NO	
CAR17		NO	
CAR18		exon6IVSdel(−9 > −2)	Splice acceptor mutation
CAR19		88A > G	Mutation in initiator ATG
CAR20	17	578delGT	Frameshift/stop
CAR21		188delCTATT	Frameshift/stop
CAR23	17	578delGT	Frameshift/stop
CAR24		NO	
CAR25		exon8IVS + 3G > A	Splice donor mutation
CAR27		211C > T	Nonsense
CAR28		578delGT	Frameshift/stop
CAR29		578delGT	Frameshift/stop
CAR30		NO	
CAR33		1038delA	Frameshift/stop
CAR34		211C > T	Nonsense
CAR37		769C > T	Nonsense
CAR103	2		
CAR104	2		
CAR107	2		
CAR108	2	578delGT	Frameshift/stop
CAR109		653AA > CAC	Frameshift/stop
CAR110	17	769C > T	Nonsense
CAR111		799insAA	Frameshift/stop
MYXB		NO	
MYX01		618delTGAT	Frameshift/stop
MYX02		NO	
Sporadic cases			
CNC01		NO	
CNC02		NO	
CNC03		Exon2IVS − 2A > G	Splice acceptor mutation
CNC04		NO	
CNC05		NO	
CNC06		211C > T	Nonsense
CNC07		1007C > G	Nonsense
CNC08		NO	
CNC09		NO	
CNC11		Exon3IVS + 1G > C	Splice donor mutation
CNC12		NO	
CNC13		Exon3IVS + 1G > C	Splice donor mutation
CNC14		NO	
CNC15		781insT	Frameshift/stop
CNC16		NO	
CNC17		NO	
CNC18		274A > T	Nonsense
CNC19		165insTAAC	Frameshift/stop

[a] The number indicates which chromosome is mapped to the respective family.
[b] Mutation observed on members of the family or from the patient (sporadic cases). When mutations were not observed, the subject was considered normal (NO) for the *PRKAR1A* gene.
[c] The mutation characteristics resulting in: nonsense, missense, and frameshift or mutations at splices sites leading to a premature stop codon.

Sandrini F et al 2003 Mol Genet Metab 78: 83–92

FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX



Etiology

- autosomal dominant inheritance
- PRKAR1A gene, 17q22–24, mutated in more than half of patients with CNC and/or PPNAD
- unknown gene, 2p16 (CNC2 locus)

Stratakis CA et al 1996 J Clin Invest 97:699–705
Casey M et al 1998 Circulation 98:2560–2566

Kirschner LS et al 2000 Nat Genet 26:89–92
Kirschner LS et al 2000 Hum Mol Genet 9:3037–3046

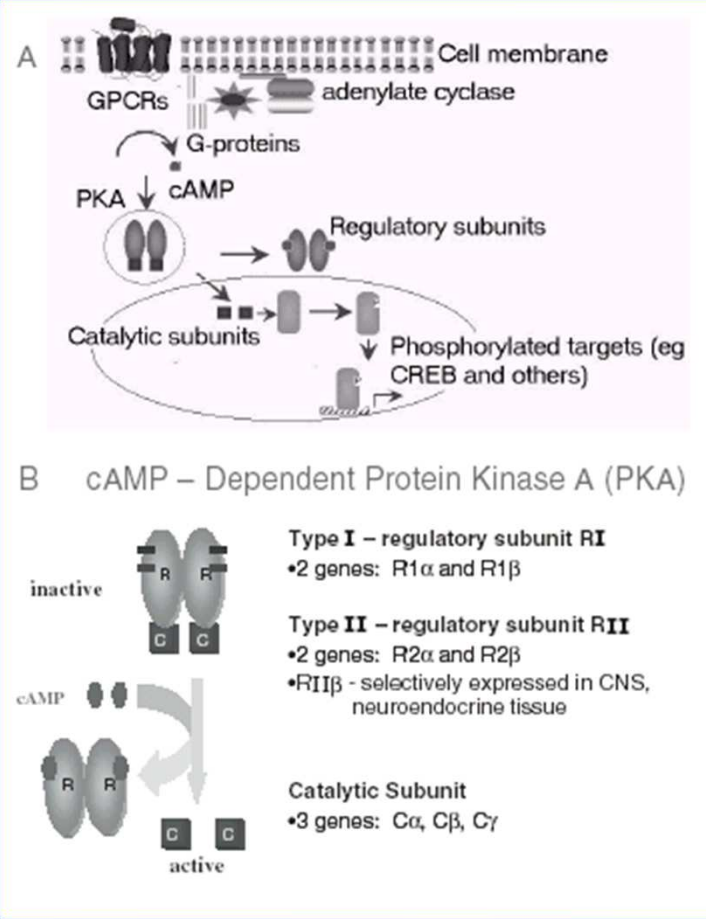
premature stop codon

unstable mutant mRNAs due to nonsense mediated mRNA decay

absent predicted mutated R1 α protein products

FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX



PRKAR1A

R1α

PKA regulatory subunit type 1α (R1α)

The predominant type of PKA isoform in a cell depends on tissue differentiation and the proliferation stage

Cellular PKA responses to cAMP can differ significantly depending on the PKA type and tissue-specific expression of phosphorylated molecular targets

Bossis I et al 2004 Endocrine-Related Cancer 11:265–280

FAMILIAL FORMS of PITUITARY ADENOMA

NORMAL CELL

PKA tetramer activation by binding of cAMP to the regulatory subunits

↓

catalytic subunits release

↓

phosphorylation of cytoplasmic targets

↓

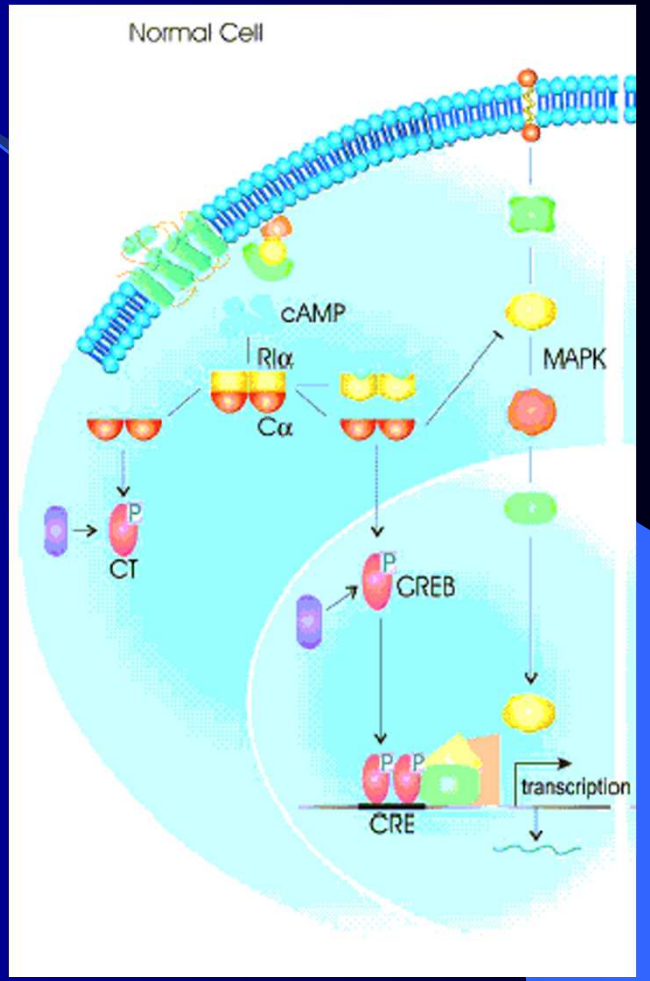
crosstalk with other intracellular signaling pathways

↓

PKA catalytic subunits phosphorylate CREB in the nucleus

↓

activation of DNA transcription of cAMP-responsive elements (CRE)-containing genes



FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

absent or ineffective R 1α

↓

compensating excess of other regulatory subunits

↓

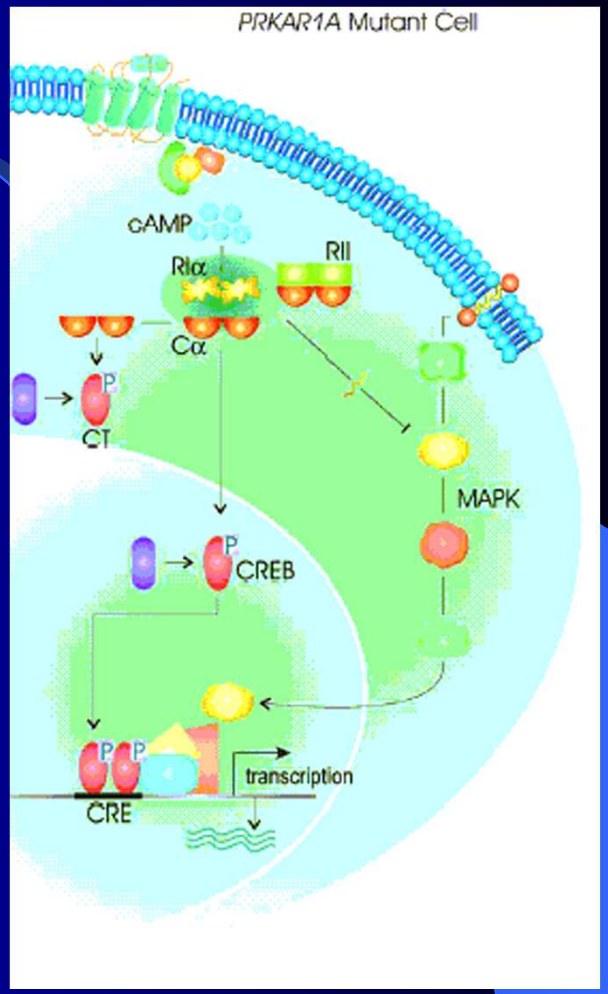
altered availability of the catalytic subunits

↓

increase in DNA transcription and
activation of multiple pathways

↓

abnormal growth and proliferation



FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

R1α haplo-insufficiency → increased cAMP stimulated PKA activity

Casey M et al 2000 J Clin Invest 106:31–38

↓

may be sufficient for tumor development

Groussin L et al 2002 J Clin Endocrinol Metab 87:4324–4329

R1α → reduced in CNC

↓

increased in several cancers

PRKAR1A could have properties of both an oncogene and an oncosuppresor gene, but satisfies the criteria for neither in human and mouse oncogenesis

Bossis I et al 2004 Endocrinology 145(12):5452–5458

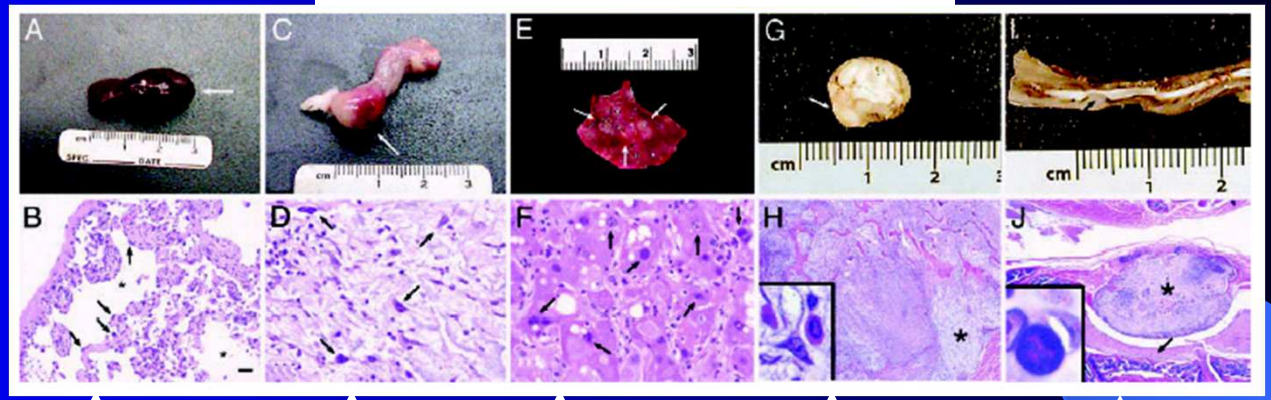
FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

Mouse model

Prkar1a haploinsufficiency in mice causes some phenotypes similar to CNC

Tumors in *prkar1a +/-* mice



spleen enlarged by splenic hemangiosarcoma

Left forelimb myxoid fibrosarcoma

Sarcoma mass involving the calvaria with distinct myxomatous areas

Extradural lumbar chondrosarcoma

multinodular hepatocellular carcinoma

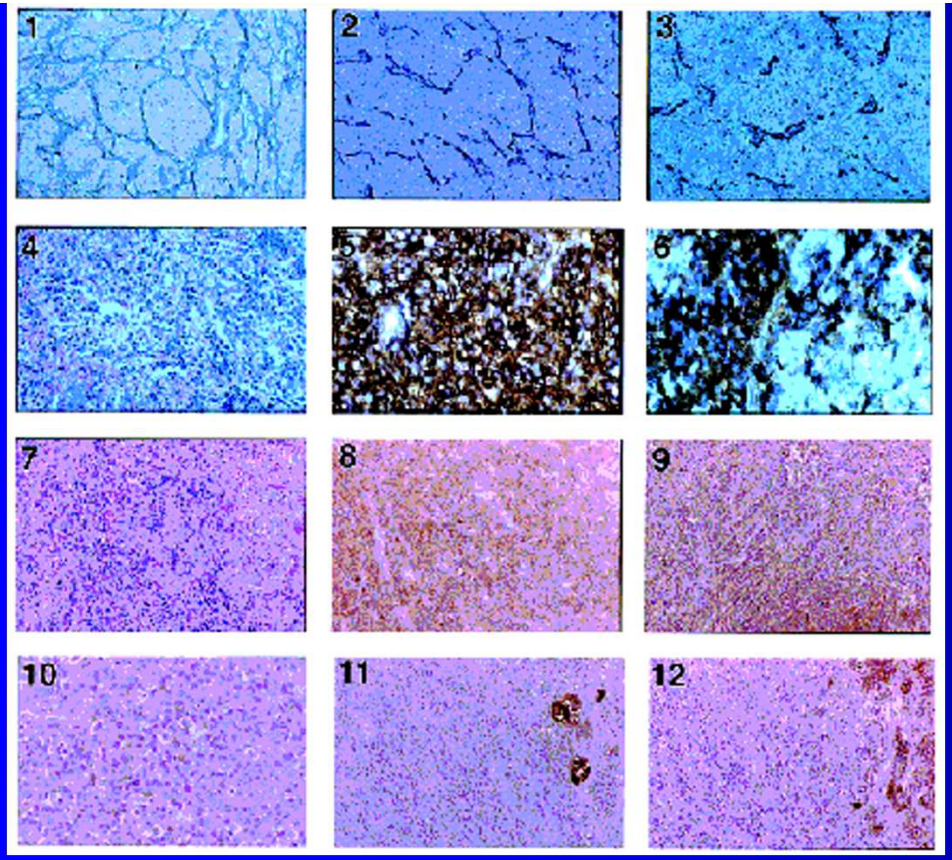
Tumors from *prkar1a +/-* mice preserve the wild-type allele, thus distinct secondary genetic events are required for tumor formation

Veugelers M et al 2004 PNAS 101:14222–14227

FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

Immunohistochemistry in pituitary tumors from patients with CNC and acromegaly



Pack SD et al 2000 J Clin Endocrinol Metab. 85:3860-5

normal pituitary

↓

pituitary hyperplasia

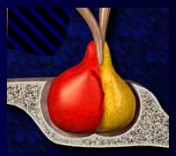
↓

pituitary adenoma

progression

FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

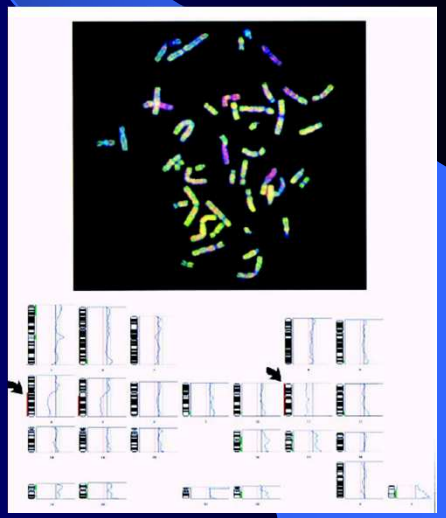


Genetic findings

by comparative genomic hybridization

multiple changes accumulating proportionally to the severity of the clinical behavior of the lesion

- gains (1p, 2q, 9q, 12q, 16, 17, 19p, 20, 22)
- losses (6q, 7q, 11)



no chromosomal changes in microadenomas

Pack SD et al 2000 J Clin Endocrinol Metab. 85:3860-5

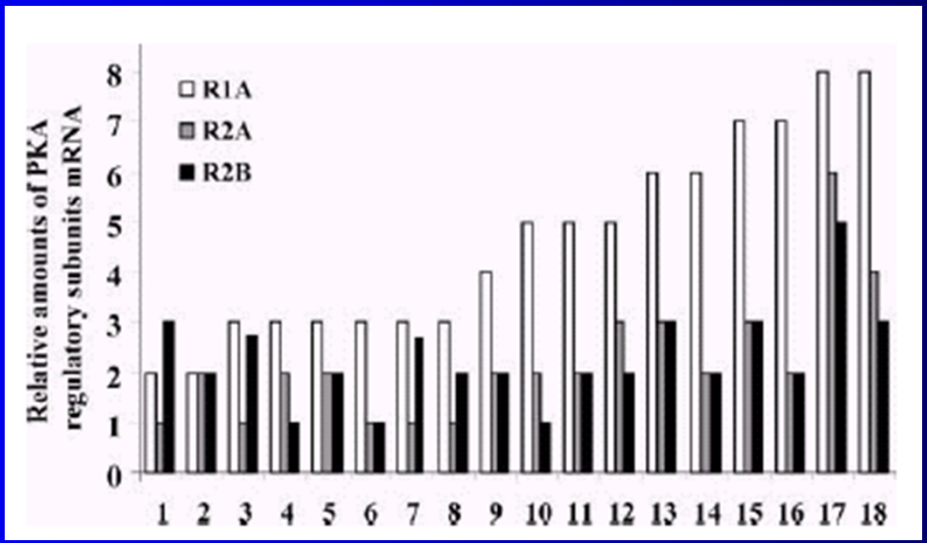
FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

PKA expression in human pituitary adenomas

analysis of the PKA regulatory subunits R1A, R2A, and R2B in 30 pituitary adenomas

- no mutation nor loss of heterozygosity of *PRKAR1A*
- R1A, R2A, and R2B mRNA present in all tumors, R1A being the most represented in the majority of samples



Lania AG et al 2004 Cancer Res 64, 9193–9198

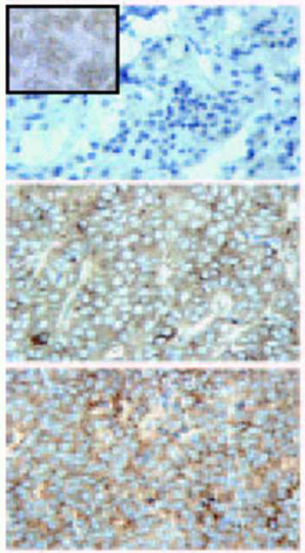
FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX

PKA expression in human pituitary adenomas

expression of the PKA regulatory subunits R1A, R2A, and R2B in 30 pituitary adenomas

→ Immunohistochemistry: low or absent R1A in all tumors
R2A and R2B highly expressed



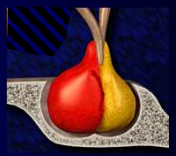
Protein	*Staining pattern*	*Staining score*			
		0	1	2	3
R1A	Diffuse cytoplasmatic	16*	14*	-	-
R2A	Diffuse cytoplasmatic Perinuclear dots	-	-	2*	28*
R2B	Diffuse cytoplasmatic	-	-	1*	29*

unbalanced
R1/R2 ratio

Lania AG et al 2004 Cancer Res 64, 9193–9198

FAMILIAL FORMS of PITUITARY ADENOMA

CARNEY COMPLEX



PKA expression in human pituitary adenomas

unbalanced R1/R2 ratio in GH3 cells and in human somatotroph adenoma

- R2-selective cAMP analog 8-Cl cAMP stimulates cell proliferation
- R1A RNA silencing increases Cyclin D1 expression

A low R1/R2 ratio promotes proliferation of transformed somatotrophs

low expression of R1A protein may favor cAMP-dependent proliferation of transformed somatotrophs

Lania AG et al 2004 Cancer Res 64, 9193–9198

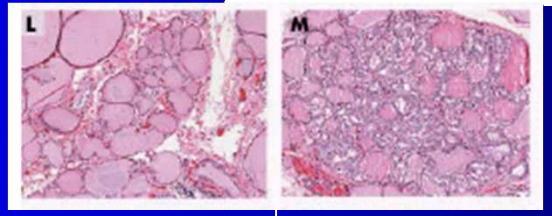
FAMILIAL FORMS of PITUITARY ADENOMA

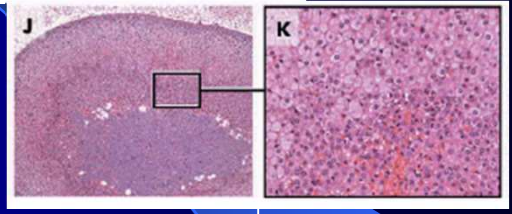
CARNEY COMPLEX

Mouse model

Transgenic mouse carrying an antisense transgene for Prkar1a exon 2 develops phenotypes similar to CNC

Tumors in tTA/X2AS mice

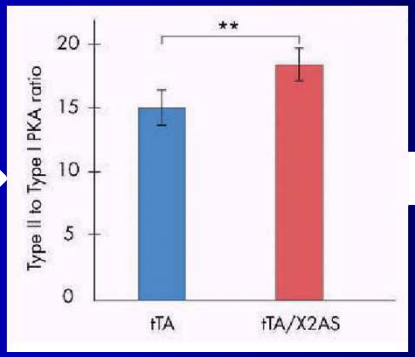




thyroid follicular hyperplasia and adenomas

adrenocortical hyperplasia and features of PPNAD

Tumors in tTA/X2AS mice display LOH of the mouse chromosome 11 Prkar1a locus, an increase in total type II PKA activity and higher RIIb protein levels



unbalanced R1/R2 ratio

Griffin K et al 2004 J Med Genet 41: 923–931

FAMILIAL FORMS of PITUITARY ADENOMA

Wermer Syndrome

FAMILIAL FORMS of PITUITARY ADENOMA

MEN-1

Definition

A consensus definition of MEN-1 is a case with 2 of the 3 main MEN1-related endocrine tumors (parathyroid adenomas, entero-pancreatic endocrine tumors, and pituitary tumor)

Familial MEN-1 is defined as at least 1 MEN-1 case plus at least 1 first degree relative with 1 of those 3 tumors

Expressions of MEN-1 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 2001 J Clin Endocrinol Metab 86: 5658–5671

endocrine cells of the stomach, duodenum, lung and thymus, adipose tissue, adrenocortical glands, follicular component of the thyroid gland

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

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

FAMILIAL FORMS of PITUITARY ADENOMA

MEN-1

Epidemiology

- MEN-1 is rare ~ 1 : 30,000
- Onset age is usually earlier in a tumor type of MEN-1 than of nonhereditary cases

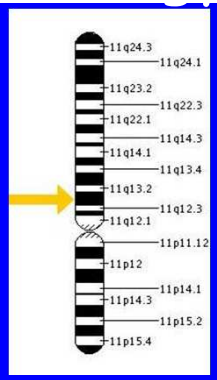
Observed penetrance and spectrum of manifestations in MEN-1

	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

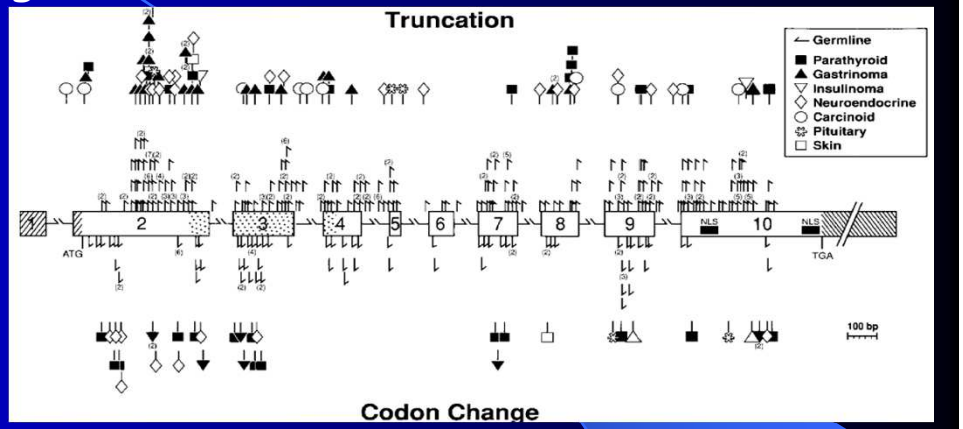
FAMILIAL FORMS of PITUITARY ADENOMA

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

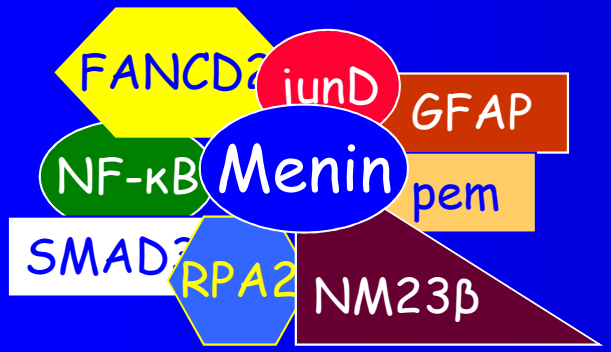
1% small indels, deletions or insertions → protein truncation and/or alter interaction with menin partners and/or favour rapid degradation

absence of genotype/phenotype relation

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

FAMILIAL FORMS of PITUITARY ADENOMA

Normal Menin



Widespread 67 kDa protein mainly nuclear

interacting with many transcription factors

Uncouples Elk-1, JunD, and c-Jun phosphorylation from MAPK activation

Represses c-fos promoter activity

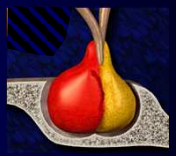
Gallo A et al 2002. Oncogene 21: 6434-6445

No physiologic role for menin interaction with any of these partners has been proved

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

FAMILIAL FORMS of PITUITARY ADENOMA

MEN-1



Mouse model

Men1 +/- mice

Homozygous Men1 -/- mice die in utero at embryonic days 11.5-12.5

Heterozygous Men1 +/- mice develop features similar to those of the human disorder:

At 9 months — pancreatic islet hyperplasia/tumors,
parathyroid adenomas

At 16 months — larger, more numerous pancreatic islet tumors
parathyroids
thyroid
adrenal cortex
pituitary

Crabtree JS et al 2001 Proc Natl Acad Sci 98:1118-23

FAMILIAL FORMS of PITUITARY ADENOMA

MEN 1

Clinical and biochemical assessment

parathyroid adenomas

does not differ from that of sporadic forms

pituitary tumors

but

starts early in life
in gene mutation carriers

neurodocrine tumors

and

continues after first surgery
due to the high likelyhood of recurrence

Norton et al. Ann Surg 247(3):501–510

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

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%)

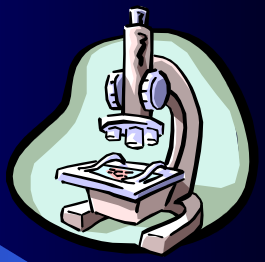
Pieterman et al. Familial Cancer 2011; 10:157–171

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

FAMILIAL FORMS of PITUITARY ADENOMA

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
  - progression
    - adenomatous lesions

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

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

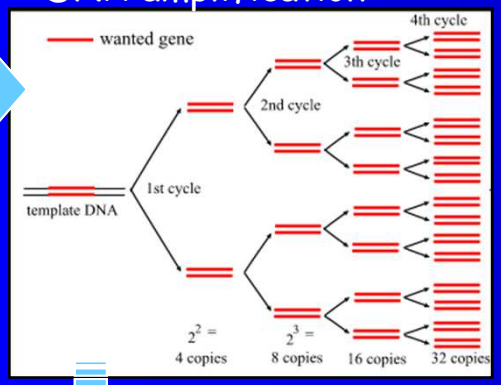
How is genetic analysis perfomed?

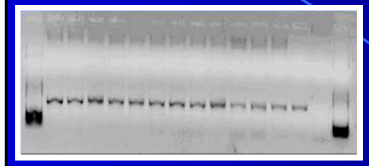
1. Patient referral for MEN or family history of MEN
2. History – evaluate family history
3. Clinical examination
4. Informed consent signature
5. Blood withdrawal (no fasting needed)
6. Sample sent to the Lab

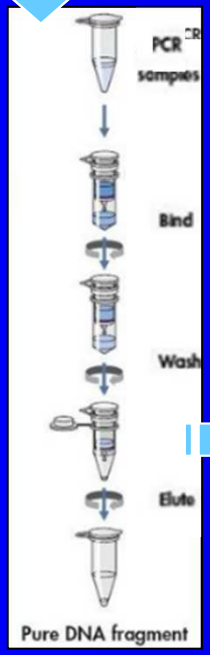
FAMILIAL FORMS of PITUITARY ADENOMA

DNA isolation

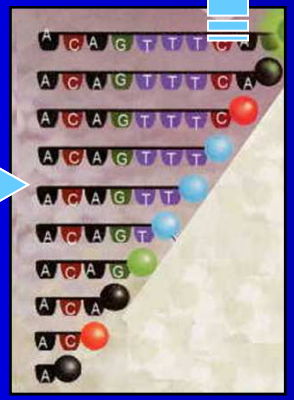
DNA amplification







purification



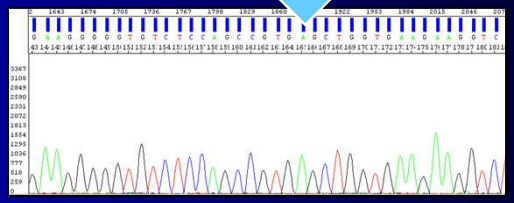
Sequencing reaction

purification

denaturation

GENOMIC DNA
DIRECT SEQUENCING

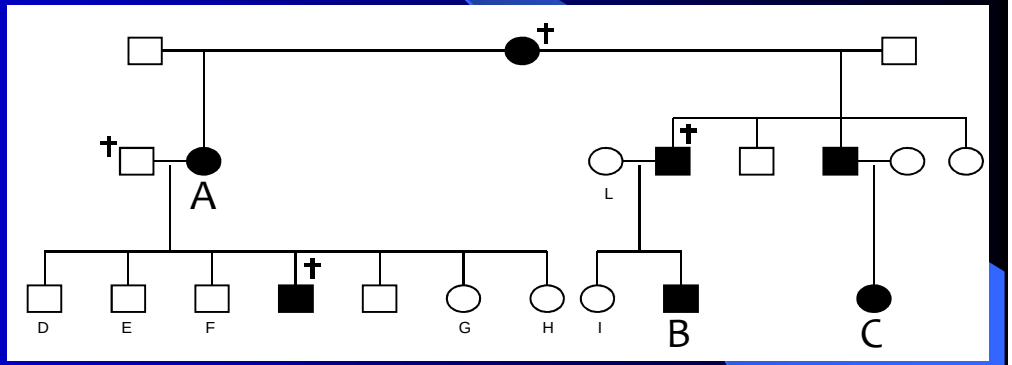
Capillary electrophoresis



FAMILIAL FORMS of PITUITARY ADENOMA

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

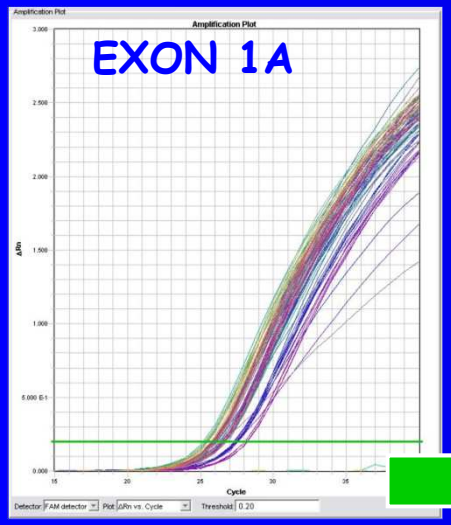
PATIENTS

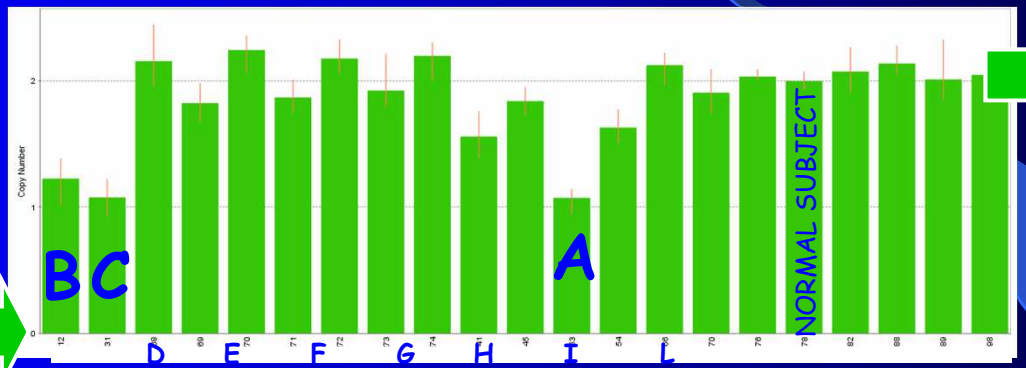


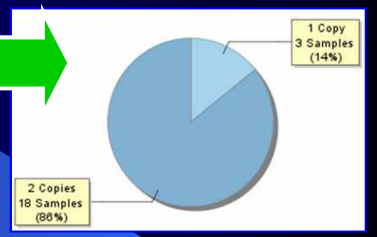
21 family members
7 affected

No SNP mutations

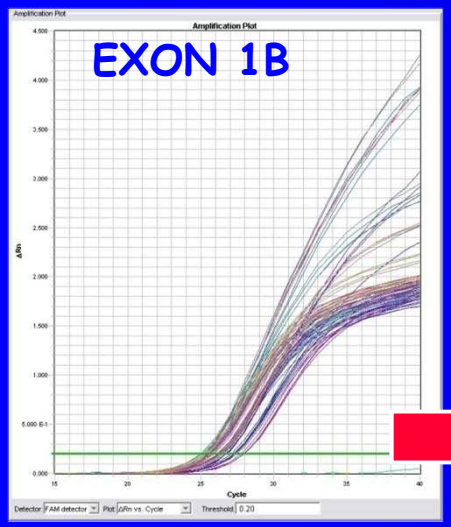
FAMILIAL FORMS of PITUITARY ADENOMA

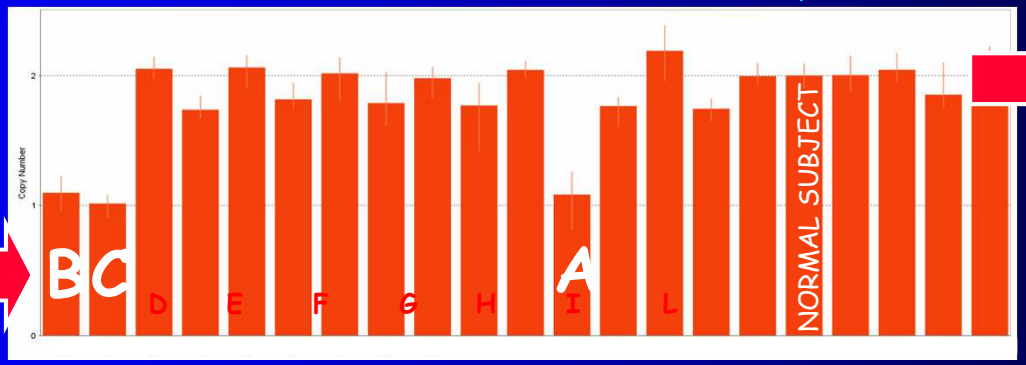


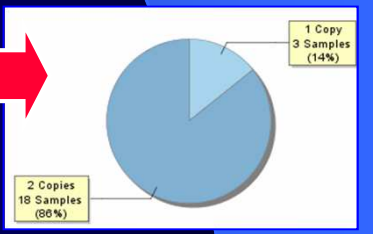




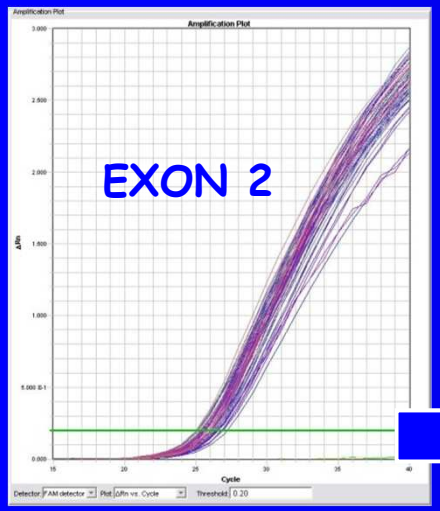
CALIBRATOR

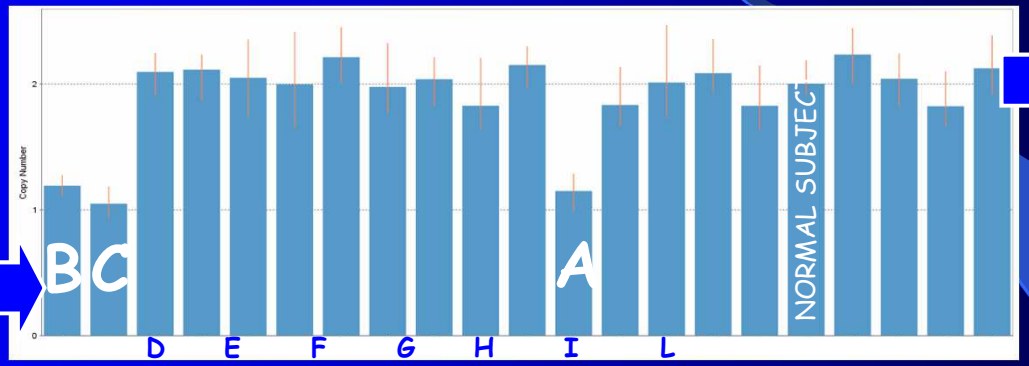


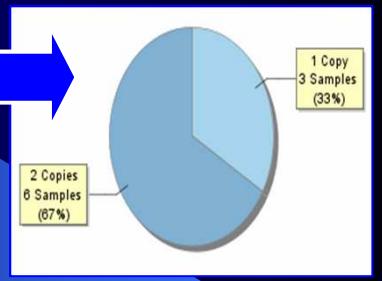




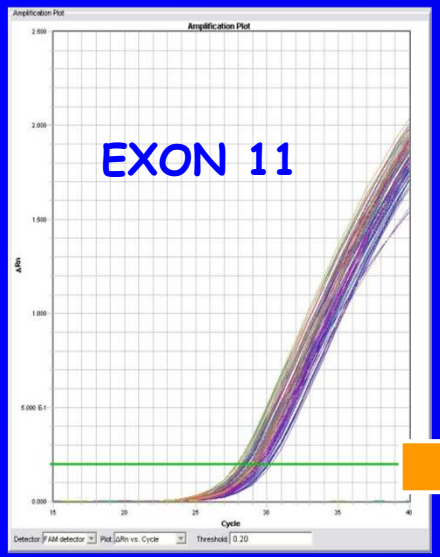
FAMILIAL FORMS of PITUITARY ADENOMA

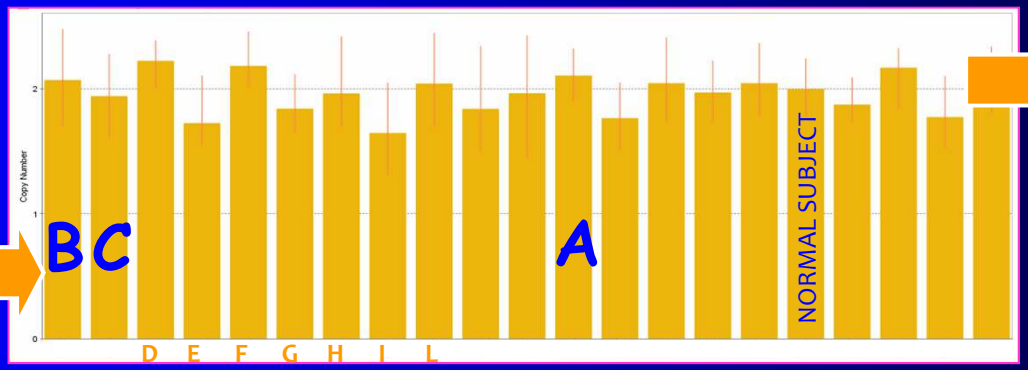


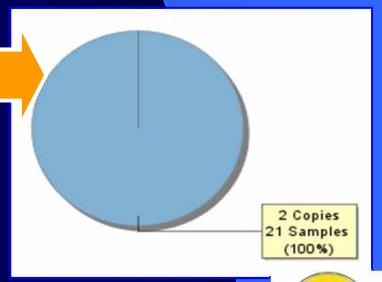




CALIBRATOR



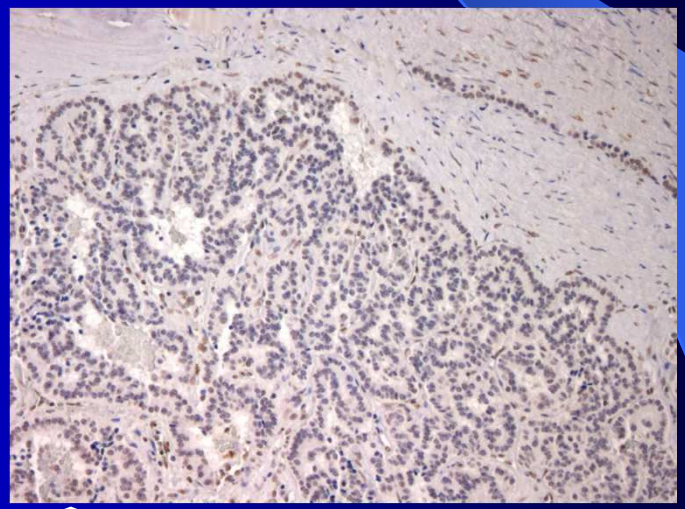




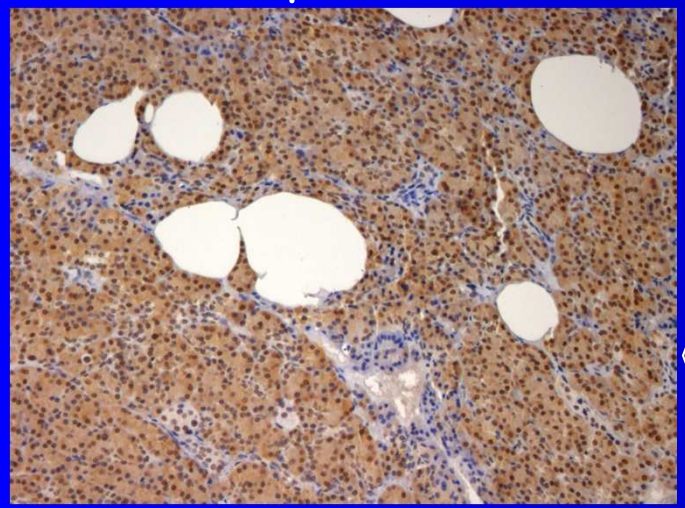
FAMILIAL FORMS of PITUITARY ADENOMA

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



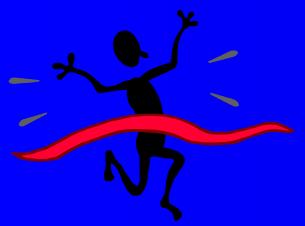
immunostaining for menin

FAMILIAL FORMS of PITUITARY ADENOMA

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



FAMILIAL FORMS of PITUITARY ADENOMA

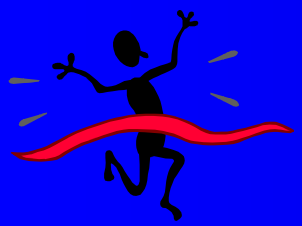
MEN 1

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



FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

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.

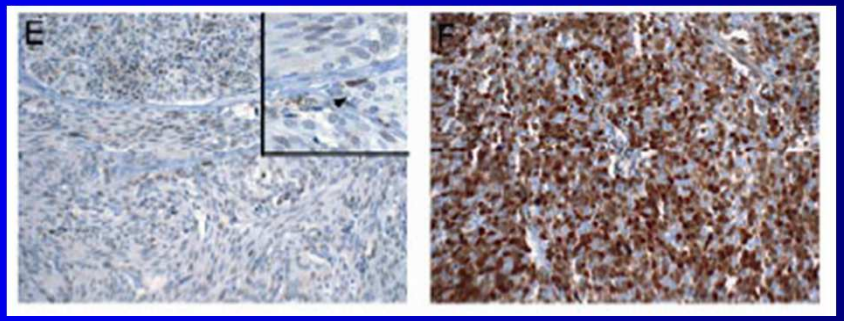
Marinoni et al. Neuroendocrinology 2011;93:19–28

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

MEN 4

p27 is a new tumor susceptibility gene for multiple neuroendocrine tumors

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

FAMILIAL FORMS of PITUITARY ADENOMA

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

FAMILIAL FORMS of PITUITARY ADENOMA

FAMILIAL ACROMEGALY

FAMILIAL FORMS of PITUITARY ADENOMA

FAMILIAL ACROMEGALY

Definition

occurrence of at least 2 cases of acromegaly or gigantism in a family that does not exhibit Carney complex or MEN-1

Frohman et al. 2004 Growth Hormone & IGF Research 14: S90–S96

Epidemiology

Reports of families with multiple affected members date back more than 100 years

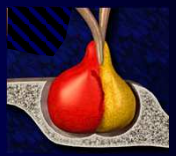
Schwoner J 1897 Ueber hereditare Akromegalie. Z Klin Med 32:202–210

Since the availability of GH measurements, reports of 38 families with 90 affected members have been published

Frohman et al. 2004 Growth Hormone & IGF Research 14: S90–S96

FAMILIAL FORMS of PITUITARY ADENOMA

FAMILIAL ACROMEGALY

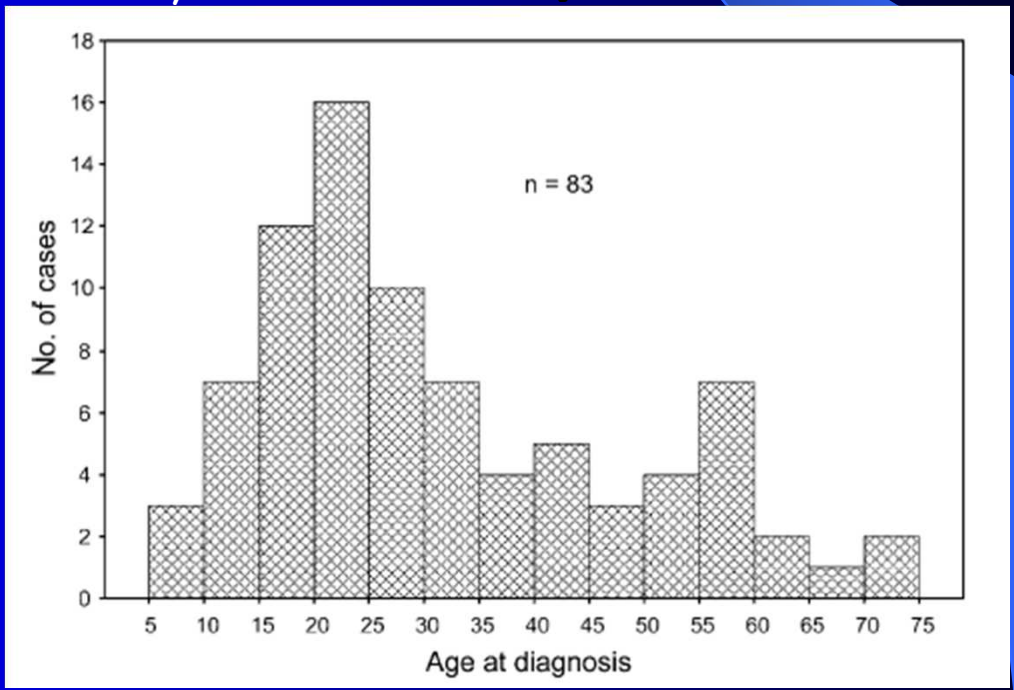


Clinical features

38 families

30 contained two affected members,
6 contained three members
1 contained four members
1 contained seven members

median age at diagnosis = 25 years (age of onset < 30 years in 64%)



Frohman et al. 2004 Growth Hormone & IGF Research 14: S90–S96

FAMILIAL FORMS of PITUITARY ADENOMA

FAMILIAL ACROMEGALY

Clinical features

In more than half of FA families the disease is not transmitted to a succeeding generation by an affected individual, probably because of the early onset of the disorder and the moderately aggressive behavior of the pituitary tumor, resulting in loss of gonadotrope function and therefore, reproductive potential, early in life

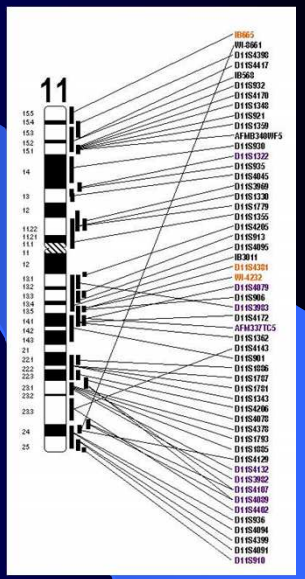
Frohman et al. 2004 GH & IGF Res 14: S90–S96

FAMILIAL FORMS of PITUITARY ADENOMA

FAMILIAL ACROMEGALY

Etiology

Gene located in a candidate region of ~10 Mb in close proximity but distinct from the MEN-1 gene



Patients exhibit absence of MEN1 germ-line mutations

Teh BT et al 1998 J Clin Endocrinol Metab 83:2621–2626

and LOH on chromosome 11q13

Gadelha MR et al 1999 J Clin Endocrinol Metab 84: 249–256

No mutations in GNAS1 or GHRH receptor genes

Jorge BH et al 2001 J Clin Endocrinol Metab 86: 542–544

Possible multiple genetic defects

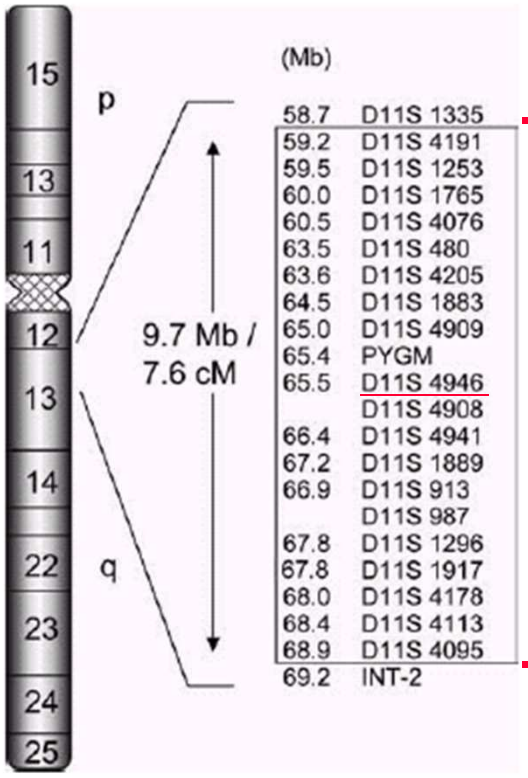
Frohman et al. 2004 Growth Hormone & IGF Research 14: S90–S96

FAMILIAL FORMS of PITUITARY ADENOMA

FAMILIAL ACROMEGALY

Genetic findings

chromosome 11



Position of markers used for meiotic recombination events and tumor deletion mapping.
The FA tumor suppressor gene candidate interval is located between markers D11S1335 and INT-2, a region of approximately 9.7 Mb/7.6 cM at 11q13.1-13.3

Marker D11S4946 is intragenic for the MEN-1 gene

Frohman et al. 2004 GH & IGF Res 14: S90–S96

FAMILIAL FORMS of PITUITARY ADENOMA

FAMILIAL ACROMEGALY

Genetic findings

CNC locus on chomosome 17q is NOT involved

suggestive linkage of chromosome 2p in one family

The gene involved in FA is still unknown

FAMILIAL FORMS of PITUITARY ADENOMA

Pituitary adenomas can occur in a familial setting in the absence of MEN1 and CNC

familial isolated pituitary adenomas (FIPA)

15% of FIPA cases display AIP mutations

AIP

Aryl Hydrocarbon Receptor Interacting Protein

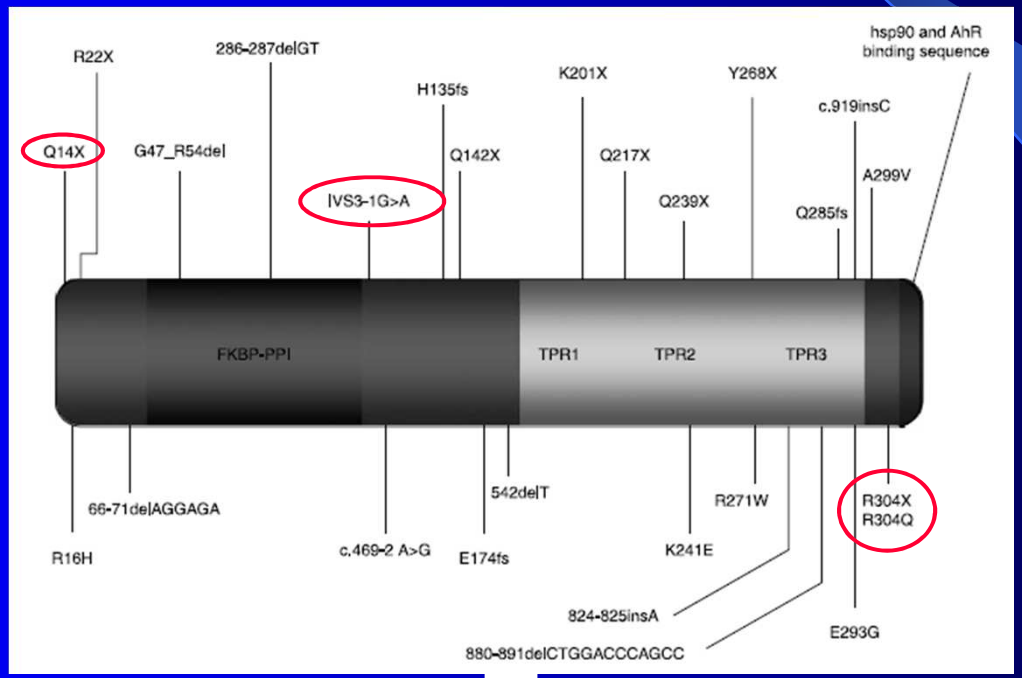
- 330 AA protein
- Encoded on chromosome 11q13.3
- Interacts with aryl hydrocarbon receptor and with heat shock 90 protein dimer
- Mediates cellular responses to environmental toxins
- Binds to and attenuates the activity of PDE4A5
- Binds to PPARα

Beckers A et al. Eur J Endocrinol. 2007 ,157:371-82

FAMILIAL FORMS of PITUITARY ADENOMA

New candidate gene for familial pituitary adenomas

Pituitary tumors display AIP LOH



protein inactivation

FAMILIAL FORMS of PITUITARY ADENOMA

New candidate gene for familial pituitary adenomas

Q14X
IVS3-1G>A } Finnish cohort

R304X } Italian family

No mutations { Turkish kindreds
German kindreds

Vierimaa et al. Science 2006, 312:1228–1230

not found in sporadic and familial adenomas from → Europe
→ Japan
→ U.S.
→ Canada

Yu et al. J Clin Endocrinol Metab 2006, 91: 5126–5129.

Barlier et al. J Clin Endocrinol Metab 2007, 92: 1952–1955.

Iwata et al. T, Clin Endocrinol 2007, 66: 499–502.

Toledo et al. J Clin Endocrinol Metab 2007, 92: 1934–1937

Digiovanni R et al. : Endocr Pathol. 2007,18:76-8

FAMILIAL FORMS of PITUITARY ADENOMA

315 patients → 10 patients → 8 patients

No MEN1 mutations
No CNC

Patient#	Sex	Age	Diagnosis
1	F	61	NFA
2	F	55	NFA
3	F	63	NFA
4	M	54	NFA
5	M	41	GH
6	F	66	NFA
7	M	31	GH
8	M	74	NFA

4 F, 4 M
Mean age= 55.6 ± 5.3 yr
Median age = 58yr
6 NFA, 2 GH-omas

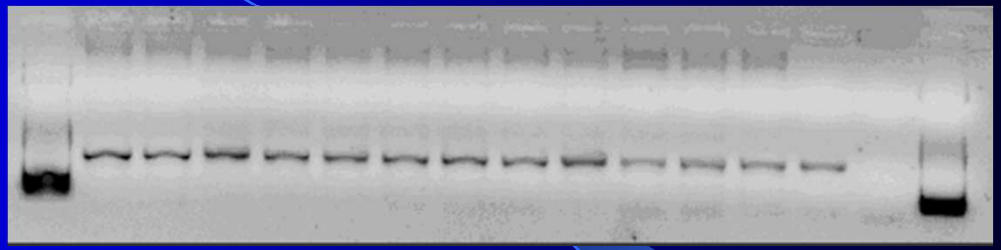
↓ Blood withdrawal

↓ Genomic DNA isolation

↓ Specific PCR

FAMILIAL FORMS of PITUITARY ADENOMA

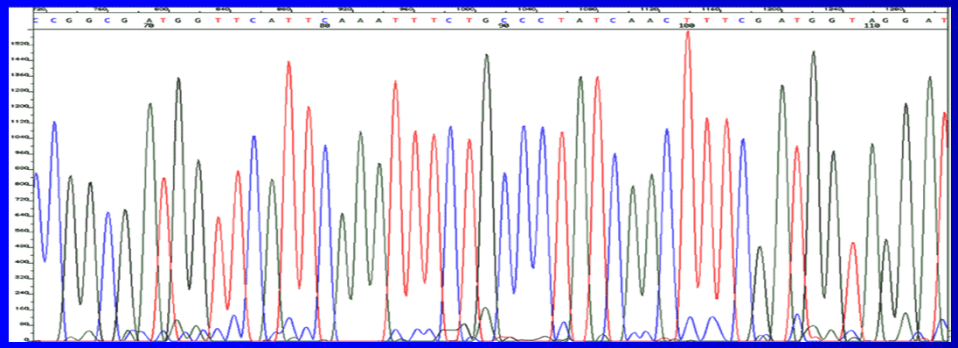
PCR product purification



Sequencing reaction

Loading on automated Genetic Analyzer

Analysis of the electropherograms

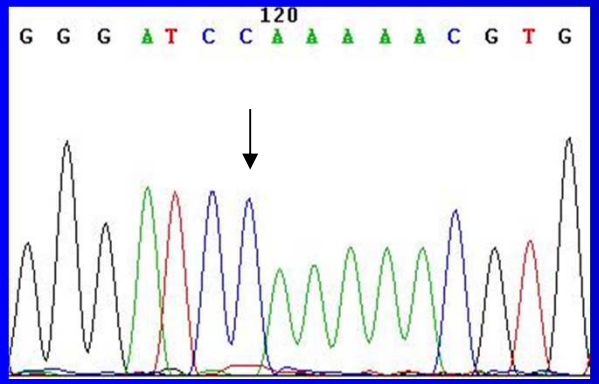


FAMILIAL FORMS of PITUITARY ADENOMA

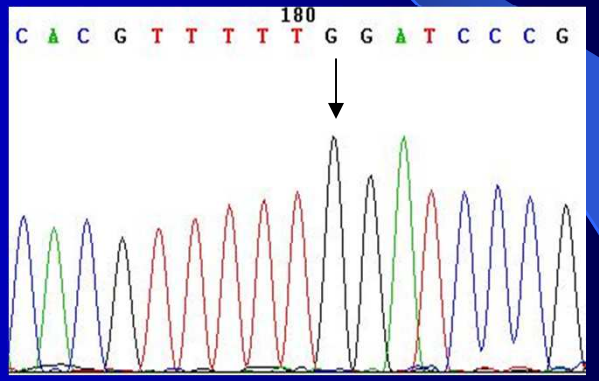
No Q14X and IVS3-1G>A mutations were detected in the examined samples

Q14X (exon 1)

forward

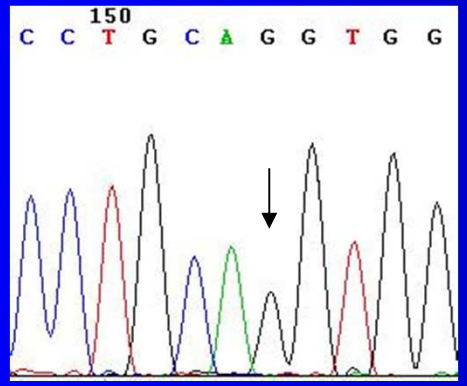


reverse

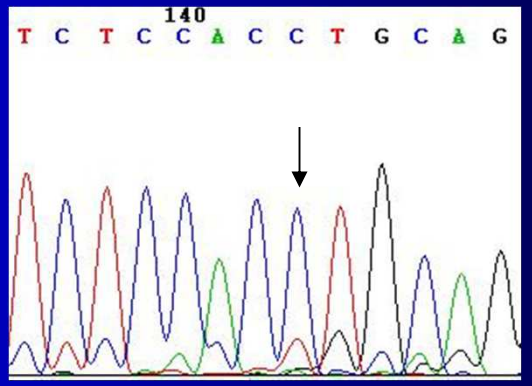


IVS3-1G>A (exon 4 splice acceptor site)

forward



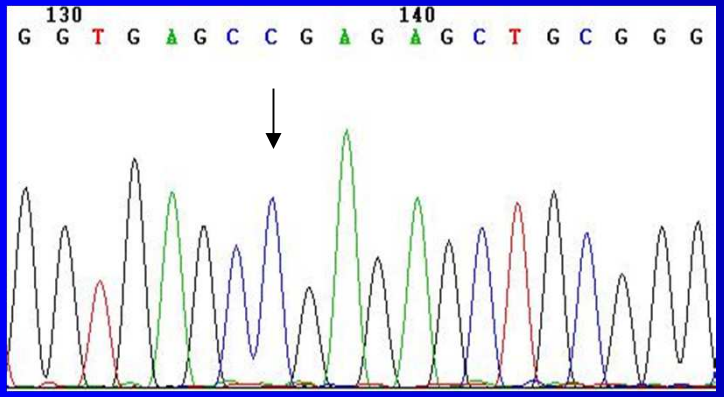
reverse

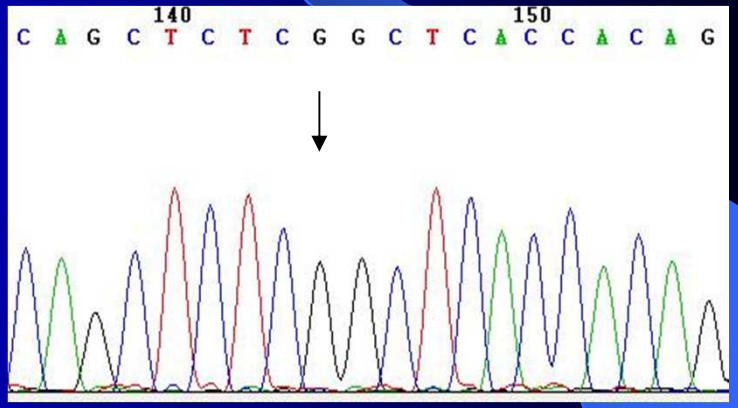


FAMILIAL FORMS of PITUITARY ADENOMA

No R304X mutations were detected
in the examined samples

R304X
(exon 6)





FAMILIAL FORMS of PITUITARY ADENOMA

CONCLUSIONS

In our population of patients with
familial pituitary adenomas
we did not find any of the reported AIP mutations

suggesting that

1) mutation frequence in our population is very low

2) AIP mutations do not predispose to the development of pituitary adenomas in our population