

Quels marqueurs génétiques dans les maladies du pancréas ?

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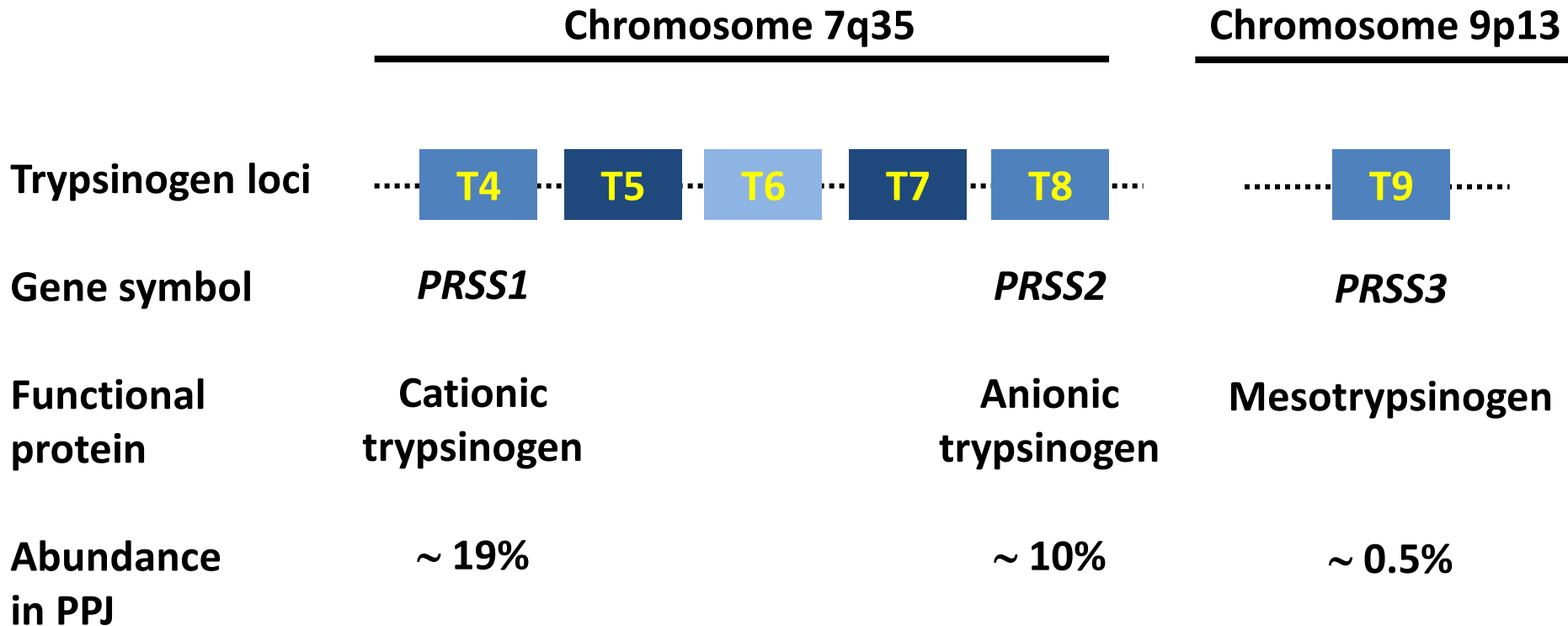
Hôpital Erasme - Bruxelles



Genetic markers in pancreatic diseases

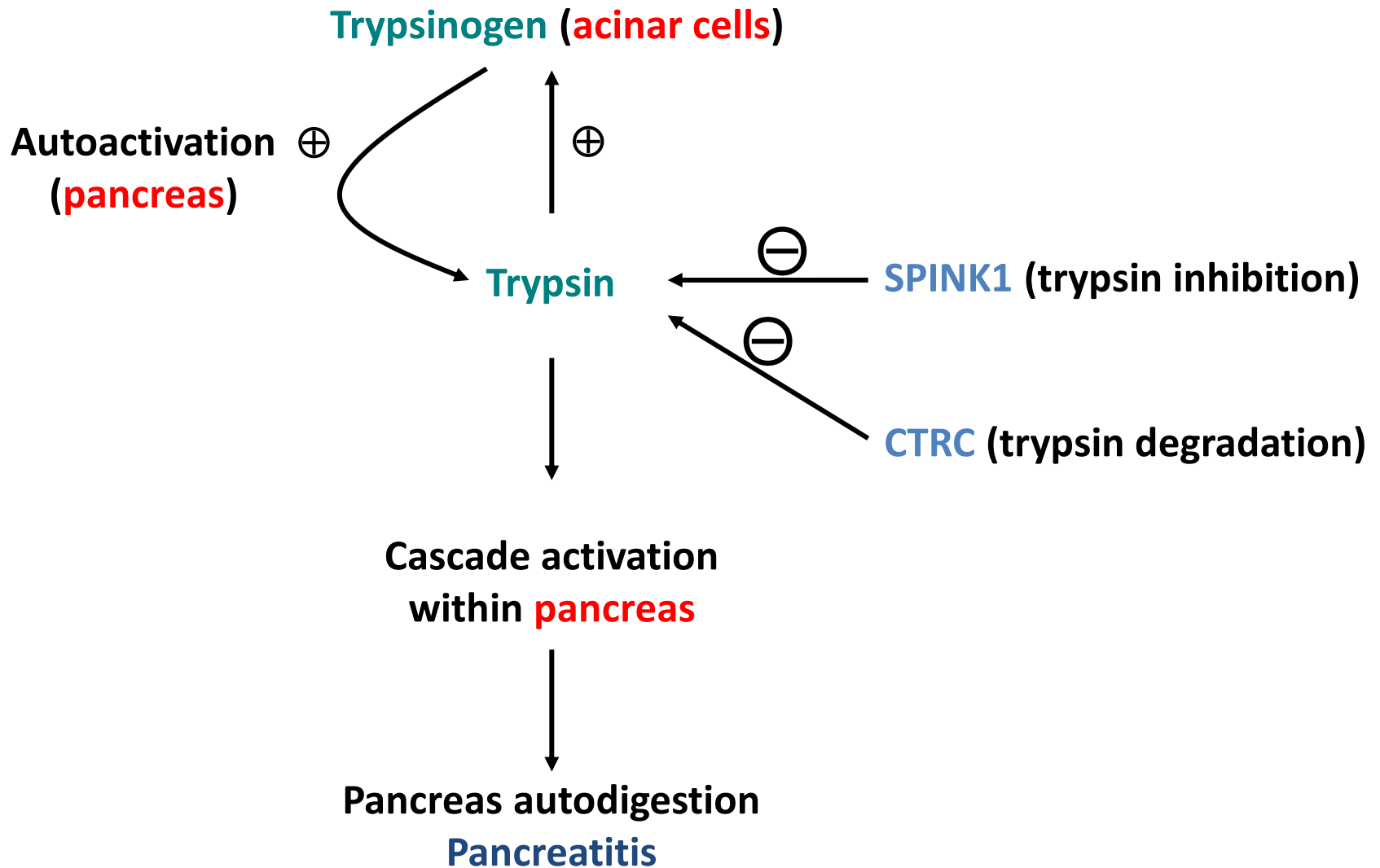
- **Pancreatitis (acute, RAP, CP)**
- **Pancreatic carcinoma**

The trypsinogen cascade

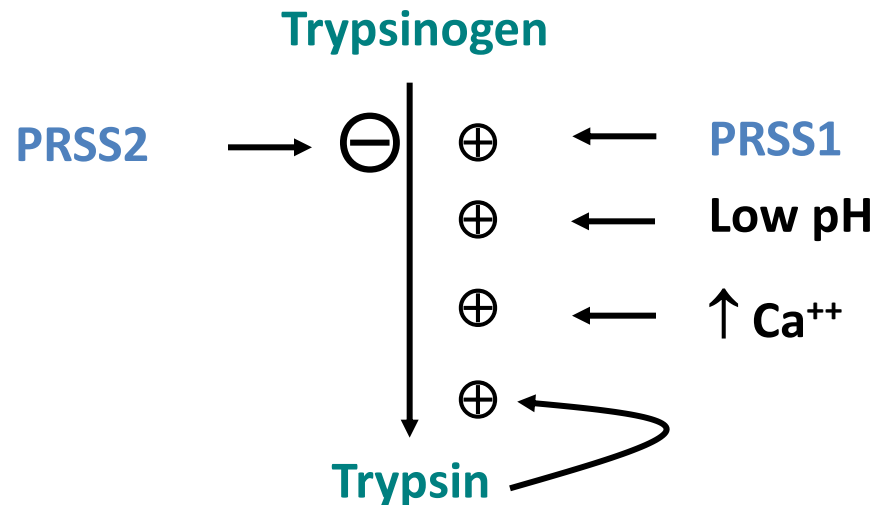


→ ~ 30% of human pancreatic secretory proteins

The trypsinogen cascade



Genetic factors influencing trypsinogen activation



Trypsinogen genes (high-penetrance genes)

– gain-of-function mutations in the PRSS1 gene

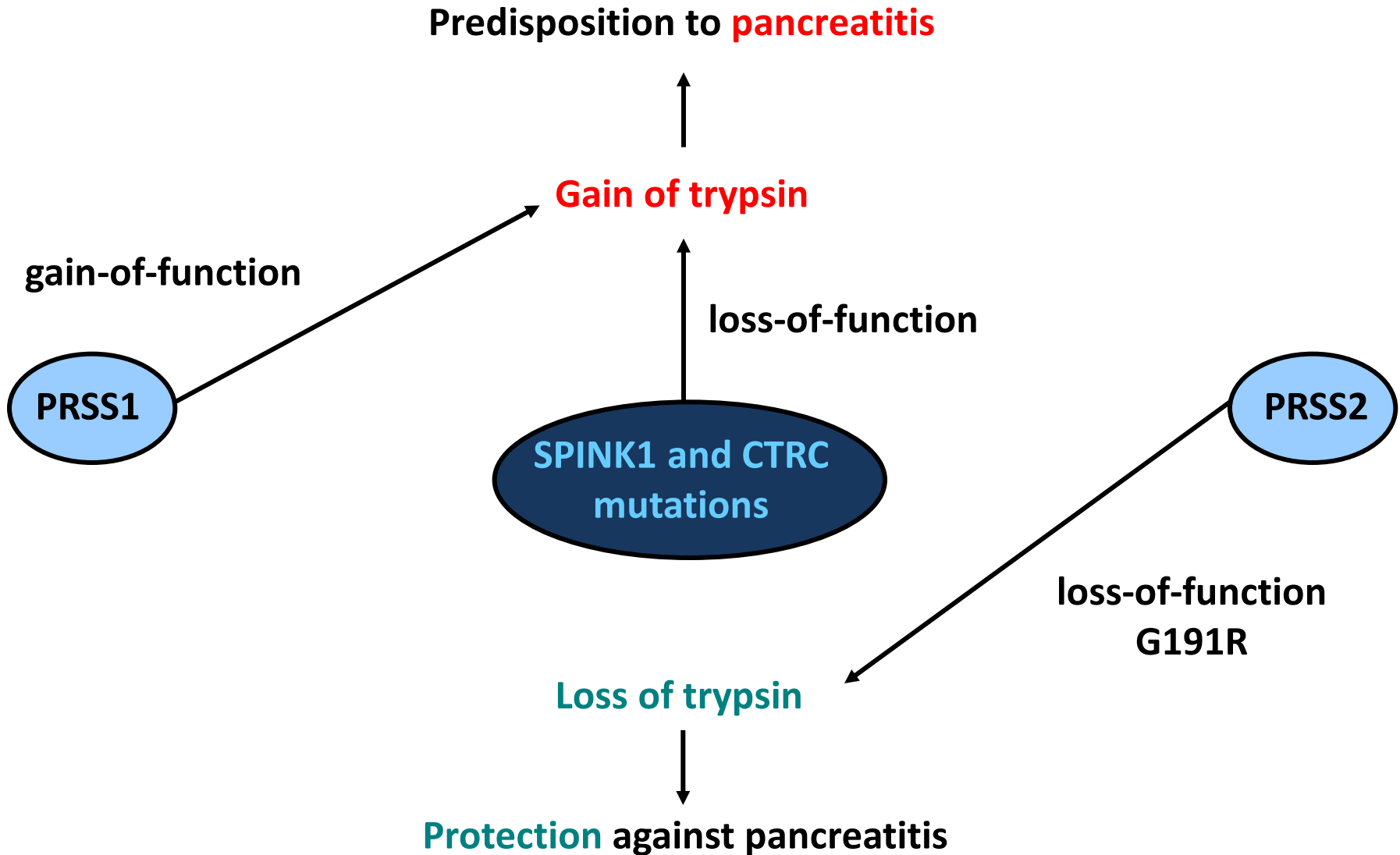
ex : R112H, N29I, A16V

– loss-of-function mutation in the PRSS2 gene

ex : G191R (→ new trypsin cleavage site → trypsin degradation)

	CP	Controls	
G191R	0.7 – 1.3%	3.4 – 5.4%	p=0.01
→ protection against CP			

Genetic factors influencing trypsinogen inactivation



Genetic factors influencing trypsinogen inactivation

SPINK1 (Serine protease inhibitor Kazal 1) (intermediate penetrance gene)

↳ *PSTI*

- acute phase protein, expressed in acinar cells
- specific trypsin inhibitor (~ 20% of potential trypsin)

		CP	Controls
N34S	heterozygosity	12.6%	1.9%
	homozygosity	3.6%	0%

- phenotype independent of genotype
 - no effect on protein expression / function
- pathogenic role?

Genetic factors influencing trypsinogen inactivation

CTRC

(chymotrypsinogen C) (low-penetrance gene)

↳ *Chymotrypsin C*

- produced by acinar cells
- degradation of prematurely activated trypsin
- 2nd line of defense

variants → loss-of-function

ex : R254 W → ↑ risk of CP x 5

Genetic factors influencing trypsinogen inactivation

CASR (calcium-sensing receptor) (low-risk susceptibility gene)

- membrane-bound G protein-coupled receptor
- role in Ca^{++} homeostasis
- expressed in human pancreatic acinar and ductal cells

	in acinar cells / MPD	
	$\uparrow\uparrow \text{Ca}^{++}$	$\downarrow\downarrow \text{Ca}^{++}$
trypsinogen activation	\uparrow	\downarrow
trypsin inactivation	\downarrow	\uparrow

ex : Mutation R990G associated with CP: OR 2 p=0.015

Overall effect small

Clinically significant in presence of additional risk factors

Genetic factors influencing trypsinogen inactivation

CFTR (Cystic Fibrosis Transmembrane conductance Regulator)

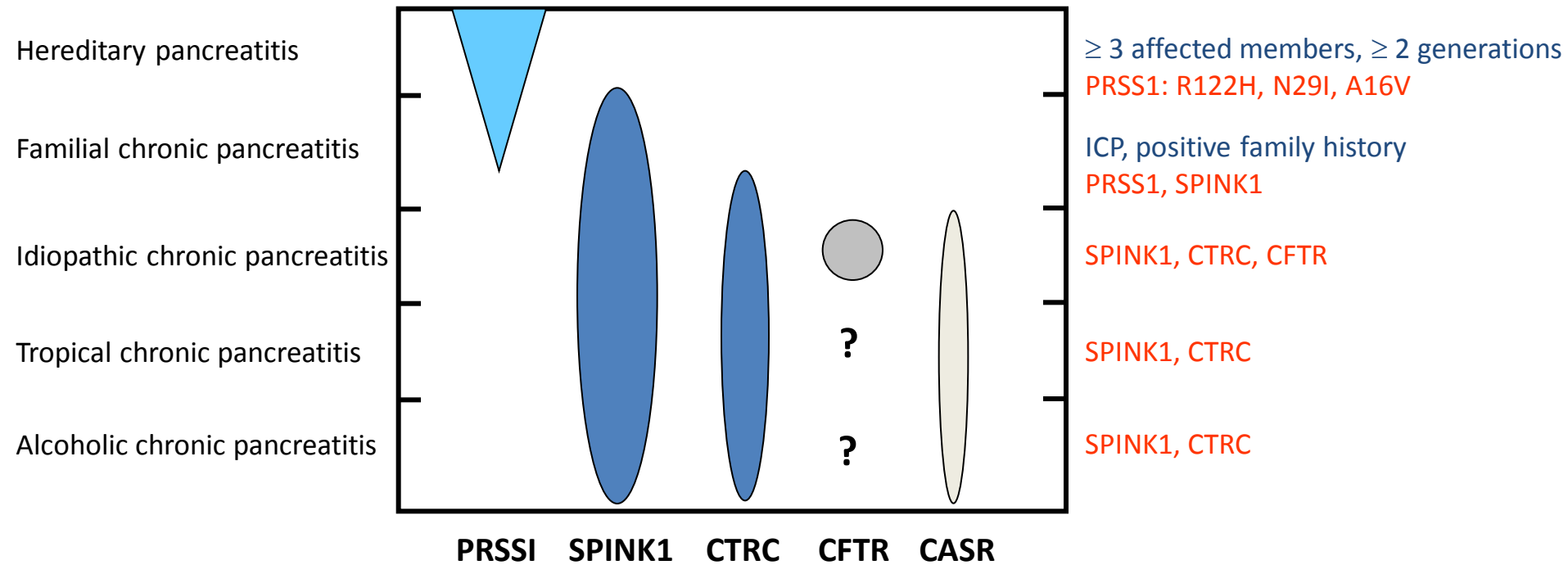
(= disease susceptibility gene)

- cAMP-activated Cl channel
- extra-acinar cell mechanism of trypsin elimination

Mutations / polymorphisms → ↓ trypsinogen/trypsin wash-out

Genotype CFTR mutations	Functional CFTR protein	Phenotype
Maj / Maj	< 1%	Typical cystic fibrosis CF + pancreatic sufficiency Atypical CF N
Min / Min	5%	
- / Maj	10%	
- / Min	50%	
- / -	100%	

CP-associated genes in chronic pancreatitis



Gene-environment interactions in CP

Majority of alcoholics → no CP

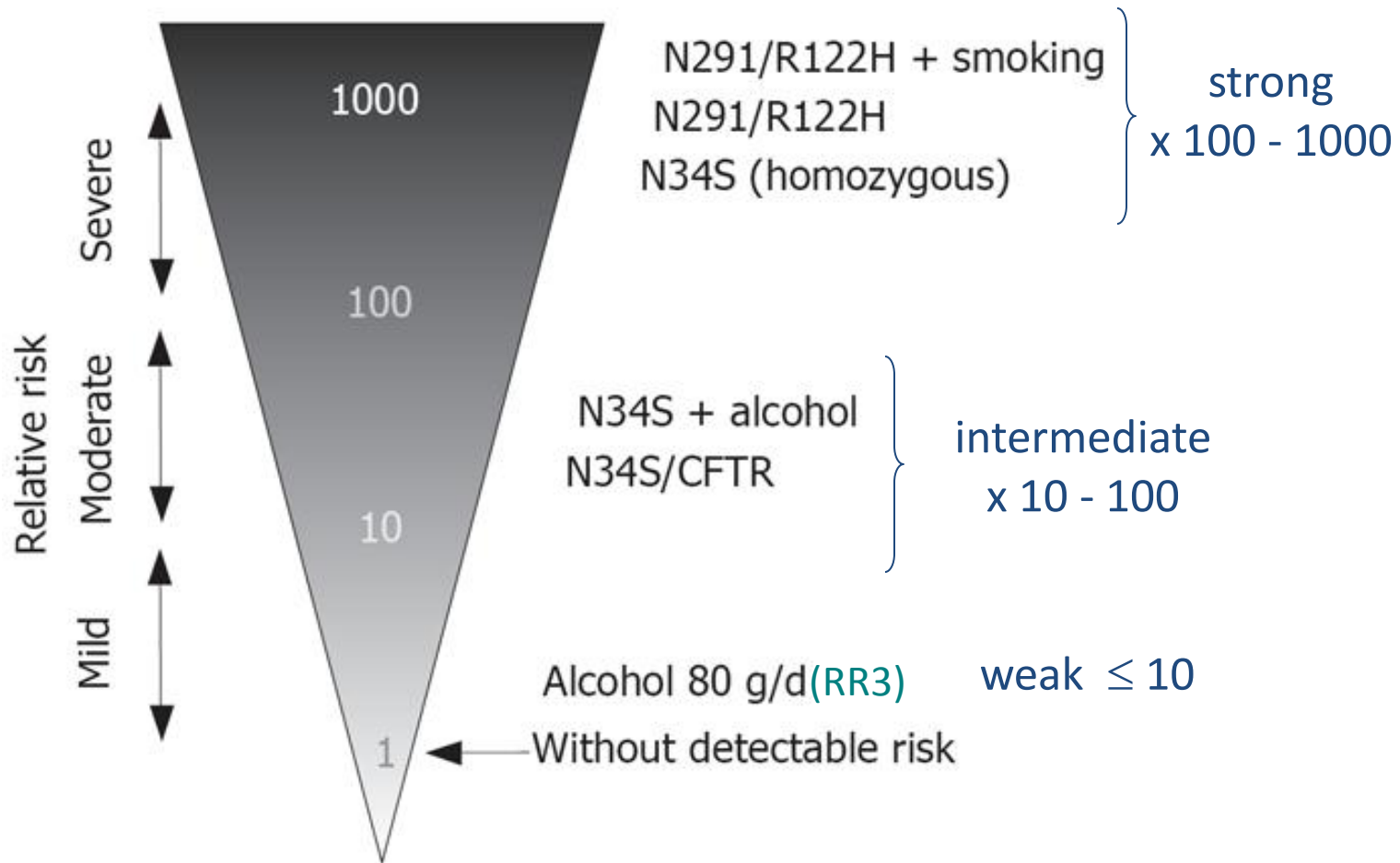
protective genetic factors

- loss-of-function mutations in PRSS1?
- PRSS2 G191R variant

predisposing genetic factors

	ACP	Alcoholics without CP	Controls
• SPINK1 N34S	n = 274 5.8%	n = 98 1%	n = 540 0.8%
<i>Witt H, JAMA 2001</i>			
• CTRC R254W	n = 348 2.9%	n = 422 ALD 0.7%	
<i>Rosendahl J, Nat Genet 2008</i>			
• CFTR mutations (NS)	8.9%	3%	3.2%
<i>Perri F, Eur J Hum Genet 2003</i>			

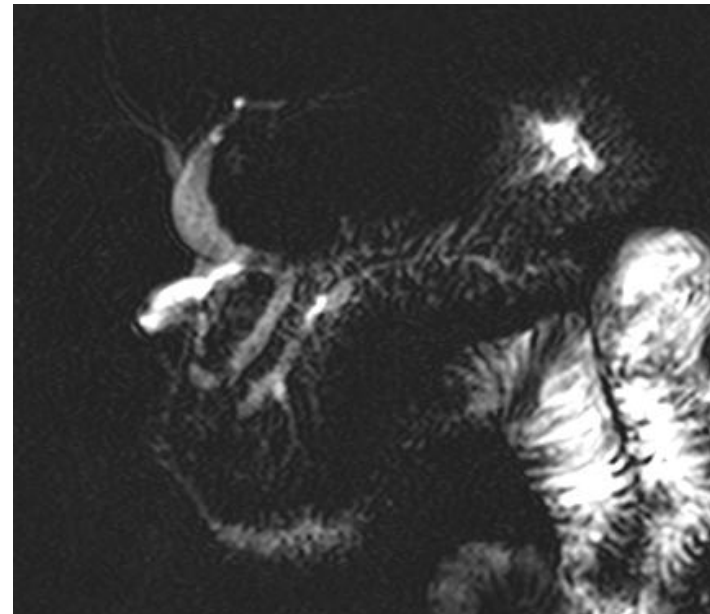
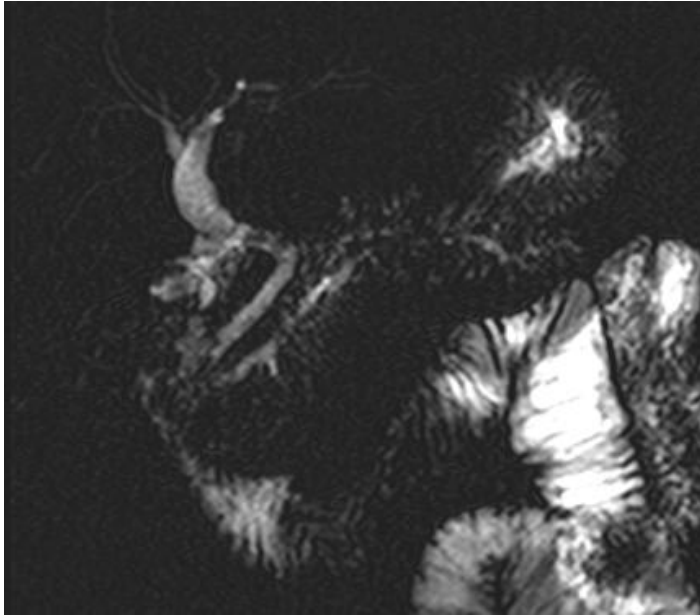
Strength of genetic and environmental risk factors of CP



Genetic markers in different groups of CP

	% OF ALL PATIENTS	% WITH MUTATION		% WITHOUT MUTATION
Alcoholic	75 – 80	SPINK1 (N34S) CFTR	5 10	85
Idiopathic	20	PRSS1 (R122H, N29I) (A16V) SPINK1 (N34S) CFTR	<< 1 < 5 20 – 40 20 – 40	15 – 50
Familial	3	PRSS1 (R122H, N29I) (A16V) SPINK1 (N34S) (other)	15 < 5 30 5	50
Hereditary	1	PRSS1 (R122H, N29I) (other)	70 – 80 10 – 20	10 – 20

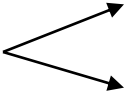
Chronic pancreatitis



Genetic markers of sentinel AP vs RAP

	Sentinel AP n = 116	RAP n = 72	Controls n = 670
SPINK1 (N34S)	0.9%	11.1%	2.8%
		OR 19.1	

→ **SPINK1 N34S** not associated with ↑ risk of sentinel AP
↑ risk of RAP

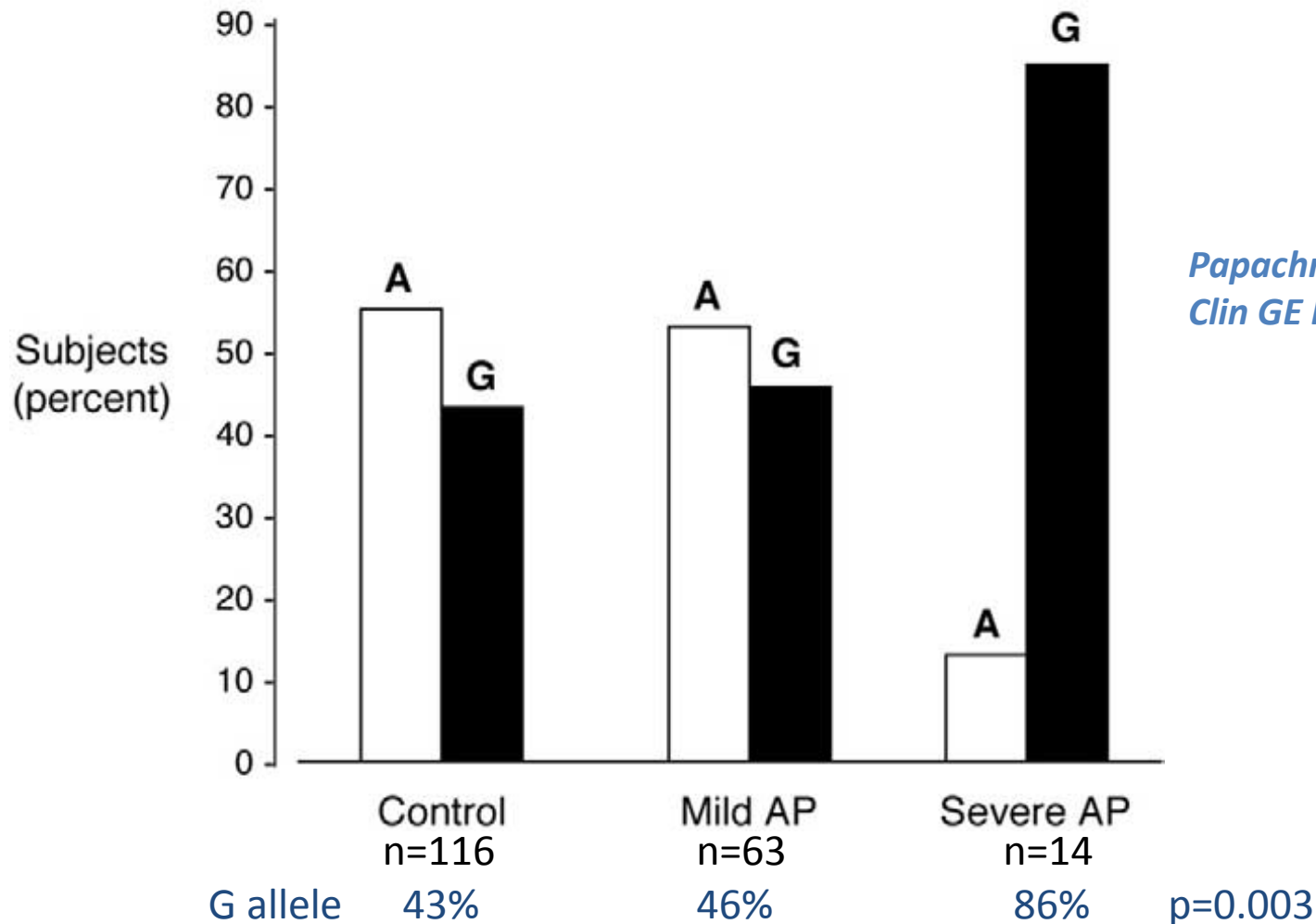
initial attack of AP  activate immune system
upregulate SPINK1 expression → protection
against RAP

Genetic testing of initial attack of AP of unknown origin

→ identification of patients with additional genetic risk factor for RAP¹⁷

Single nucleotide polymorphism in Monocyte Chemotactic Protein-1 at position -2518 A/G

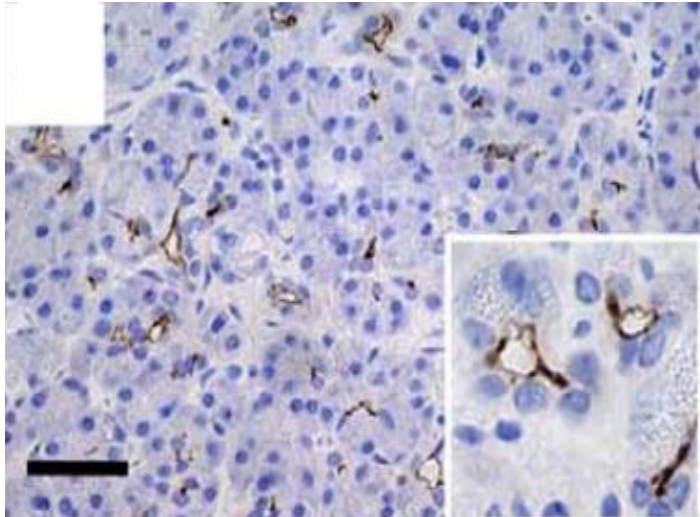
Genotypes: A only (A/A) vs G (A/G or G/G)



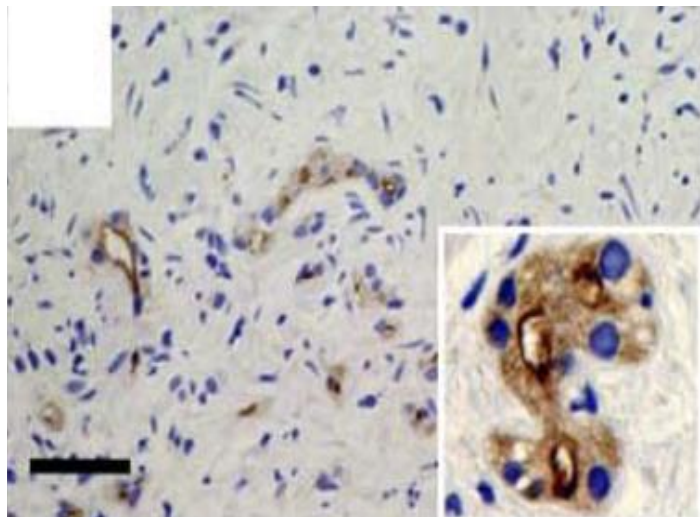
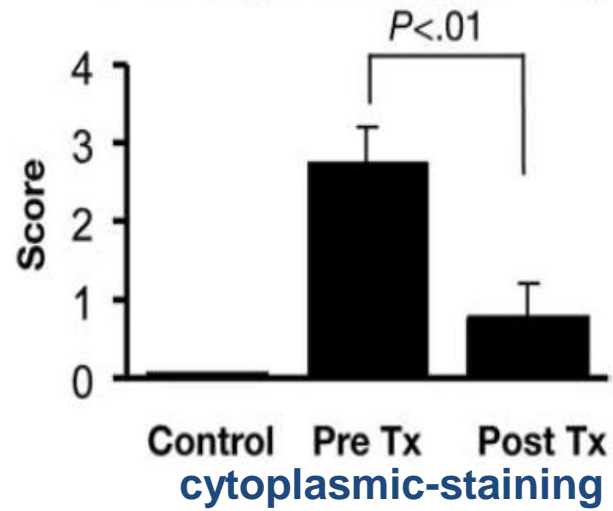
→ presence of G allele (genotypes A/G + G/G) predisposes to SAP (OR 7.8) ¹⁸

Effects of steroids on the pancreas in AIP

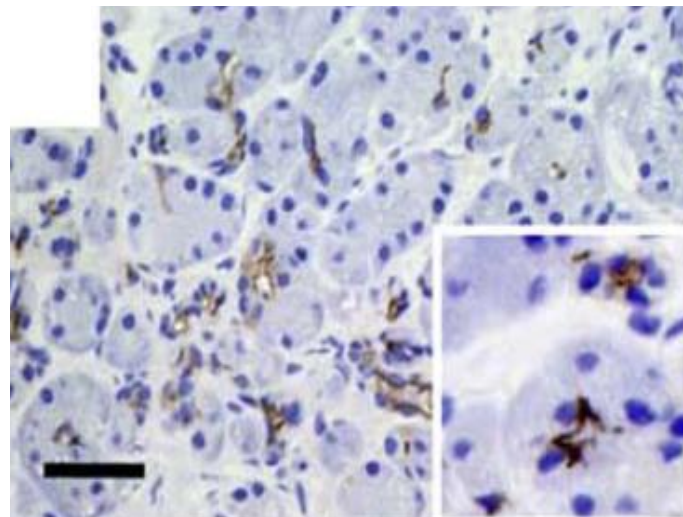
Normal



Histologic scoring (CFTR)

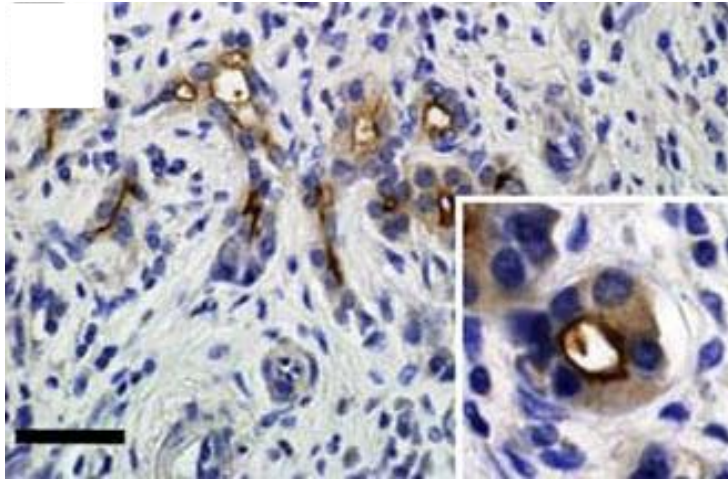


Before treatment

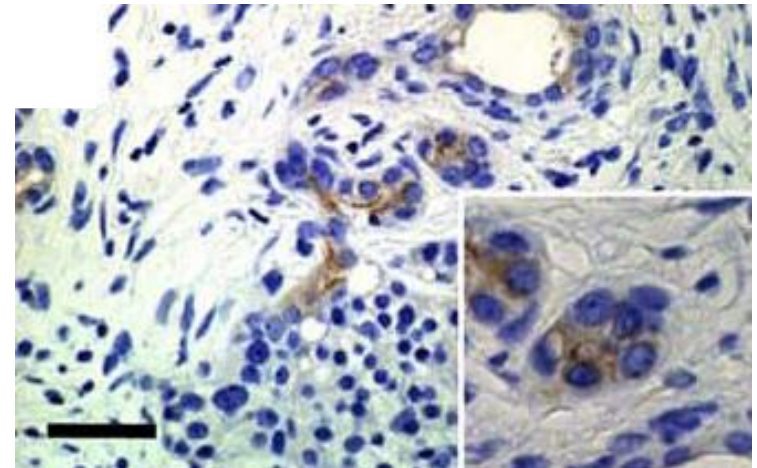


After treatment

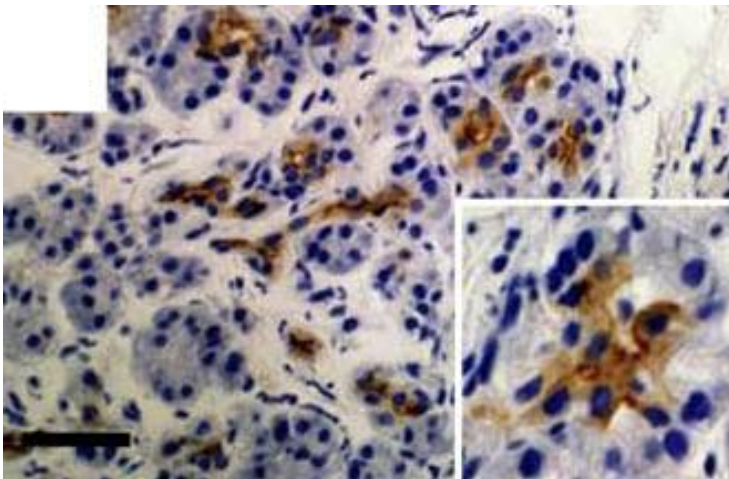
CFTR mislocalization in pancreatitis



Alcoholic CP n = 6

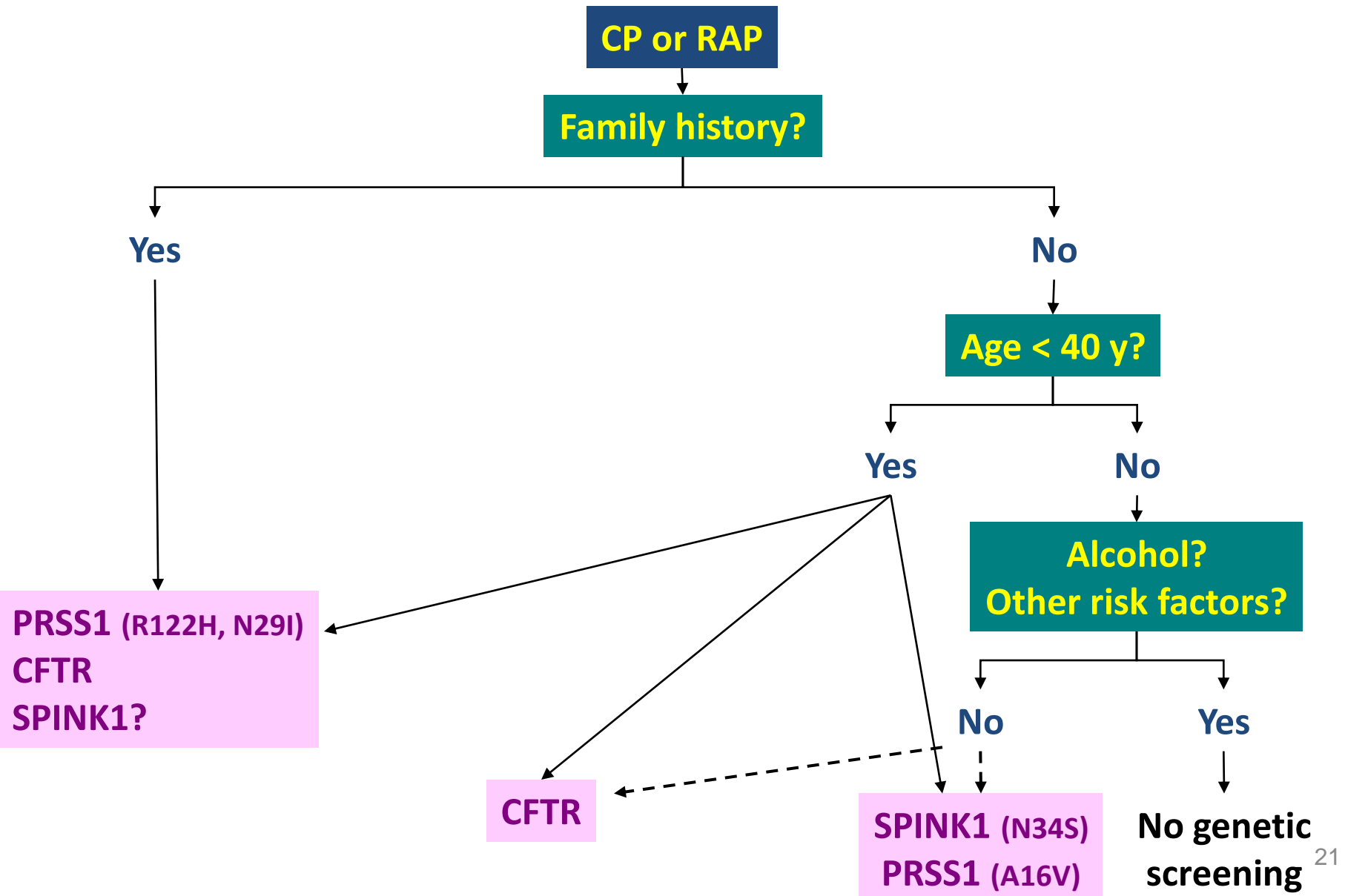


Obstructive CP n = 2

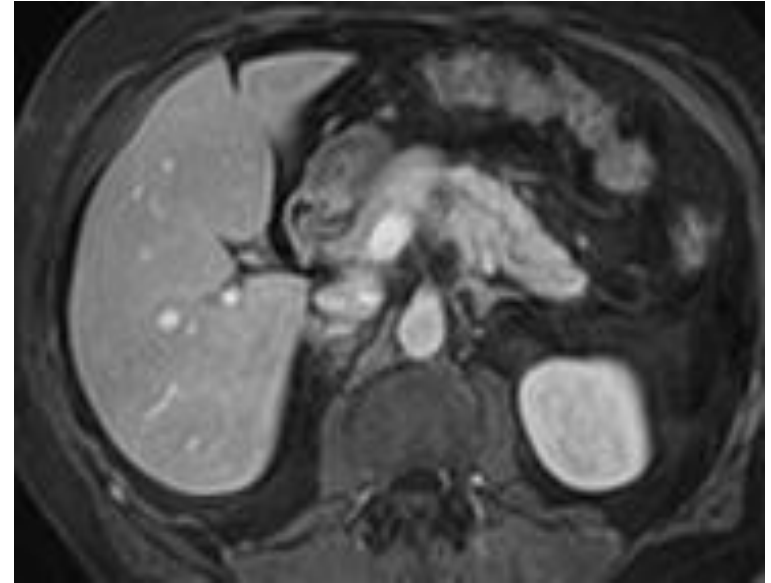
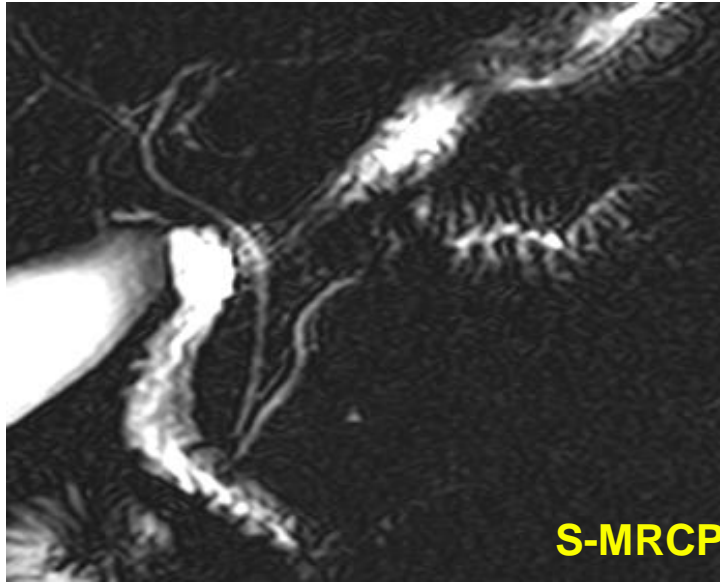


Idiopathic CP n = 3

Genetic screening in pancreatitis



Pancreatic carcinoma



Prevalence: 8 – 12/100,000

If RR = 2 → risk ~ 20/100,000 = 0.02%

Lifetime risk for breast carcinoma = 12.7%

Clinical risk factors

- Diabetes > 5 y RR x 2
new onset within past 3 y OR: 7.94
- Smoking
 - current smokers RR x 2.5
 - 2 y after quitting smoking RR ↓ 50%
 - 10 y after quitting smoking Return to baseline
- Advancing age > 40 y

- **Primary screen = identify high risk individuals (RR > 10)**
- **Secondary screen = identify early asymptomatic cancer
→ curative surgery**

Hereditary cancer syndromes associated with increased pancreatic cancer risk

Syndrome	Gene	Estimated RR of pancreatic carcinoma	Lifetime risk (%)
PEUTZ - JEGHERS syndrome (GI polyps & cancers, pigmented lesions, breast, ovarian cancers)	STK11	132	36
HP	PRSS1	20 – 75	40
FAMMM Familial atypical multiple mole melanoma (melanoma, endometrial, breast, lung cancers)	CDK2A/p16	9 – 47	17
HNPCC Hereditary nonpolyposis colorectal cancer (colorectal/extracolonic cancers) Lynch syndrome I / II	MLH1	8.6	
FAP Familial adenomatous polyposis	APC	4.5	
HBOC Breast and ovarian cancer	BRCA1 BRCA2	3 – 10	7

Familial Pancreatic Cancer

- No CP
- No other cancer

Number of affected relatives		RR	Lifetime risk %
≥ 3	1st DR	32	40
2	1st DR	6.4	8 – 12
1	1st DR	4.6	6
≥ 1	2nd DR	1.28	1.7
1	3rd DR	1.09	1.4
1	4th DR	1.06	1.4
General population		1	1.3

Genetic anticipation	G1	G2	G3
Median age of death	70 (59 – 77)	64 (57 – 69)	49 (44 – 56)

- **Very high risk ($RR \geq 10$):** PJS, FAMMM, HP, FPC (≥ 3 FDR)
- **High risk ($RR > 4.5$):** HNPCC, HBOC (BRCA2),
FPC (2 FDR or 1 FDR +
smoking)
- **Intermediate risk ($RR > 3.5$):** FAP, FPC (1 FDR),
non-hereditary CP
- **Low risk ($RR > 2$):** > 7 y CP, HBOC (BRCA1), CFTR carriers,
young onset of CP, diabetes, smoking
- **Likely no risk:** alcohol, coffee

Results of screening for pancreatic cancer in high risk patients

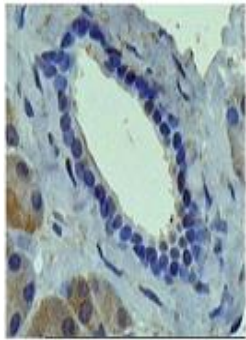
Author	Study population	Screening modality	Number of patients		Findings with final diagnosis
			In study	With findings	
Brentall, 1999	High risk (> 2 cases in 2 generations)	EUS, ERCP, CT, CA 19-9, CEA	14	11	6 with wide spread dysplasia
Canto, 2004	Peutz-Jeghers syndrome, high risk	CT, EUS	78	17	8 Pancreatic adenocarcinoma (n=1), IPMN, PanIN 2-3
Langer, 2009	High risk	MRI, EUS	182	28	7 PanIN 1-2, IPMN, SCN, CP
Verna, 2010	Average to high risk	EUS, ERCP, MRI, CT	51	45	10 Pancreatic adenocarcinoma (n=2), IPMN, PanIN 2, ovarian cancer, carcinoid, thyroid
Ludwig, 2011	High risk	EUS, MRI	109	18	9 Pancreatic adenocarcinoma (n=1), IPMN, PanIN 2-3, CP
TOTAL			434	119	40

→ Diagnostic yield = 27% (17 – 88)

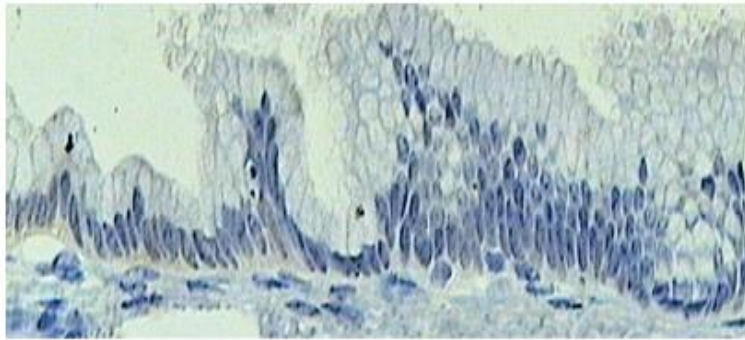
→ Pathologic yield = 9% (4 – 43)

{ diagnosis of an occult cancer is rare
no survival benefit demonstrated

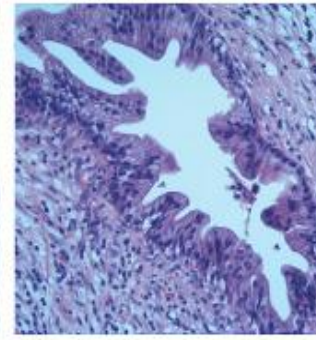
Pancreatic carcinogenesis



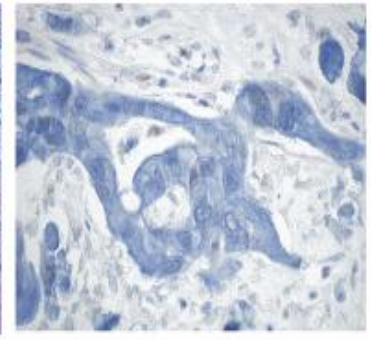
Benign ductal
cells



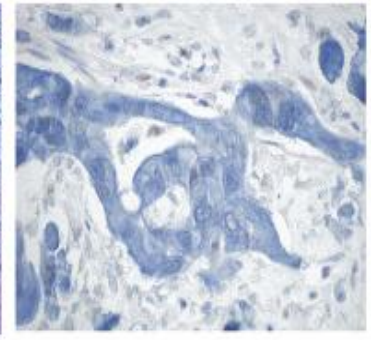
PanIN-1



PanIN-2



PanIN-3



Invasive
carcinoma

- **Genomic alterations**

ex : single point mutation = single base substitution in DNA sequence
K-ras mutation → ↑ mitogenic activity

- **Genetic instability in specific regions encoding microRNAs**

micro RNAs = small non coding RNAs involved in negative regulation of mRNAs translation

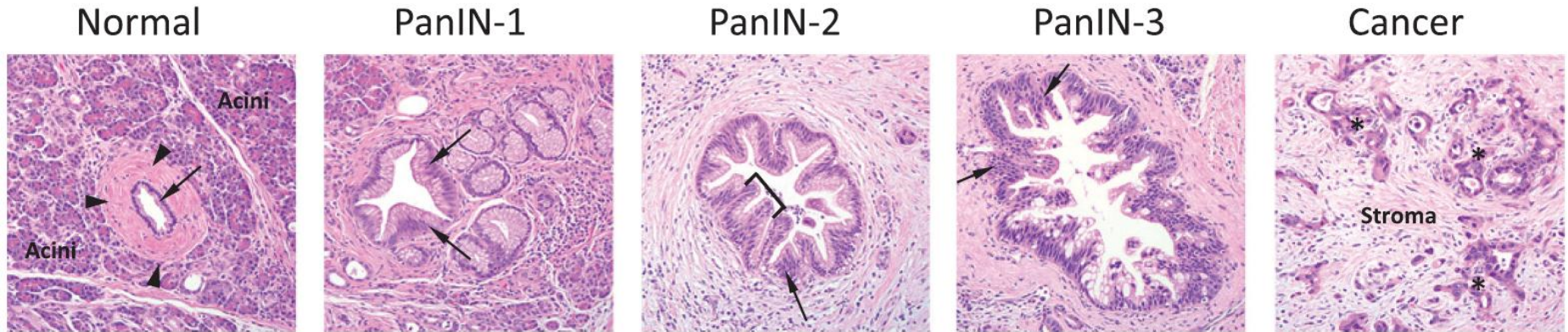
→ oncogene loss of repression

→ tumor suppressor gene downregulation

- **Epigenetic alterations**

ex : DNA methylation → inactivation of tumor suppressor genes

Pancreatic carcinogenesis



early events

Telomere shortening chromosomal breakage / fusion / bridging in dividing cells
 ↓
 gene deletions / amplifications

Kras 2 activating mutations → ↑ cell proliferation / survival

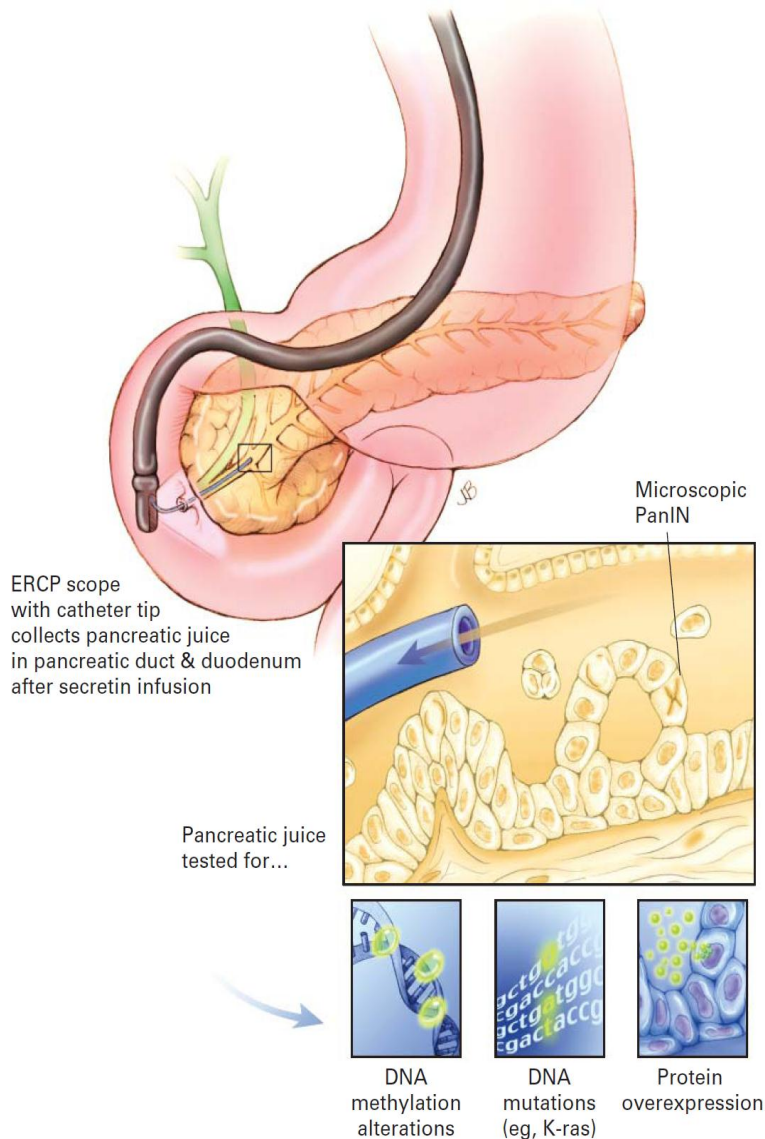
intermediate events

CDKN 2A loss of function → loss of inhibition of cell cycle progression (G1-S)

late events

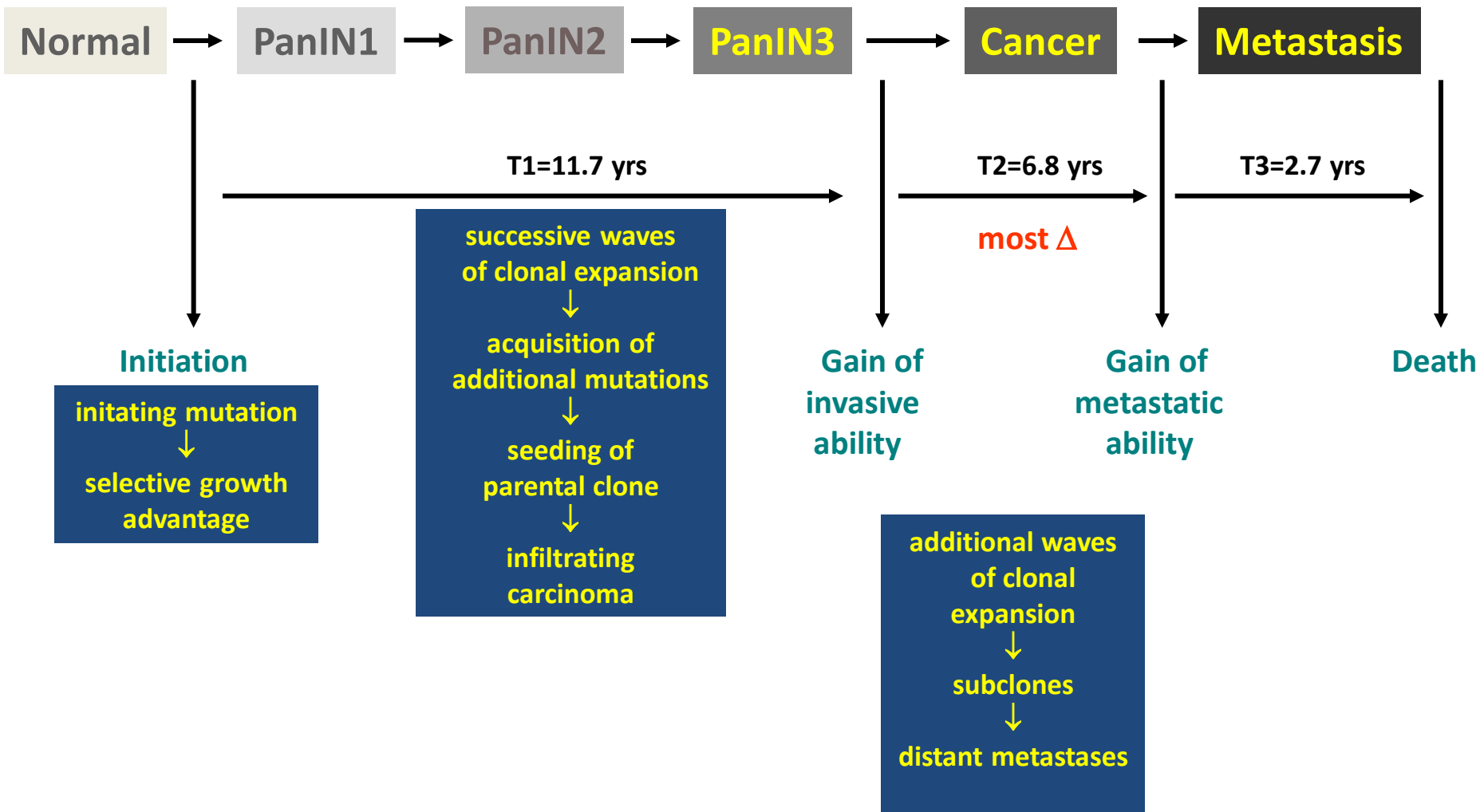
TP53 loss of function → ↑ cell survival (by ↓ apoptosis)

SMAD4 loss of function → escape from TGF-β-induced growth inhibition

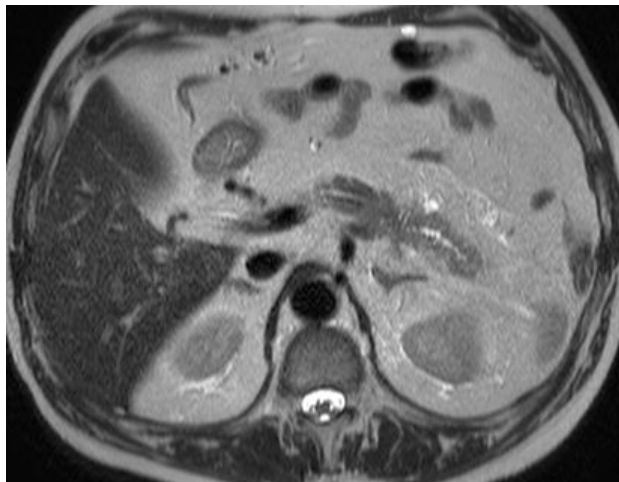
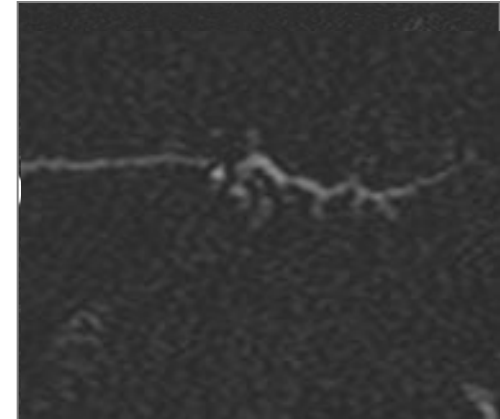
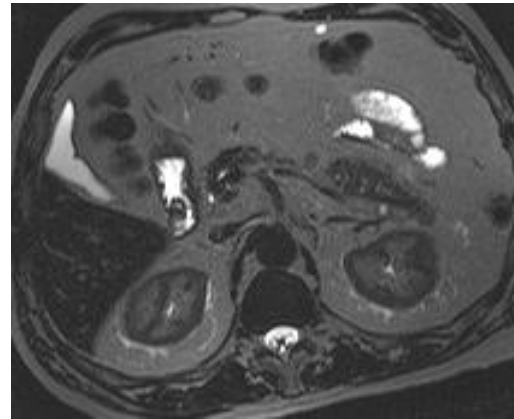
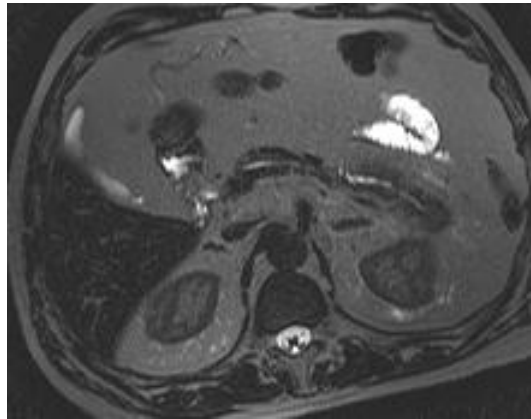


Molecular markers PPJ, brush cytology

Pancreatic carcinogenesis



Early detection of small lesions



Screening for pancreatic carcinoma

	Genetic marker	Environment	Family history	Symptoms
Surveillance if anyone factor is present	PJS FAMMM (p16) PRSS1		≥ 2 affected 1 = FDR	
Surveillance if combination of factors	BRCA2 (1) HNPCC FAP	Smoking Carcinogens	≥ 1 FDR	Weight loss Pain New onset diabetes

Screening for pancreatic carcinoma

- **Begin screening**
 - at 30 y (PJS)
 - at 40 y (FPC – HP)
 - 10 y before the age of the youngest relative affected

- **Stop screening**
 - at ≥ 70 y

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