

Genetic variants of MYOSIN IXB and PARD3 predispose to acute pancreatitis

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Acute Pancreatitis Werkgroep Nederland
www.pancreatitis.nl

Background (1): pathogenesis of acute pancreatitis



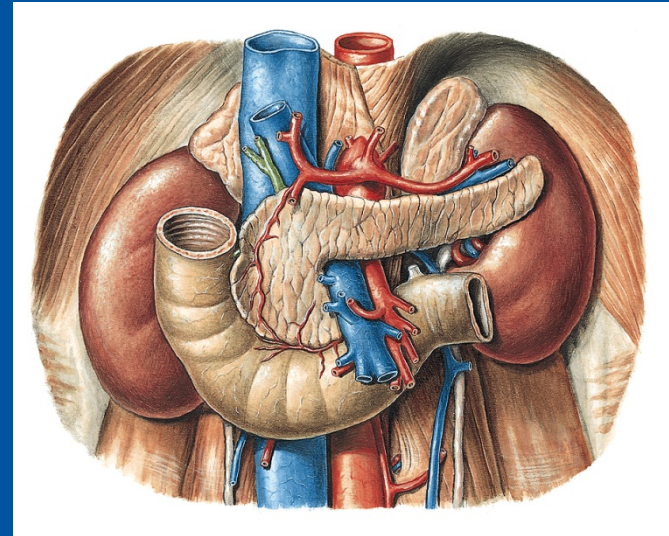
Preliminary activation of trypsin
within pancreas



Autodigestion and local
inflammation



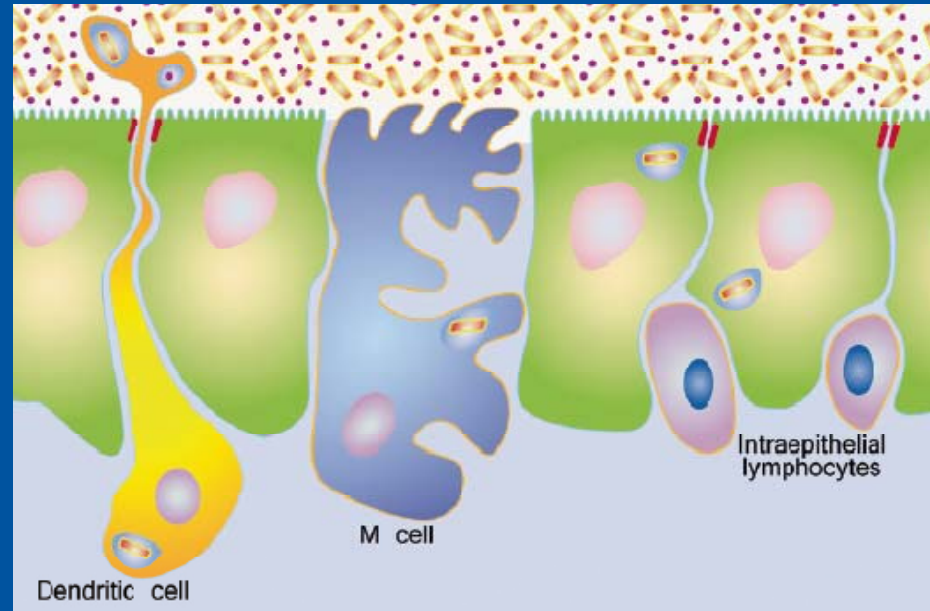
Systemic reaction: SIRS
Organ failure



Background (2): impairment of mucosal barrier function



- Acute pancreatitis:
 - *Impaired mucosal barrier function*
 - *Bacterial translocation*
 - *Infectious complications*



- Early phase of acute pancreatitis
 - *Mouse model → disruption of occludin and claudin-1: early event¹*

¹ Schmitt *et al.*, Pancreas, 2004

Background (3): celiac disease & inflammatory bowel disease



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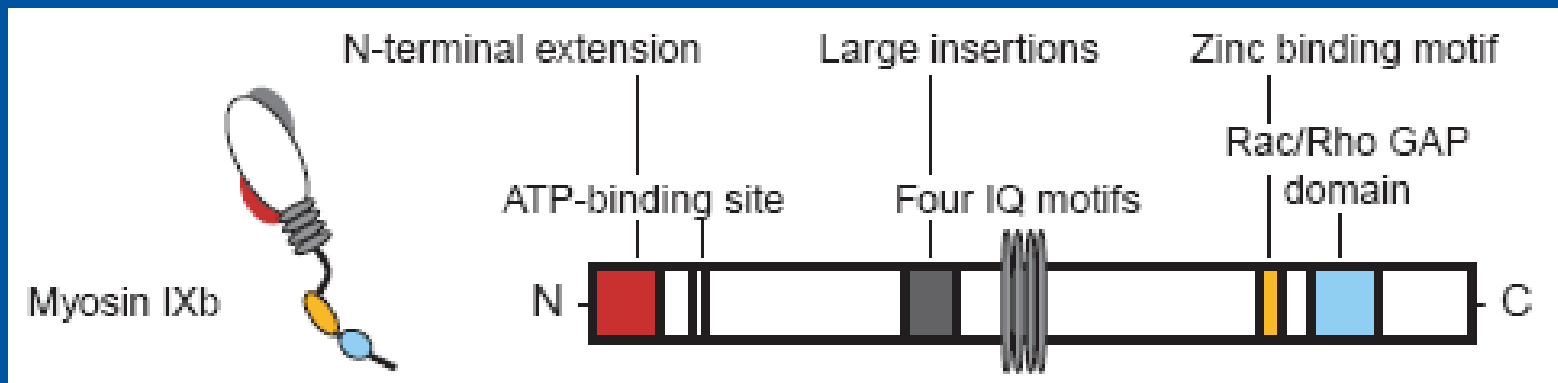
Celiac disease
Inflammatory bowel disease



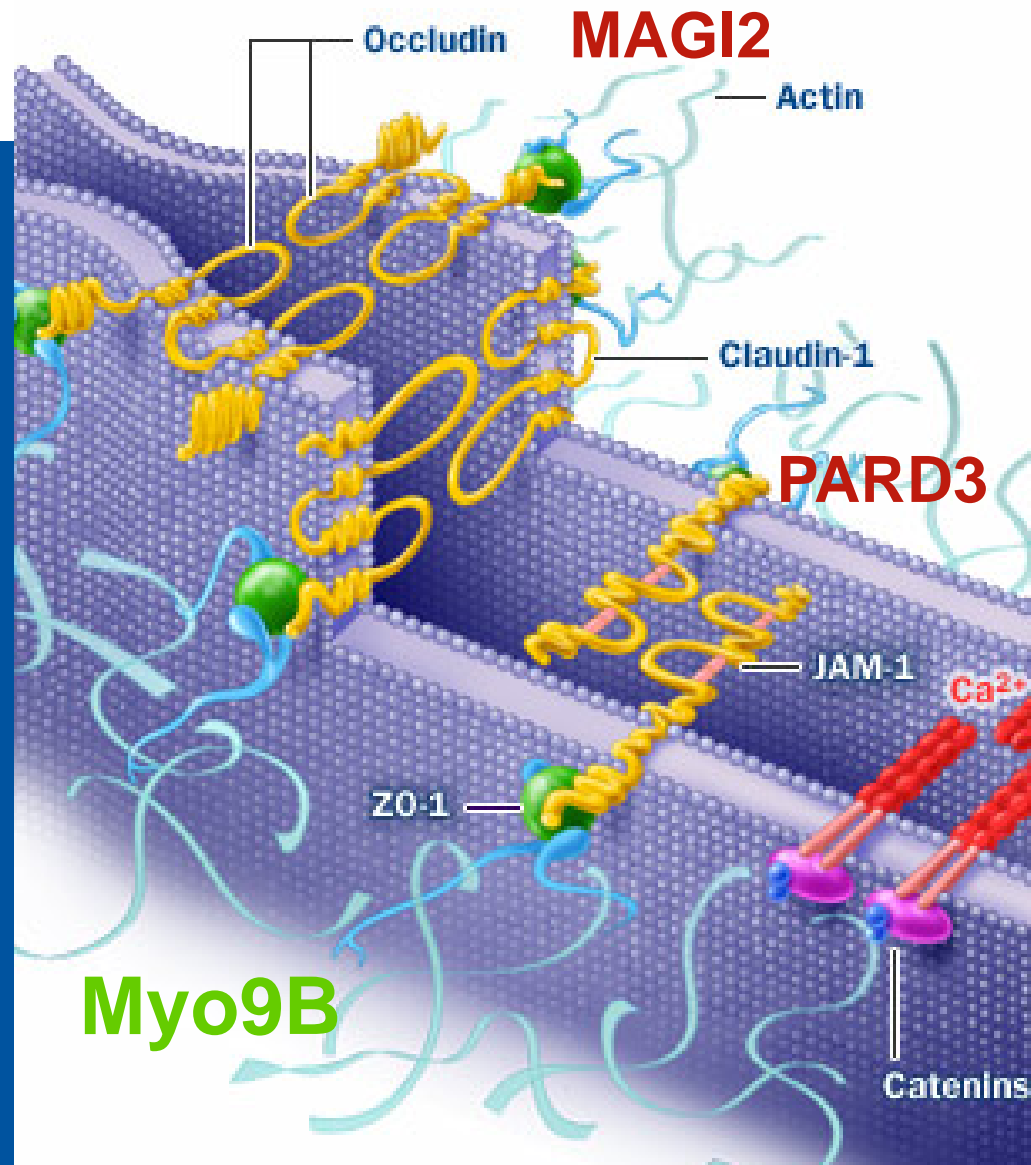
- MYOSIN IXB
- PARD3
- MAGI2

Background (4): MYOSIN IXB

- Motor protein with a Rho-GTPase activating domain
- Rho-GTPase:
 - *Remodeling cytoskeleton*
 - *Assembly of tight junction complex*



Pard3 en Magi2 → tight junction adaptor genes

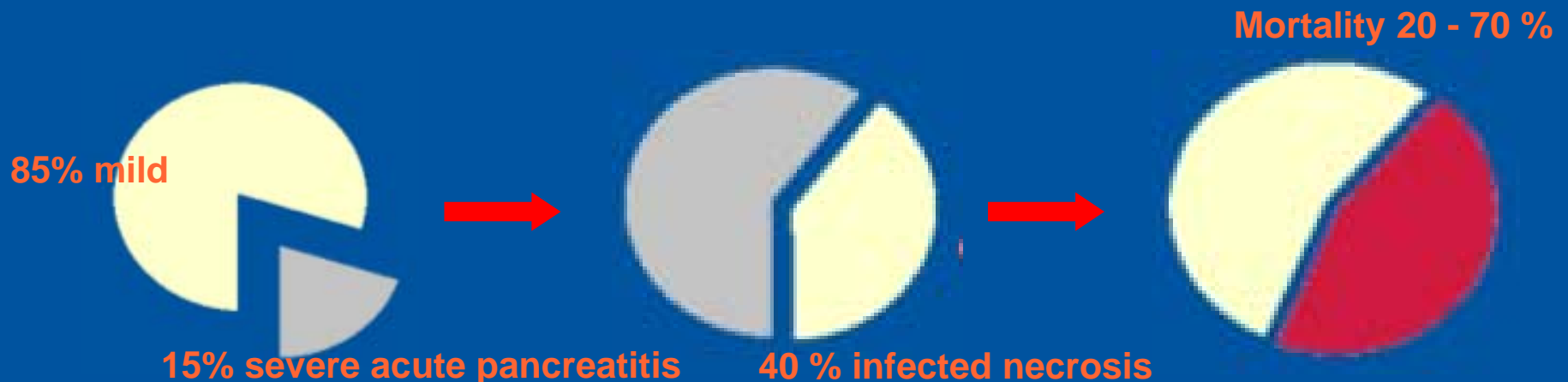


Monsuur *et al.*, Nature Genetics 2005; Van Bodegraven *et al.*, Gastroenterology 2006

Wapenaar *et al.*, Gut 2008

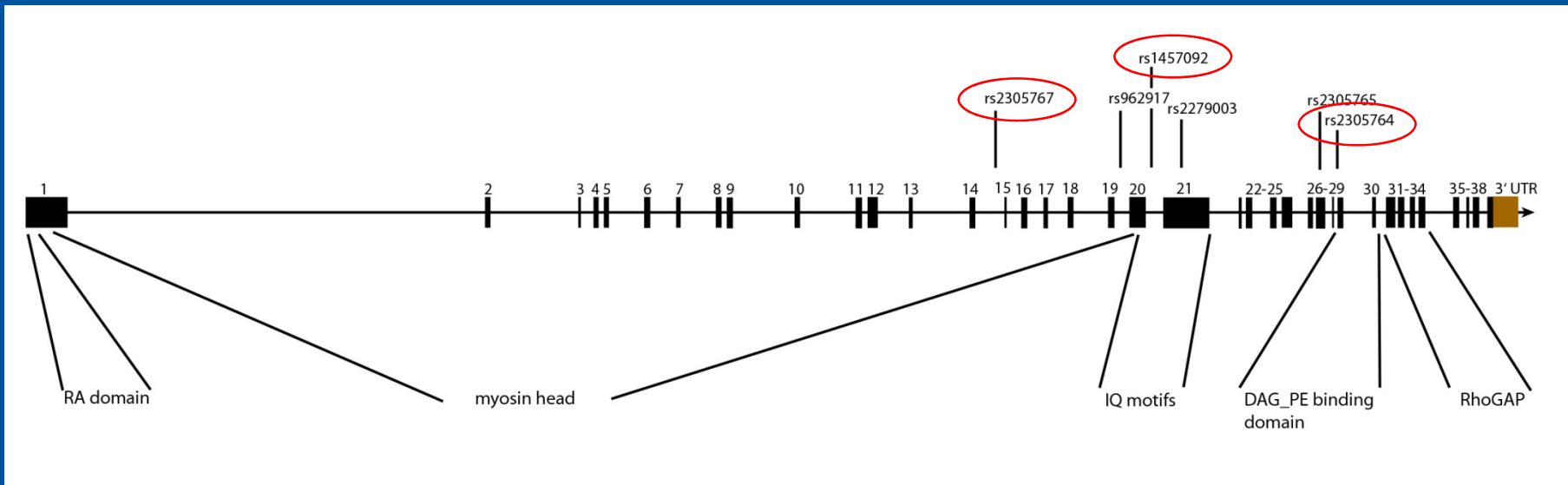
Hypothesis & research questions

- Genetic variants could play a role in:
 - *Susceptibility to acute pancreatitis*
 - *Course of acute pancreatitis*
1. Are genetic variants of *Myo9B*, *Pard3* en *Magi2* associated with the disease acute pancreatitis?
 2. Are genetic variants of *Myo9B*, *Pard3* en *Magi2* associated with the course of acute pancreatitis?



Patients & Methods

- Patients: both severe and mild acute pancreatitis → N = 409
- Controls:
 - *MYO9B*: N = 1624
 - *TJ genes*: N = 929
- DNA isolation (Magna Pure LC – Roche)
- SNP genotyping (TaqMan, Applied Biosystems)



Genetic variants of MYO9B are associated with acute pancreatitis



	Acute pancreatitis		Controls		P value ¹	OR	95%-CI
	Associated allele	Associated allele (freq)	Associated allele (freq)				
rs7259292	T	34 (0.044)	89 (0.028)	0.0193	1.64	1.10-2.45	
rs2305767	A	486 (0.620)	1764 (0.559)	0.0020	1.29	1.10-1.51	
rs1545620	C	351 (0.447)	1143 (0.358)	0.000004	1.45	1.24-1.69	
rs1457092	T	315 (0.398)	1041 (0.330)	0.0003	1.34	1.14-1.58	
rs2305764	A	1204 (0.433)	1204 (0.381)	0.0075	1.24	1.06-1.45	

Amino acid change: Alanine → Serine

¹ Two-tailed *P* values calculated

Haplotypes of MYO9B carrying the associated C allele are associated with acute pancreatitis



	Acute pancreatitis	Controls			
	Presence (freq)	Presence (freq)	P value ¹	OR	95%-CI
CGACG	289.5 (0.363)	1396.5 (0.433)	(ref)	1	
CACAA	307.6 (0.385)	1043.6 (0.324)	0.0001	1.42	1.19-1.70
CAACG	122.2 (0.153)	495.8 (0.154)	0.149	0.84	0.66-1.06
CAACA	26.0 (0.033)	160.3 (0.050)	0.267	1.24	0.81-1.91
TACCG	32.4 (0.041)	78.5 (0.024)	0.001	2.02	1.32-3.09

¹ Two-tailed *P* values calculated by χ^2 analysis of allele counts

The order of the SNPs is: rs7259292, rs2305767, rs1545620, rs1457092, rs2305764.

Genetic variants of *Pard3* are associated with acute pancreatitis



		Acute pancreatitis		Controls		P value ¹	OR	95%-CI
		Associated allele	Associated allele (freq)	Associated allele	Associated allele (freq)			
<i>Pard3</i>	rs10763976	A	377 (0.478)	782 (0.435)	0.0430	1.19	1.01-1.41	
	rs4379776	A	291 (0.369)	560 (0.312)	0.0047	1.29	1.08-1.54	
<i>Magi2</i>	rs6962966	G	391 (0.496)	840 (0.467)	0.1663	1.13	0.95-1.33	
	rs9640699	A	317 (0.400)	685 (0.381)	0.3428	1.09	0.92-1.29	
	rs1496770	G	462 (0.594)	1062 (0.590)	0.8559	1.02	0.86-1.20	

¹ Two-tailed *P* values calculated by χ^2 analysis of allele counts

1 Haplotype of Pard3 is associated with acute pancreatitis



	Acute pancreatitis	Controls			
	Presence	Presence	P value ¹	OR	95%-CI
GG	373.4 (0.469)	936.3 (0.520)	(ref)	1	
AA	253.3 (0.318)	482.1 (0.268)	0.005	1.32	1.09-1.60
AG	128.2 (0.161)	303.2 (0.168)	0.631	0.94	0.74-1.19
GA	41.1 (0.052)	80.4 (0.045)	0.216	0.77	0.52-1.14

¹ Two-tailed *P* values calculated by χ^2 analysis of allele counts
The order of the SNPs is: rs10763976, rs4379776.

Myo9B variants are associated to infected pancreas necrosis and organ failure



Disease severity	NS	
Mortality	NS	
Infectious complications	NS	
Infected pancreatic necrosis	OR = 2,65	95% CI = 1,25 – 5,63
Early organ failure	OR = 1,87	95% CI = 1,17 – 2,99

Conclusion



- *MYO9B* and *Pard3* → association with acute pancreatitis
- *MYO9B* → associated with complications
- *MYO9B* and *Pard3* are involved in mucosal barrier function
- The genetic make-up of patients with gallstones or alcohol abuse can increase susceptibility to acute pancreatitis

Discussion



- Practical implications:
 - *Not useful for prediction*
 - *Insight in pathogenesis*

- MYO9B:

A common gene for pathology of the digestive tract?

Acknowledgements



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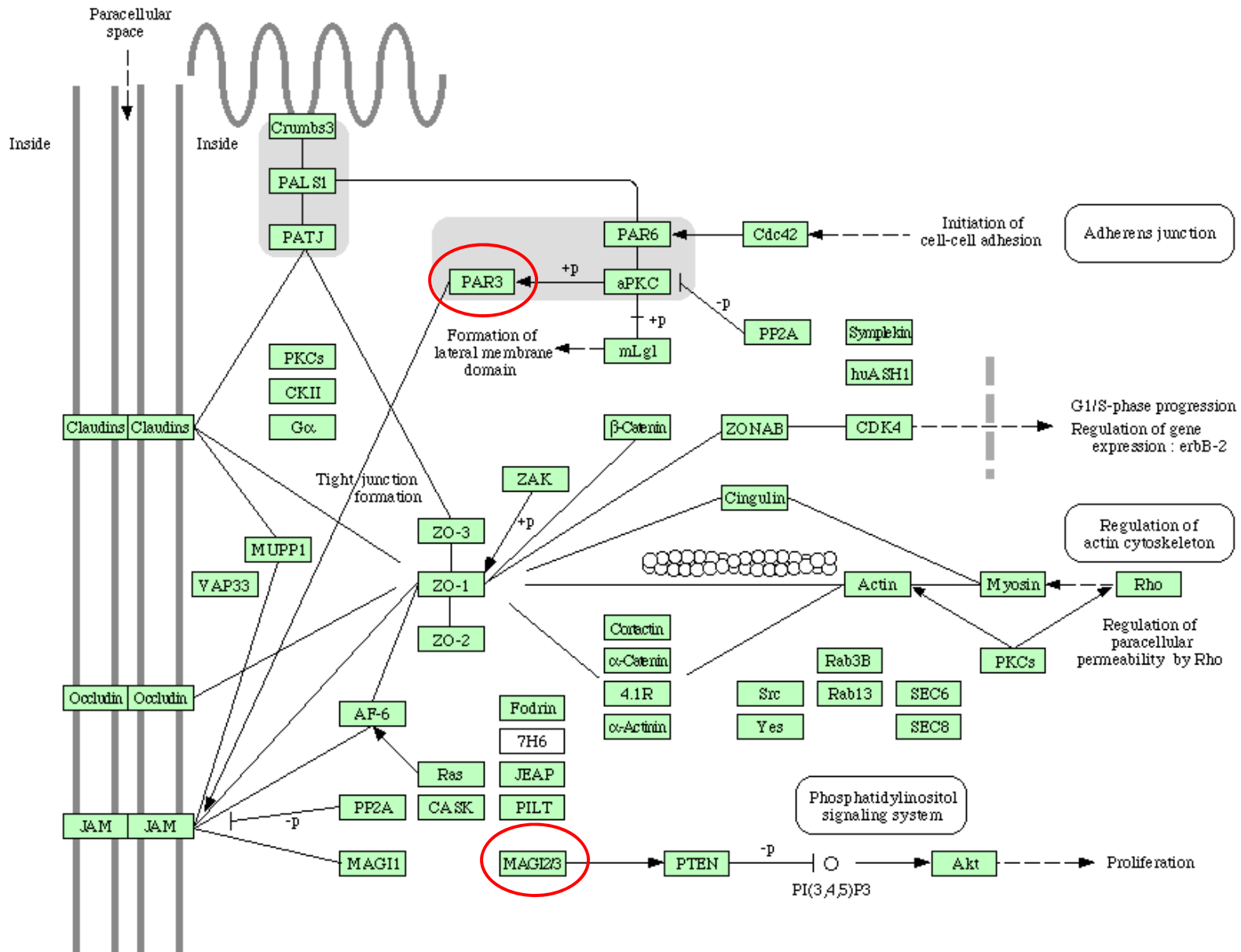
Potentiële consequenties van een causale variant



SNPs komen voor op verschillende plaatsen van het DNA:

- Coding regions:
 - *Non-synonymous verandering (aminozuur verandering → invloed op eiwit sequence, structuur, functie)*
 - *Splicing*
 - *Expressie levels (meer / minder mRNA)*
- Non-coding regions
 - *Regulatoire consequenties???*

TIGHT JUNCTION



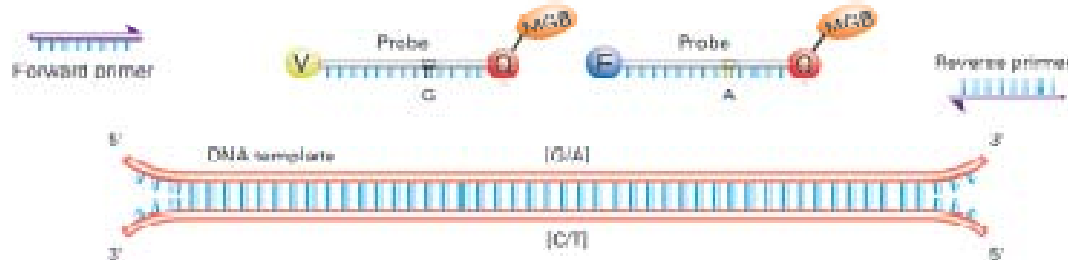
Discussie (2)



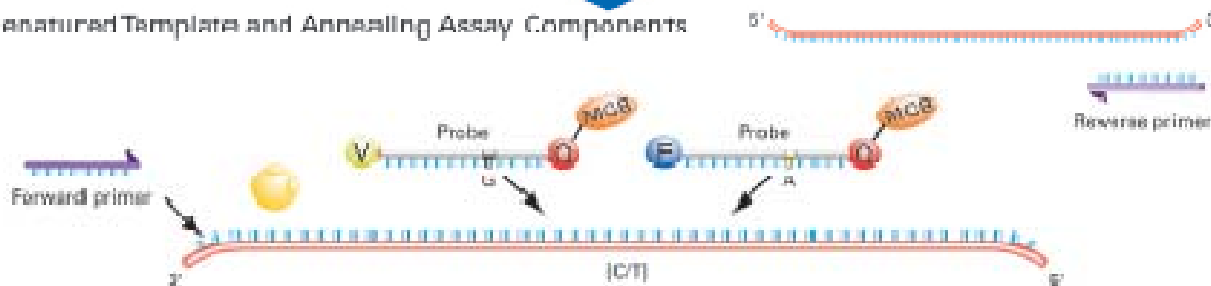
- Associatie met reumatoïde artritis en SLE → gen voor auto-immuunziekten?
- Associatie met schizofrenie?!!
- Becker (2004): Common variant / multiple disease hypothesis

SNP typing: methode

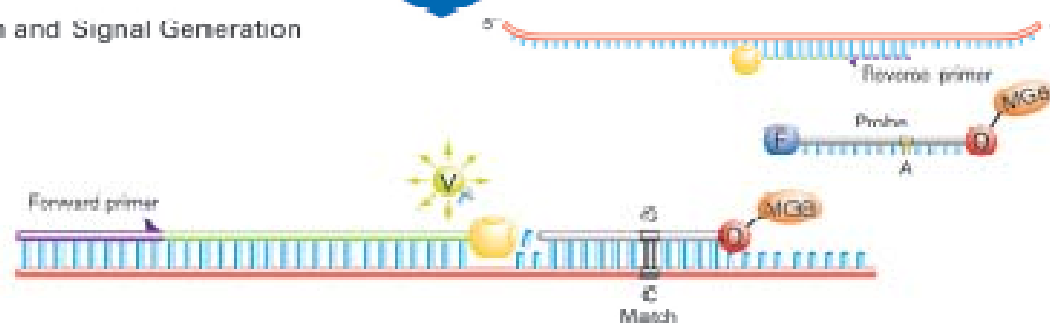
1. Assay Components and DNA Template



2. Denatured Template and Annealing Assay Components

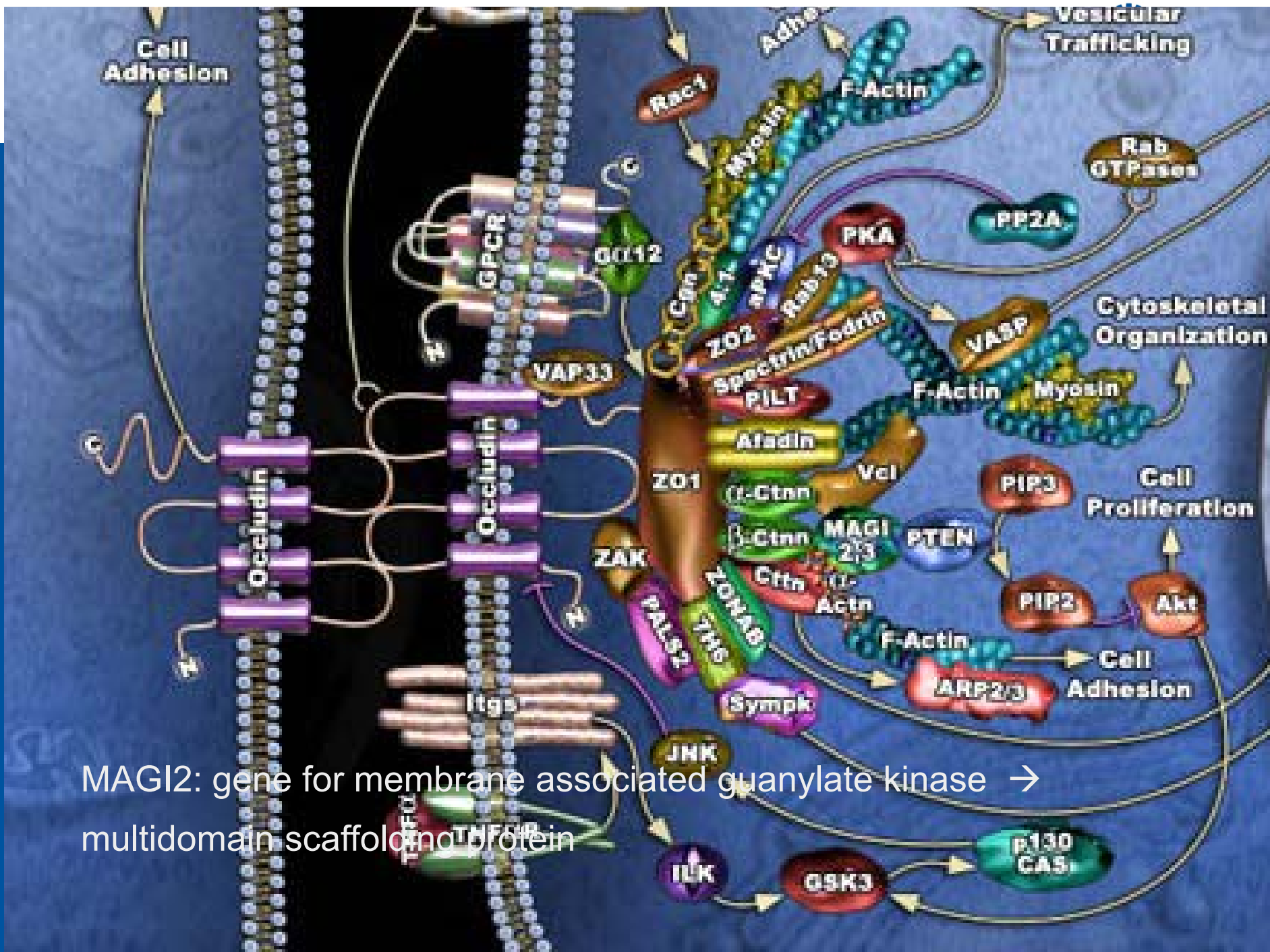


3. Polymerization and Signal Generation

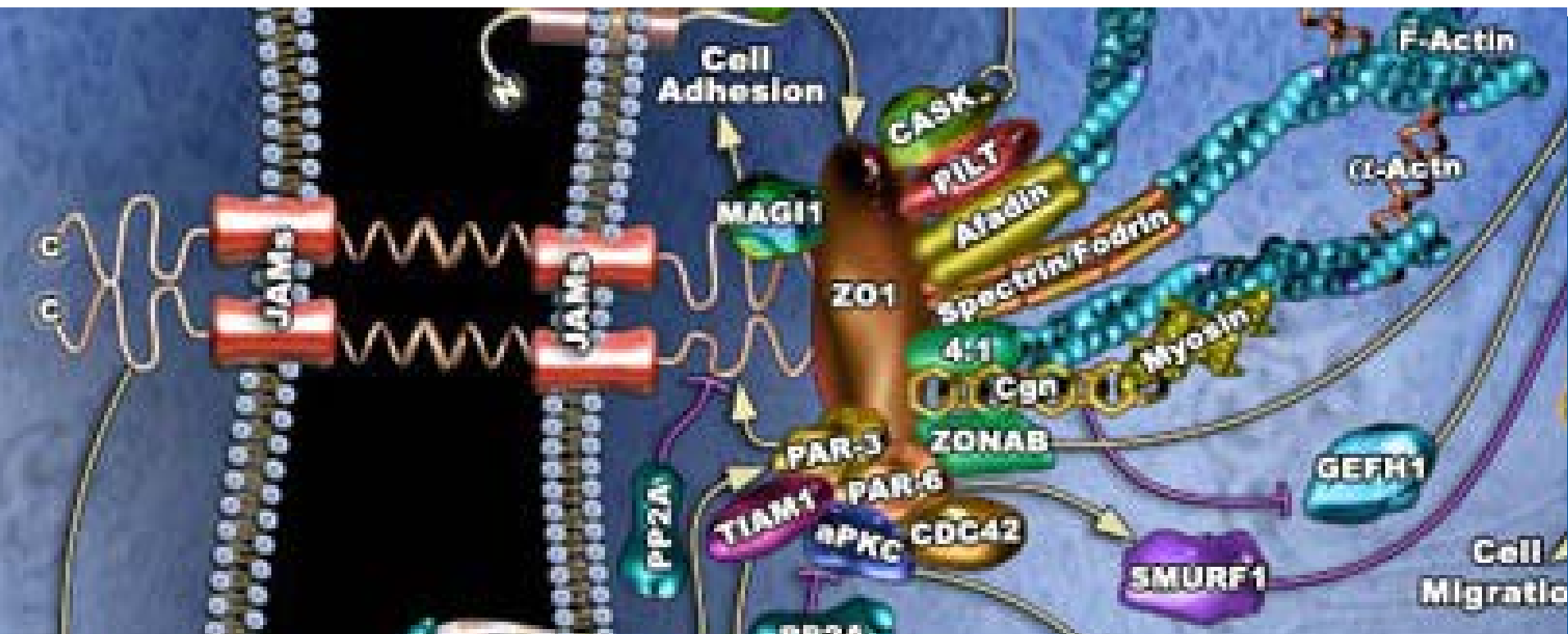


LEGEND

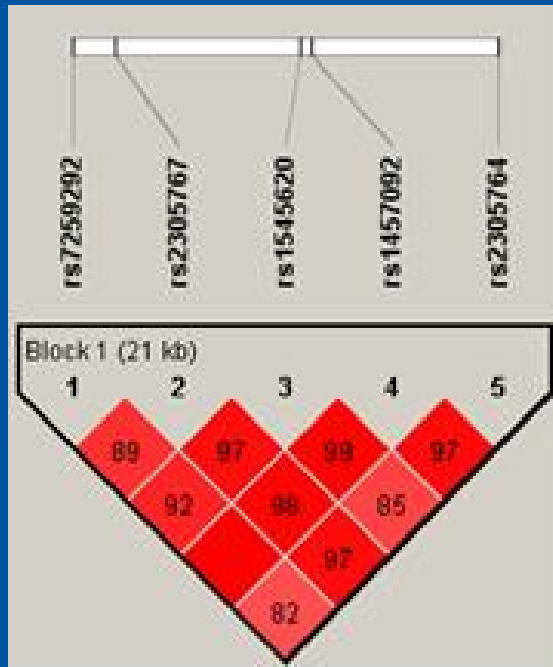
-  VIB dye
-  FAM™ dye
-  Quencher
-  Minor Groove Binder
-  AmpliTaq Gold® DNA Polymerase
-  Probe
-  Primer
-  Template
-  Extended Primer



MAGI2: gene for membrane associated guanylate kinase → multidomain scaffolding protein



PARD3 = gene for partitioning defective protein 3 →
epithelial TJ assembly



Case-control study

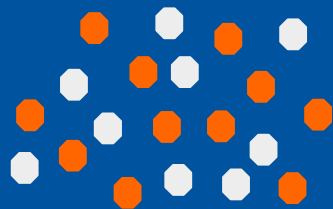


Voorbeeld:
C = 45% en A = 55%

Ergens op het genoom

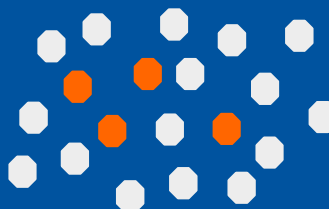


Verschil in allel frequentie tussen:



Controls

&



Cases

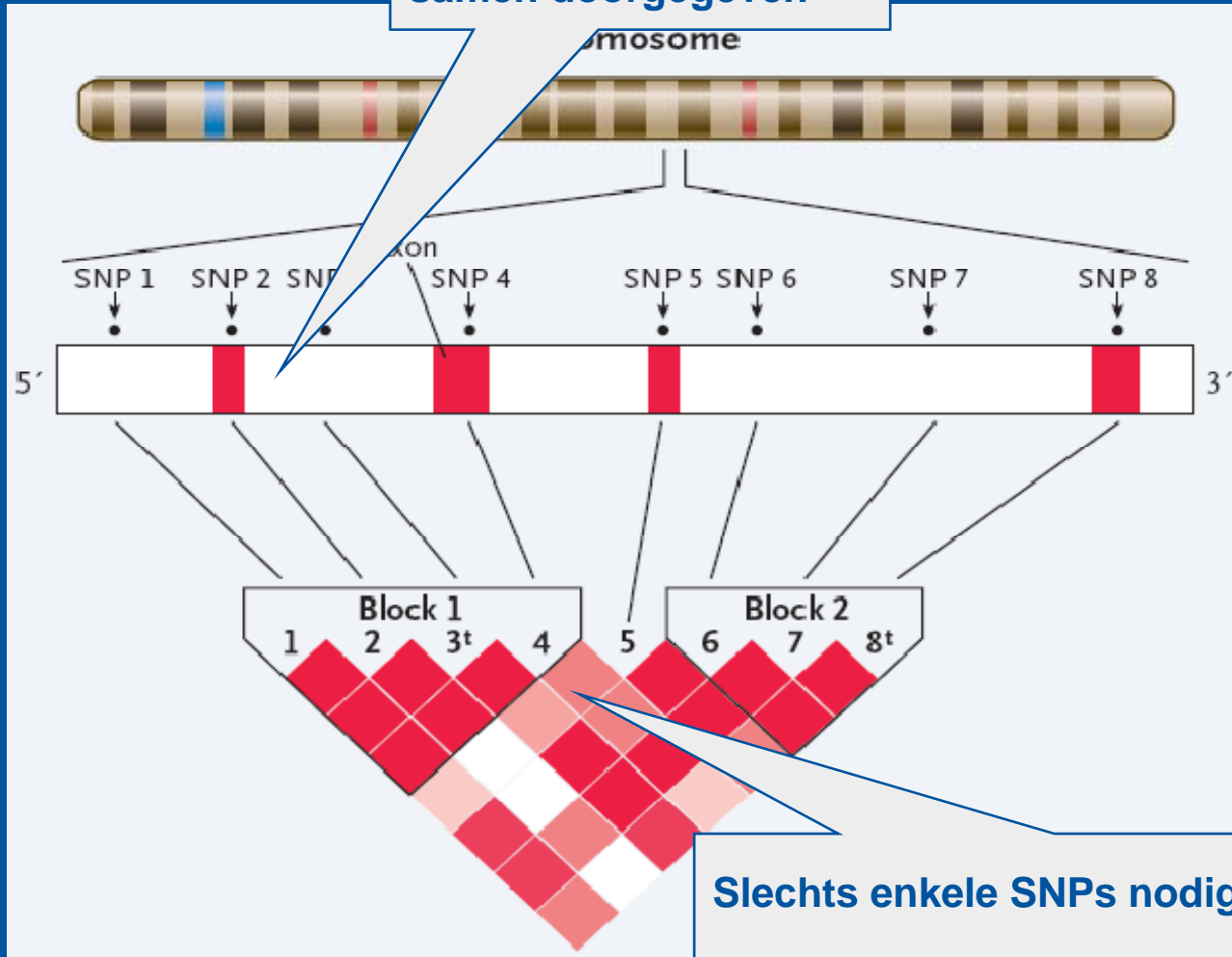
● A allel aanwezig

● C allel aanwezig

Haplotype



SNPs die fysiek
dichtbij elkaar liggen →
samen doorgegeven



Slechts enkele SNPs nodig voor
informatie over alle SNPs van gen

