

Genetic tools to address individual radiosensitivity and their limitations

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Overview of clinical studies addressing SNPs and normal tissue radiosensitivity

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Editorial

Searching for genetic determinants of normal tissue radiosensitivity – Are we on the right track?

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Overview of clinical studies addressing SNPs and normal tissue radiosensitivity

47 studies

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Sample size 25 – 778 pts. Median 144 pts.

Overview of clinical studies addressing SNPs and normal tissue radiosensitivity

47 studies

Candidate gene approach

Overview of clinical studies addressing SNPs and normal tissue radiosensitivity

47 studies

ATM BCL2 BRCA1 BRCA2 XRCC1 XRCC2 XRCC3 XRCC5 APEX OGG1
XPF XPD hHR23A RAD50 RAD51 RAD52 NBS MRE11 DNA lig IV
ERCC2 ERCC4 ESR1 NBN MSH6 NR3C1

GSTP1 GSTM1 GSTT1 GSTA1 SOD2 CAT MPO eNOS

TGFB1

CYP1A1 CYP2C9 CYP2C19 CYP3A5 CYP2D6 CYP11B2 CYP17A1
DHFR CX3CR1 Hyl-1 MS HTHFR

Suga T, 2007: 999 SNPs in 137 genes (399 patients)

Isomura M, 2008: 3,144 SNPs in 494 genes (156 patients)

Suga T, 2008: 450 SNPs in 118 genes (179 patients)



Overview of clinical studies addressing SNPs and normal tissue radiosensitivity

47 studies

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DHFR CX3CR1 Hyl-1 MS HTHFR

Kerns SL, 2010: First GWAS in clinical radiobiology (79 patients)



Overview of clinical studies addressing SNPs and normal tissue radiosensitivity

47 studies

Approximately 2/3 of the studies have
reported significant associations

Typical conclusion of studies addressing SNPs and normal tissue radiosensitivity

may be associated with

These results suggest that SNPs in

results of this study should not be regarded as definitive evidence for or against an association between these SNPs

Large clinical studies are needed

Further investigations are strongly warranted

warrant further investigation.

Typical conclusion of studies addressing SNPs and normal tissue radiosensitivity

may be associated with

These results are

results of this

associative
between these SNPs

Results have been very conflicting

are needed

Further investigations are strongly warranted

warrant further investigation.

Typical conclusion of studies addressing SNPs and normal tissue radiosensitivity

may be associated with

These results

results of this

No unambiguously proven associations

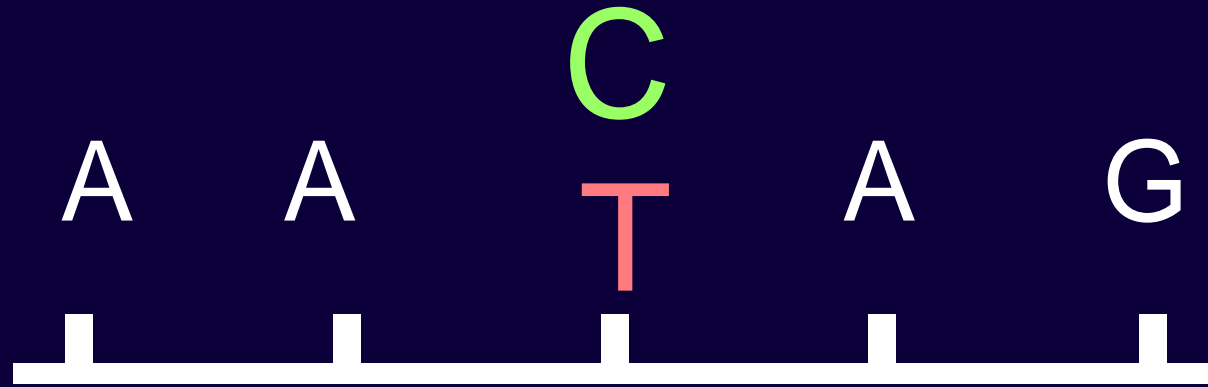
are needed

Further investigations are strongly warranted

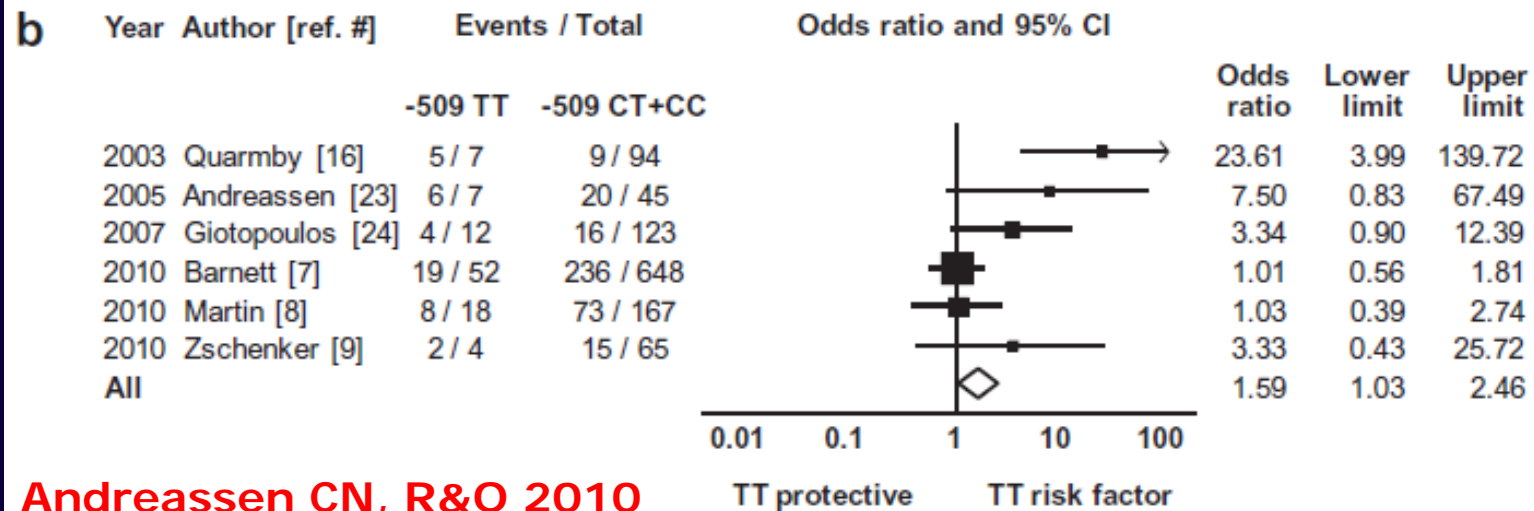
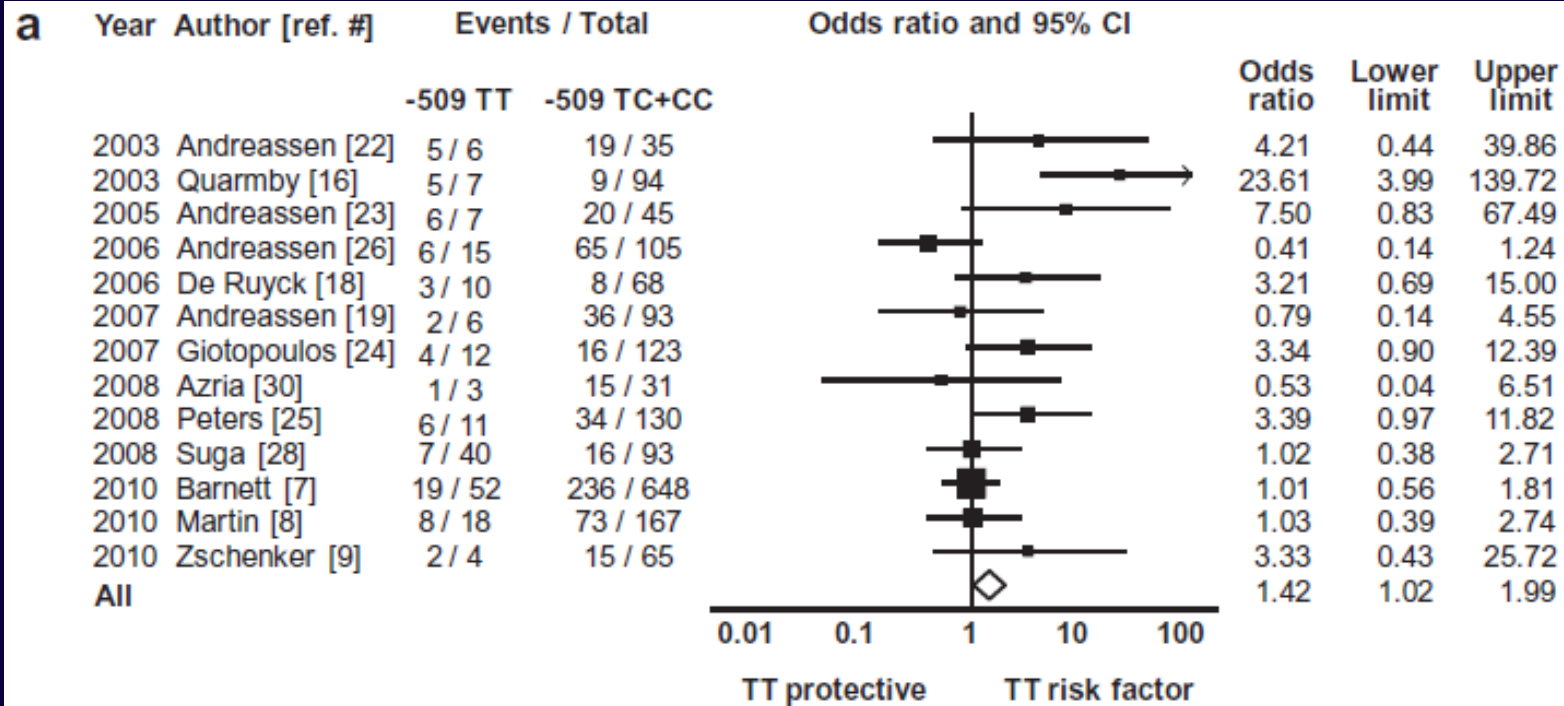
warrant further investigation.



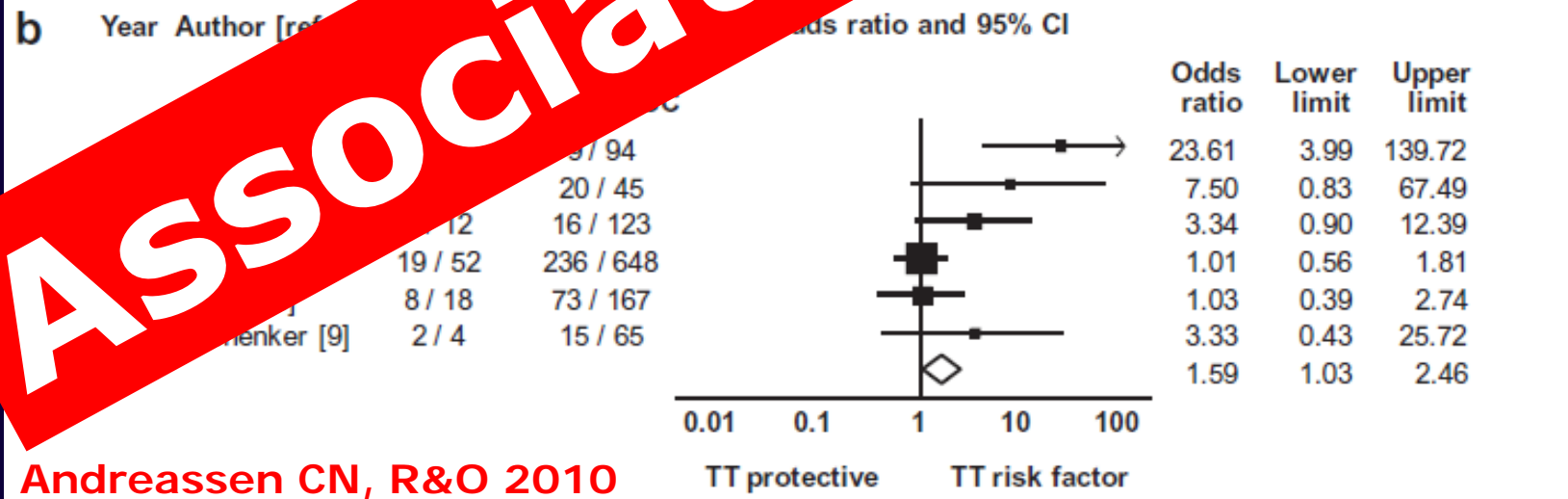
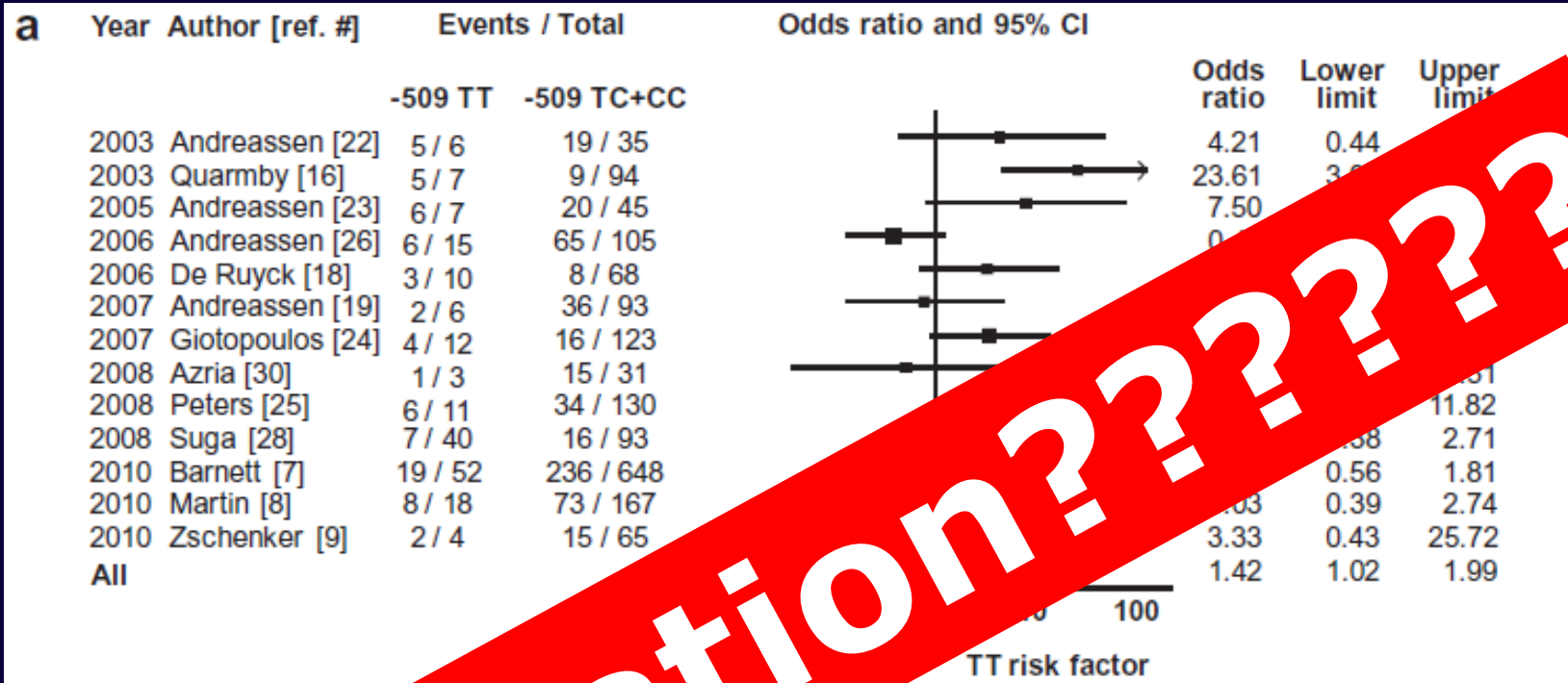
Meta analysis TGFB1 position -509 C/T and late toxicity



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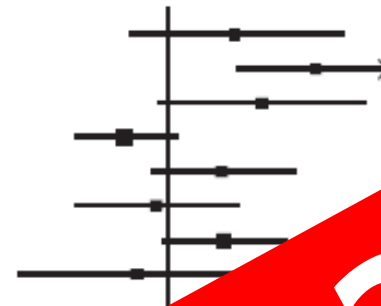
Andreassen CN, R&O 2010



Meta analysis TGFB1 position -509 C/T and late toxicity

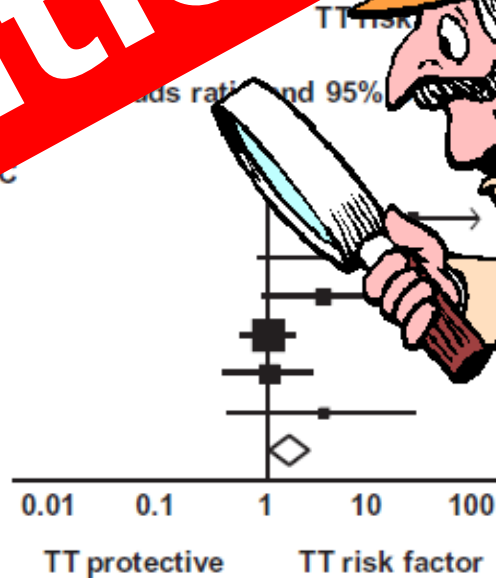
a

Year	Author [ref. #]	Events / Total		Odds ratio and 95% CI		
		-509 TT	-509 TC+CC	Odds ratio	Lower limit	Upper limit
2003	Andreassen [22]	5 / 6	19 / 35	4.21	0.44	
2003	Quarmby [16]	5 / 7	9 / 94	23.61	3.5	
2005	Andreassen [23]	6 / 7	20 / 45	7.50		
2006	Andreassen [26]	6 / 15	65 / 105	0.83		
2006	De Ruyck [18]	3 / 10	8 / 68			
2007	Andreassen [19]	2 / 6	36 / 93			
2007	Giotopoulos [24]	4 / 12	16 / 123			
2008	Azria [30]	1 / 3	15 / 31			11.82
2008	Peters [25]	6 / 11	34 / 130			2.71
2008	Suga [28]	7 / 40	16 / 93		0.56	1.81
2010	Barnett [7]	19 / 52	236 / 648	0.39	0.39	2.74
2010	Martin [8]	8 / 18	73 / 167	3.33	0.43	25.72
2010	Zschenker [9]	2 / 4	15 / 65	1.42	1.02	1.99
All						

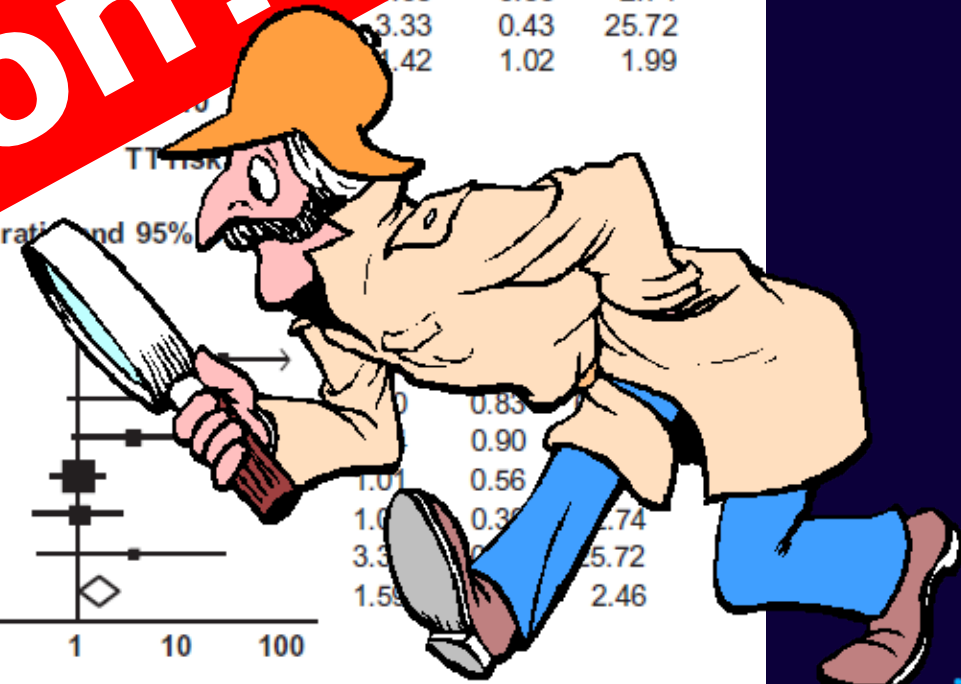


b

Year	Author [ref. #]	Events / Total		Odds ratio and 95% CI		
		-509 TT	-509 TC+CC	Odds ratio	Lower limit	Upper limit
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		8 / 18	73 / 167	1.01	0.39	2.74
	Zschenker [9]	2 / 4	15 / 65	3.33	0.43	25.72
All				1.55		2.46



Association???????



Shortcomings of the studies carried out so far

- Most studies relatively small (25-778 ptt. Median 144 pts.)

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A study of 150 subjects with a 1:2 ratio between high and low risk genotype has a power of less than 30% to detect a 1.5 fold increase in normal tissue complication risk from 20% to 30% ($\alpha = 0.05$, two tailed test)

High risk of
false
negatives

Shortcomings of the studies carried out so far

- Multiple SNPs investigated
- Several different endpoints included
- Subgroup analyses occasionally conducted
- No means taken to adjust for multiple comparisons

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In a study addressing the impact of 7 SNPs on two different normal tissue endpoints (corresponding to 14 individual tests), the probability of getting at least one positive finding by chance is more than 50% assuming a 5% significance level in each comparison

High risk of false positives

Shortcomings of the studies carried out so far

We have far too often conducted studies in which the probability of detecting the presence of a 'true' association may have been less than 30% (in each comparison) while the risk of finding something that does not exist has been above 50% (in the entire study)

Andreassen CN, R&O 2010



Shortcomings of the studies carried out so far

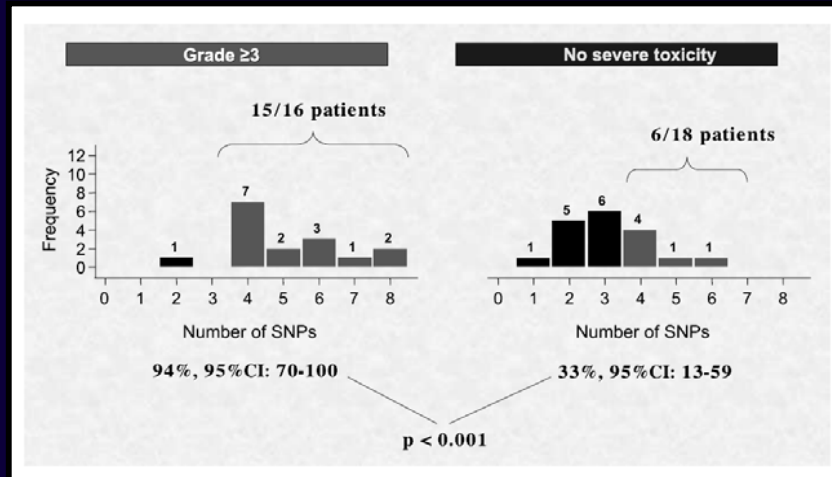
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Andreassen CN, R&O 2010

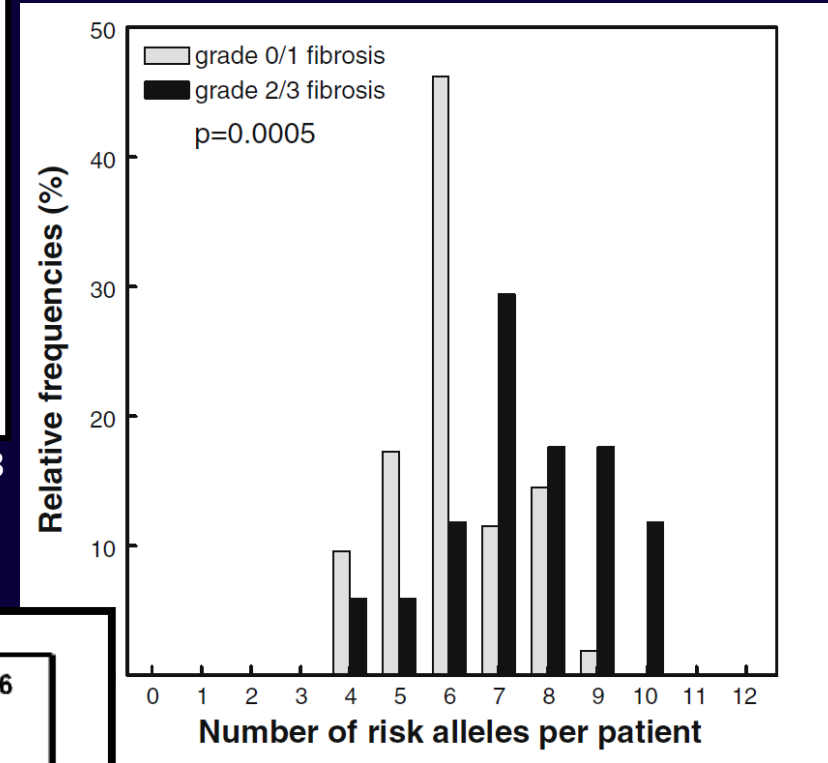
No wonder
results are
confusing



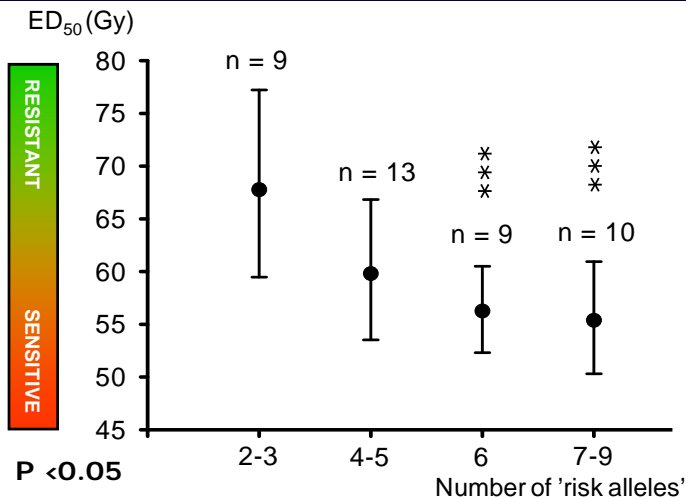
Multiple-SNP models



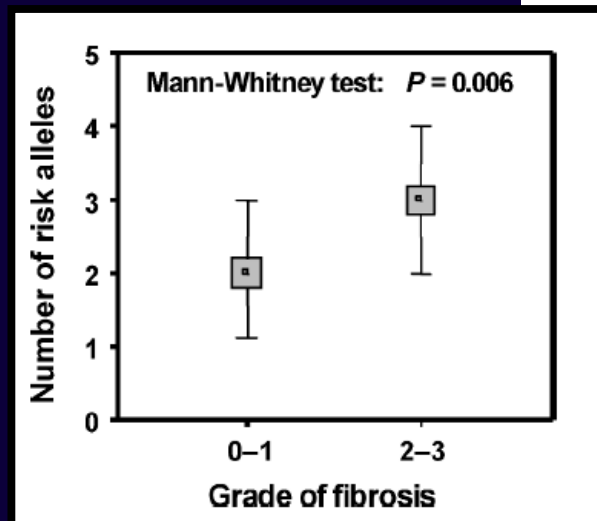
Azria D 2008



Zschenker O 2010



Andreassen CN 2003



Alsbeih G 2010



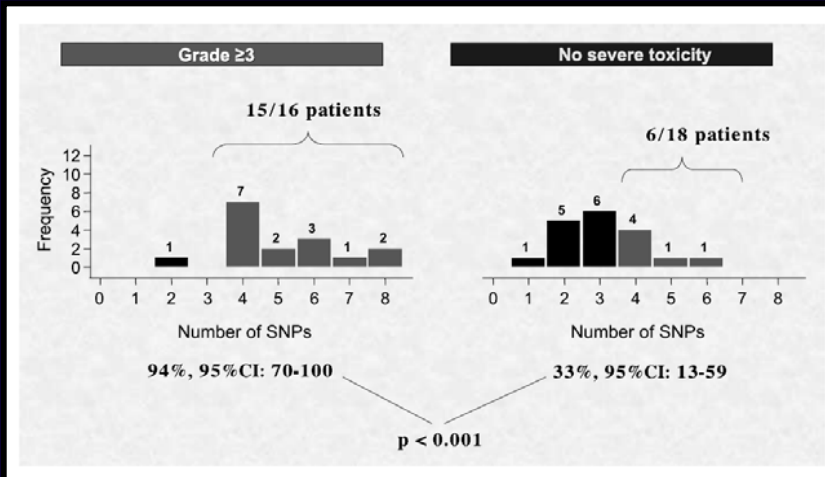
Multiple-SNP models

ATM

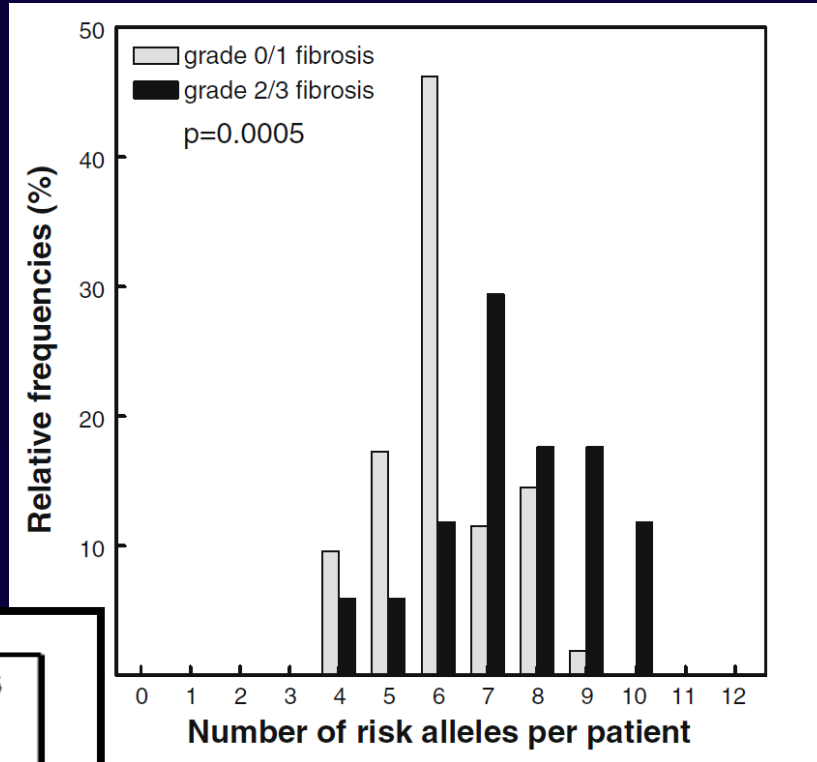
TGFB1

XRCC1

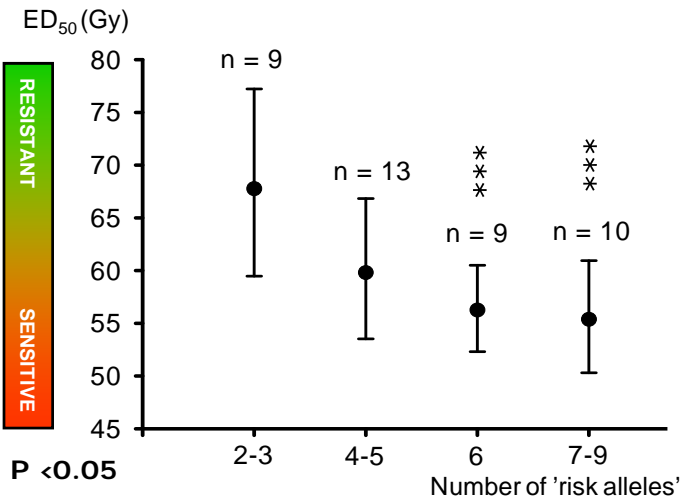
XRCC3



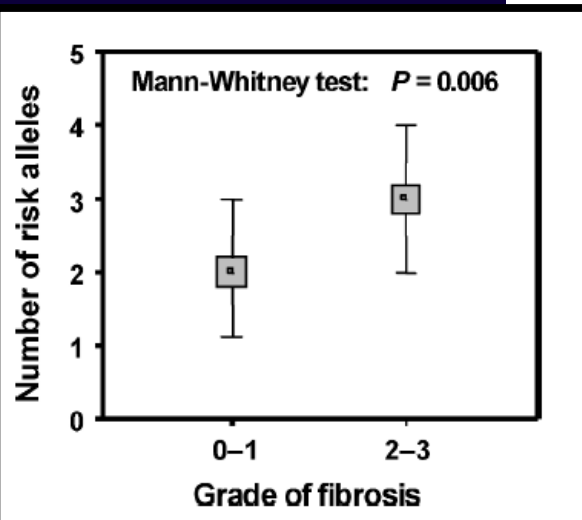
Azria D 2008



Zschenker O 2010



Andreassen CN 2003



Alsbeih G 2010



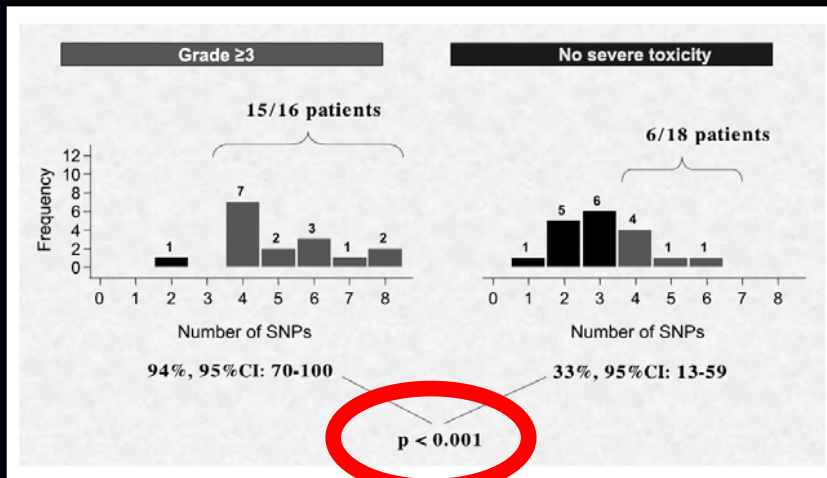
Multiple-SNP models

ATM

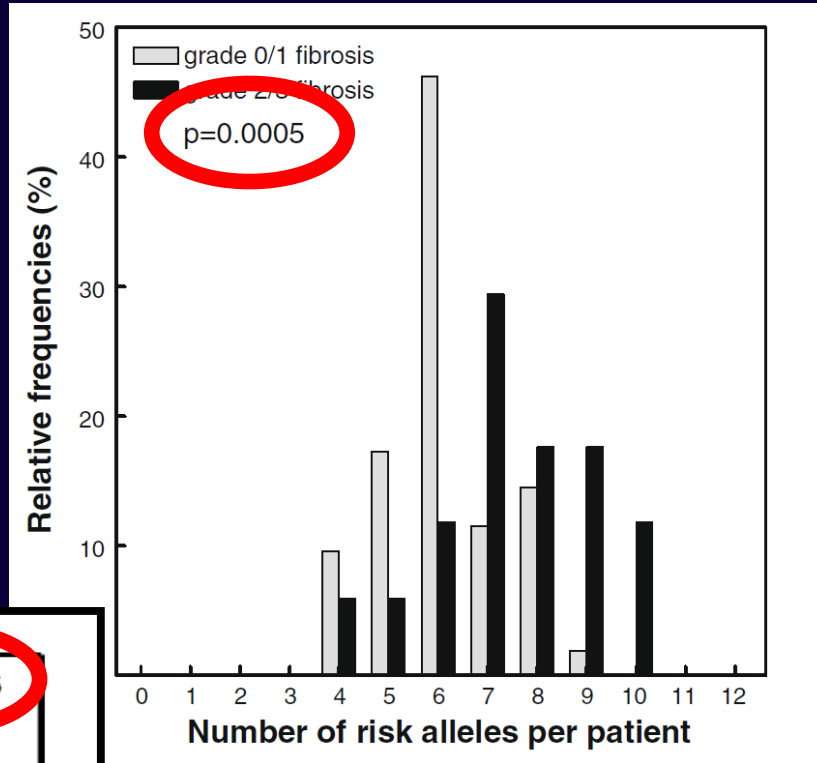
TGFB1

XRCC1

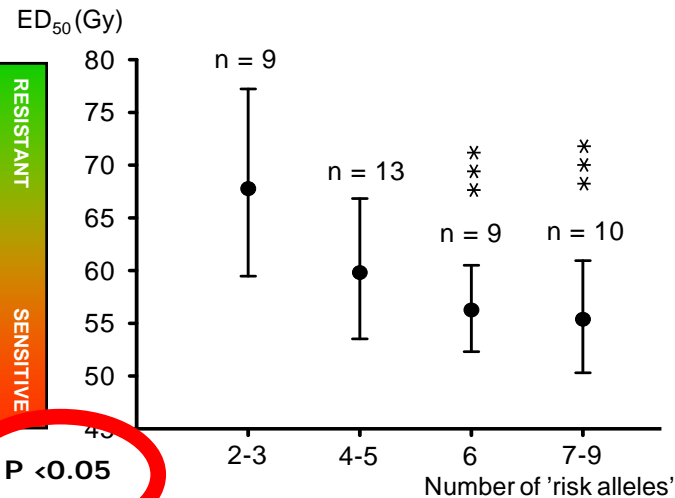
XRCC3



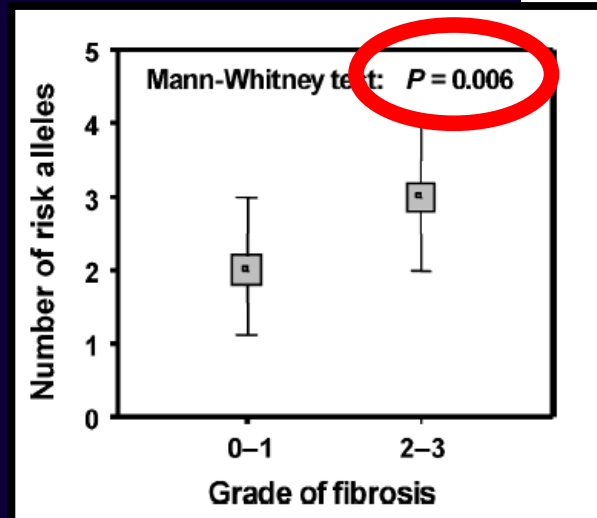
Azria D 2008



Zschenker O 2010



Andreassen CN 2003



Alsbeih G 2010



Multiple-SNP models

Author, Year	<i>ATM</i> codon	<i>TGFB1</i> codon	<i>XRCC1</i> codon	<i>XRCC3</i> codon
	1853 Asp/ Asn	10 Leu/ Pro	399 Arg/ Gln	241 Thr/ Met
Andreassen CN, 2003	-	Pro	Arg	Thr
Azria D, 2008	Asn	-	Gln	-
Alsbeih G, 2010	-	Leu	Arg	Met
Zschenker O, 2010	Asp	-	Gln	-

Andreassen CN R&O 2010



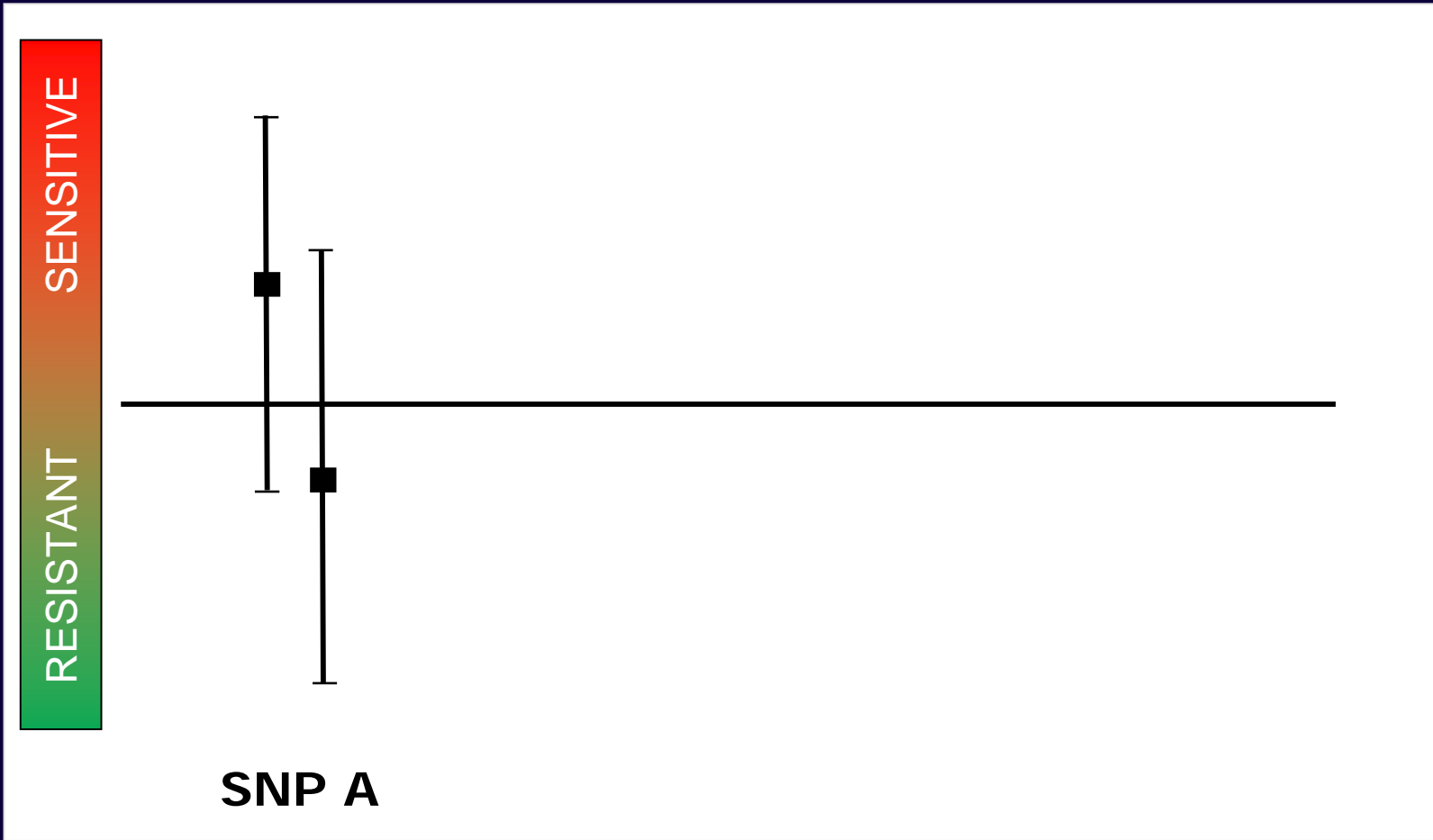
Multiple-SNP models

Author, Year	ATM codon	TCO	ES codon
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Andreassen CN, 2003	-		Thr
Azria D, 2008			Gln -
Alsbeih G, 2010			Arg Met
Zschenker		-	Gln -

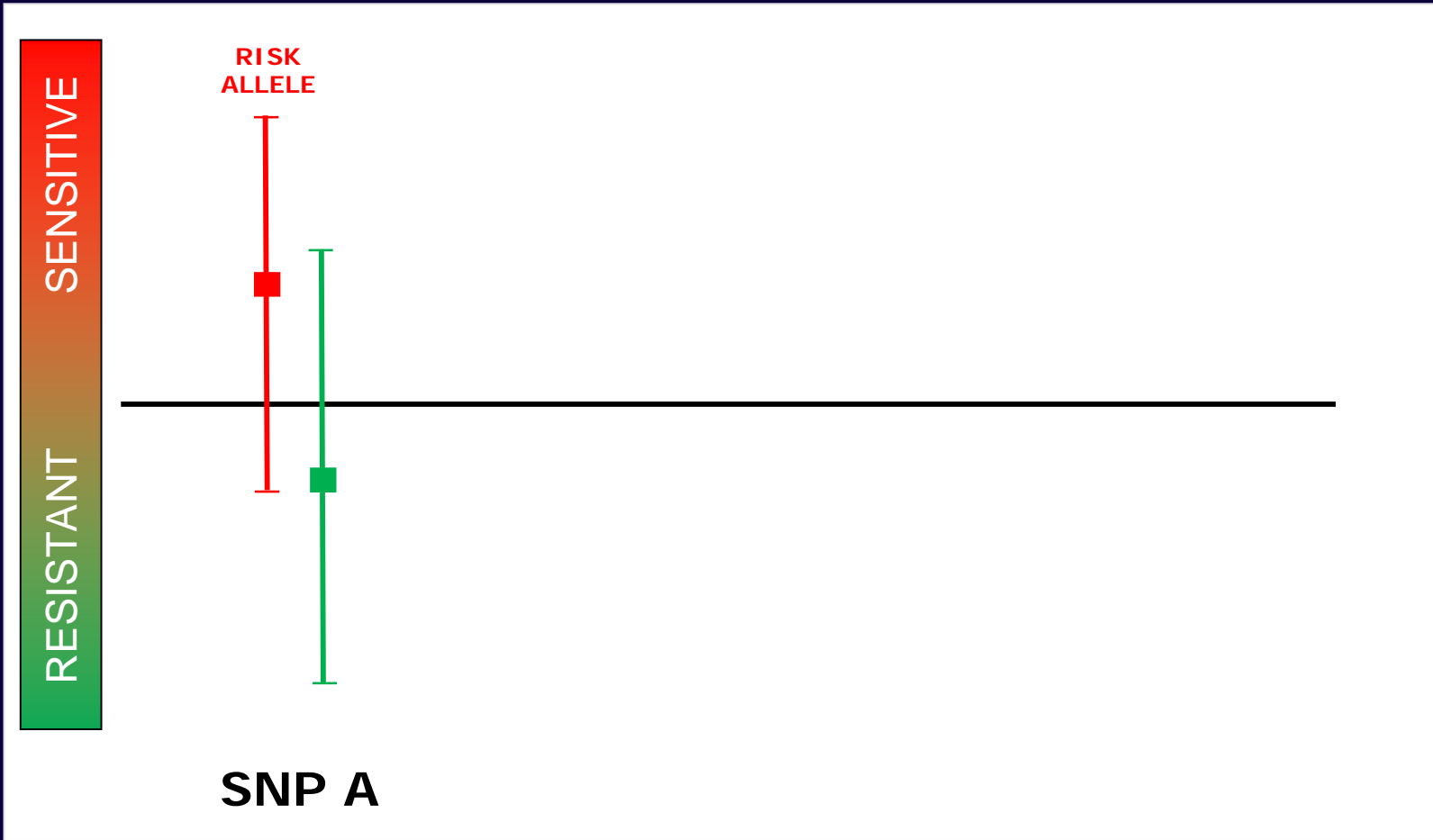
WHY?????

Andreassen CN R&O 2010

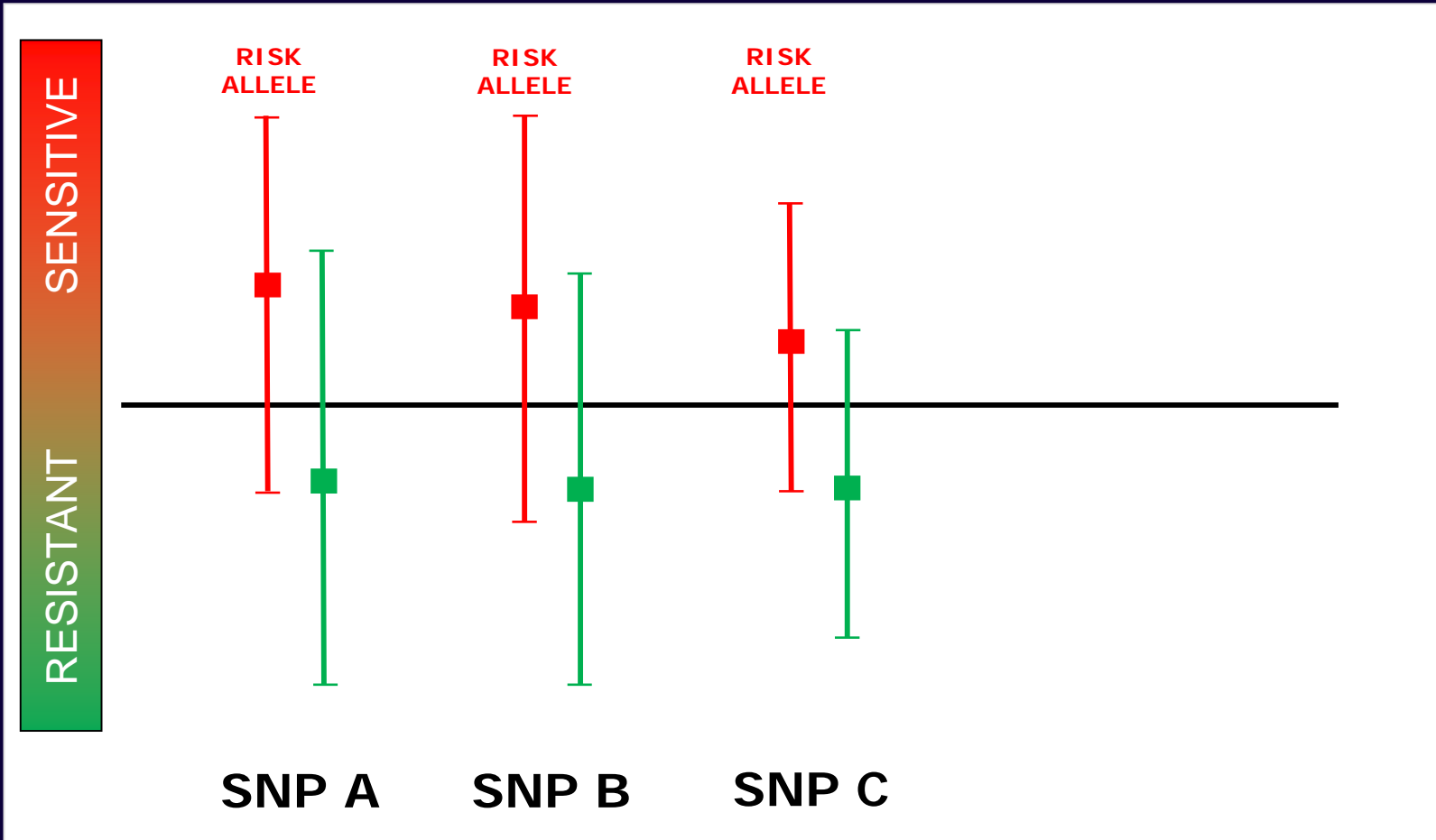
Multiple-SNP models



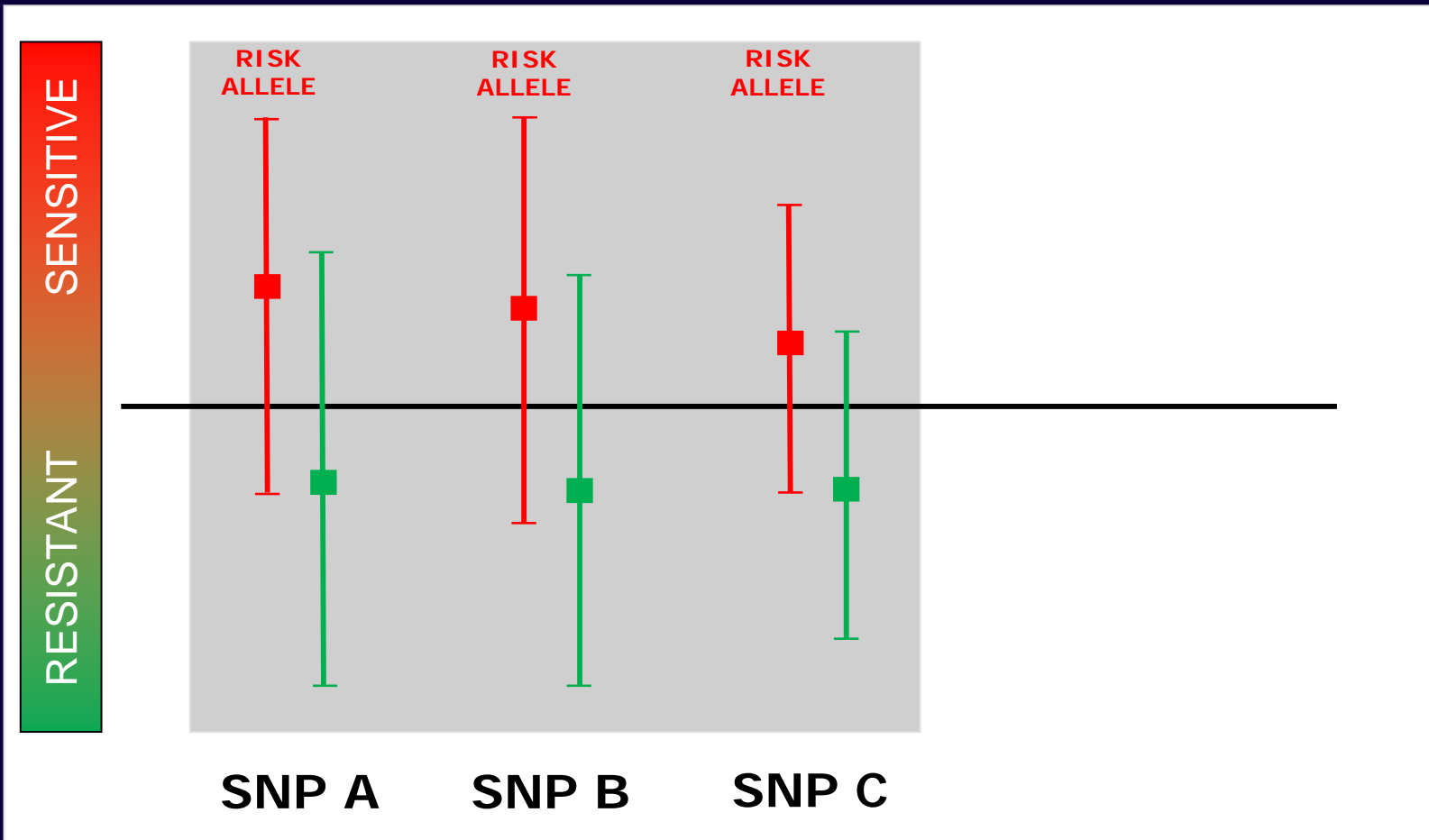
Multiple-SNP models



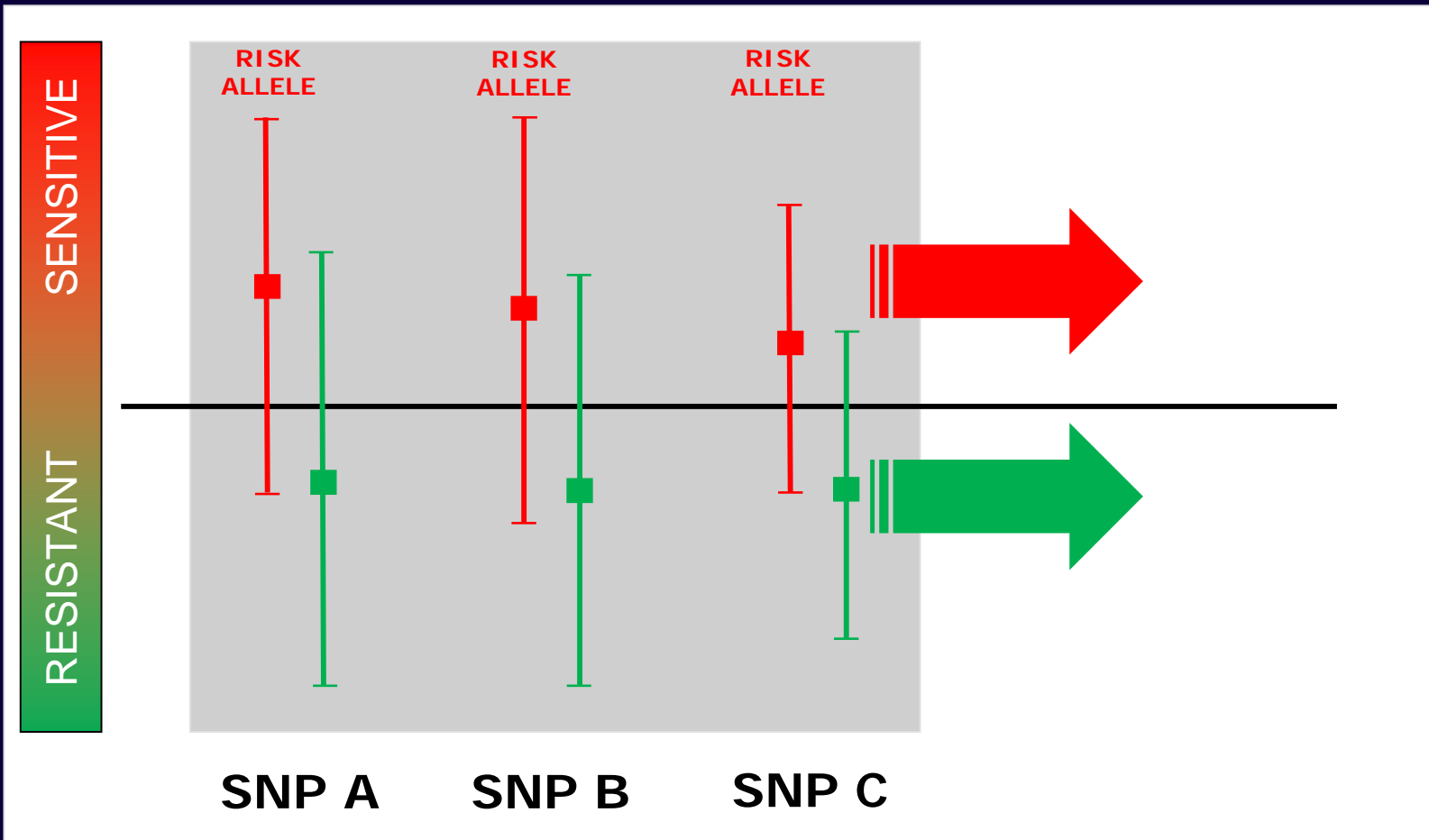
Multiple-SNP models



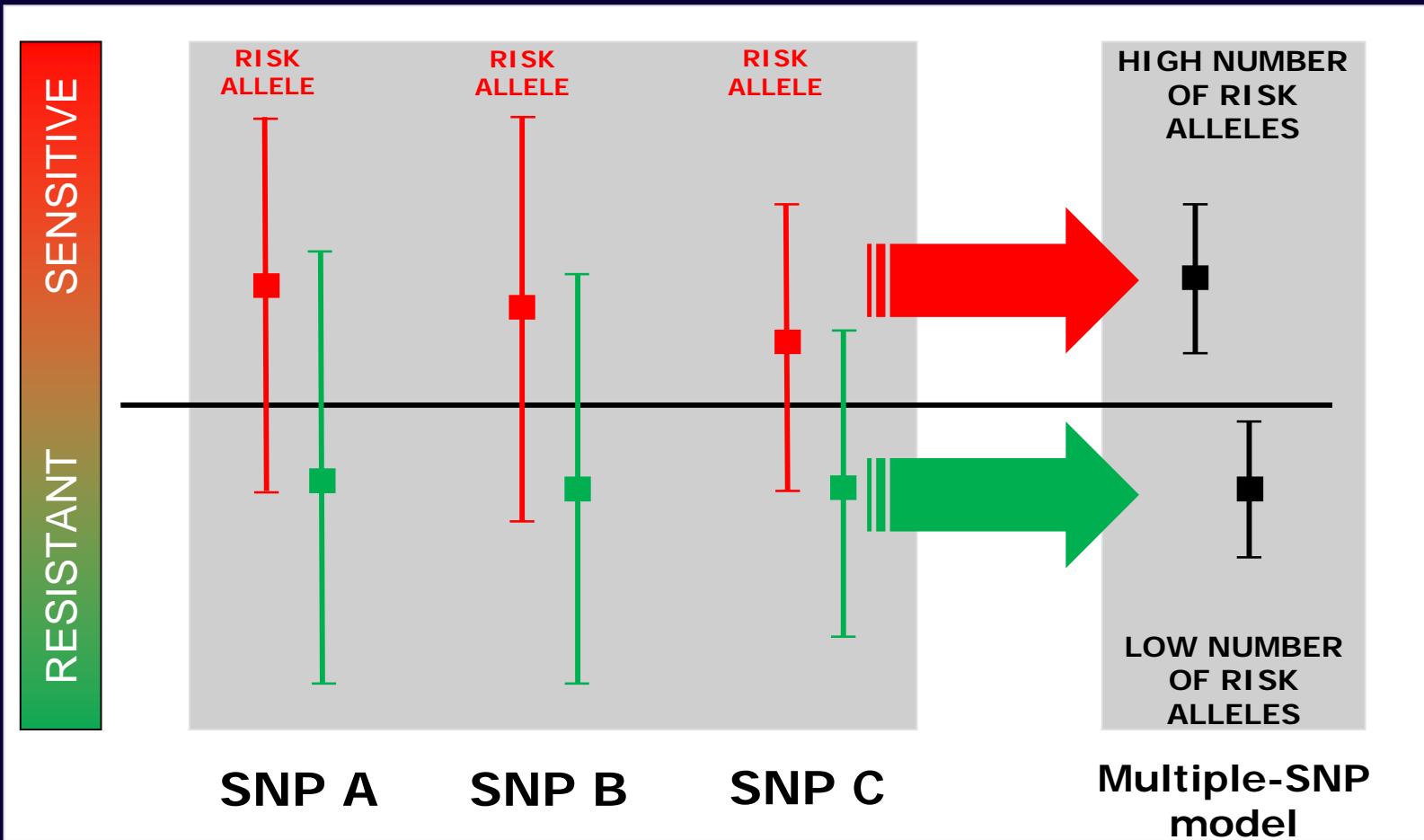
Multiple-SNP models



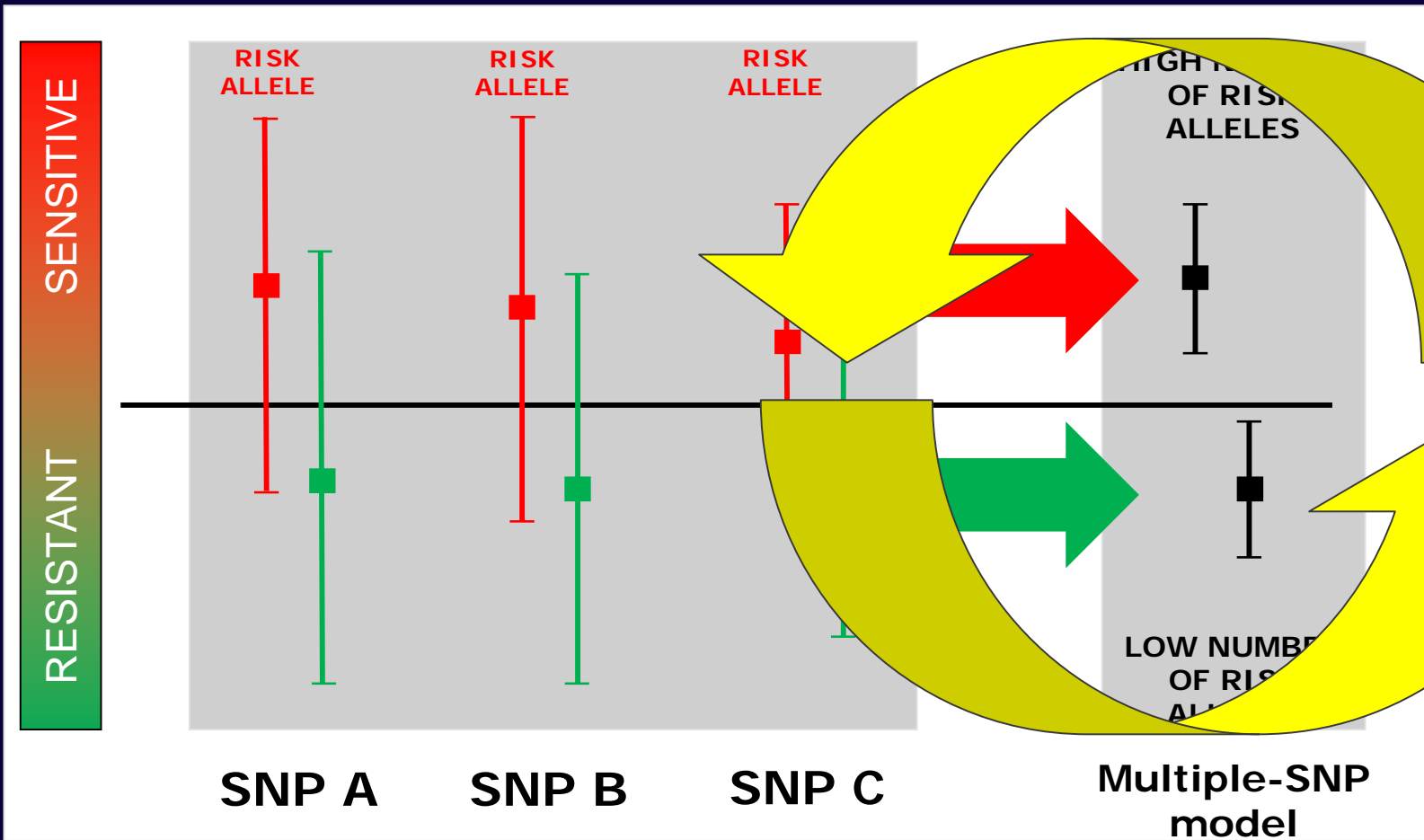
Multiple-SNP models



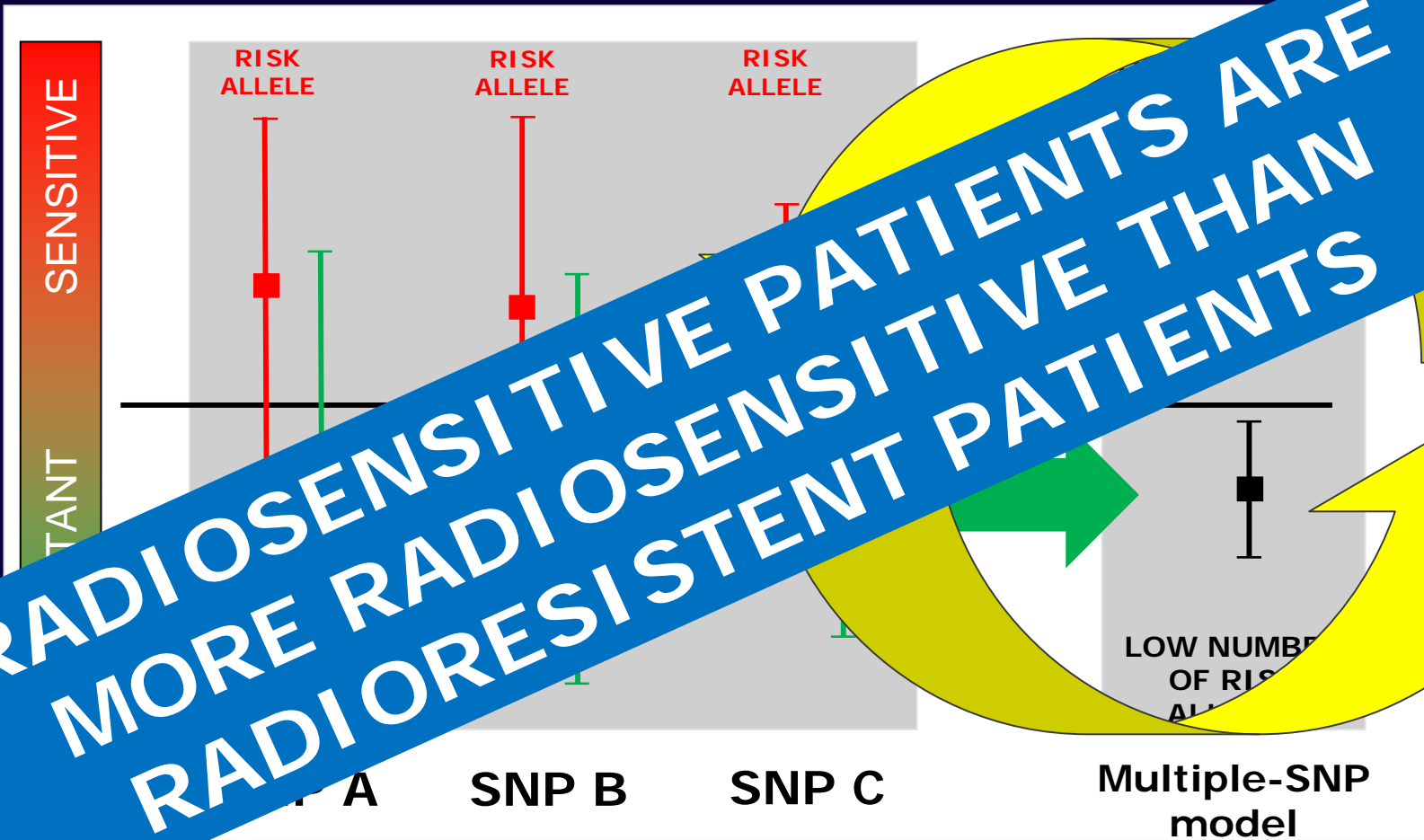
Multiple-SNP models



Multiple-SNP models



Multiple-SNP models



RADIOSENSITIVE PATIENTS ARE MORE RADIOSENSITIVE THAN RADIORESISTENT PATIENTS

**GENOME-WIDE ASSOCIATION STUDY TO IDENTIFY SINGLE NUCLEOTIDE
POLYMORPHISMS (SNPS) ASSOCIATED WITH THE DEVELOPMENT OF ERECTILE
DYSFUNCTION IN AFRICAN-AMERICAN MEN AFTER RADIOTHERAPY FOR
PROSTATE CANCER**

SARAH L. KERNS, PH.D., M.P.H.,* HARRY OSTRER, M.D.,* RICHARD STOCK, M.D.,[†] WILLIAM LI, M.D.,[‡]
JULIAN MOORE, D.O.,[†] ALEXANDER PEARLMAN, PH.D.,* CHRISTOPHER CAMPBELL, B.S.,*
YONGZHAO SHAO, PH.D.,[§] NELSON STONE, M.D.,^{†||} LYNDY KUSNETZ, B.A.,[†]
AND BARRY S. ROSENSTEIN, PH.D.^{†¶}

IJROBP 2010

N=79



**Erectil
dysfunction**

**Association with
FSHR SNP**

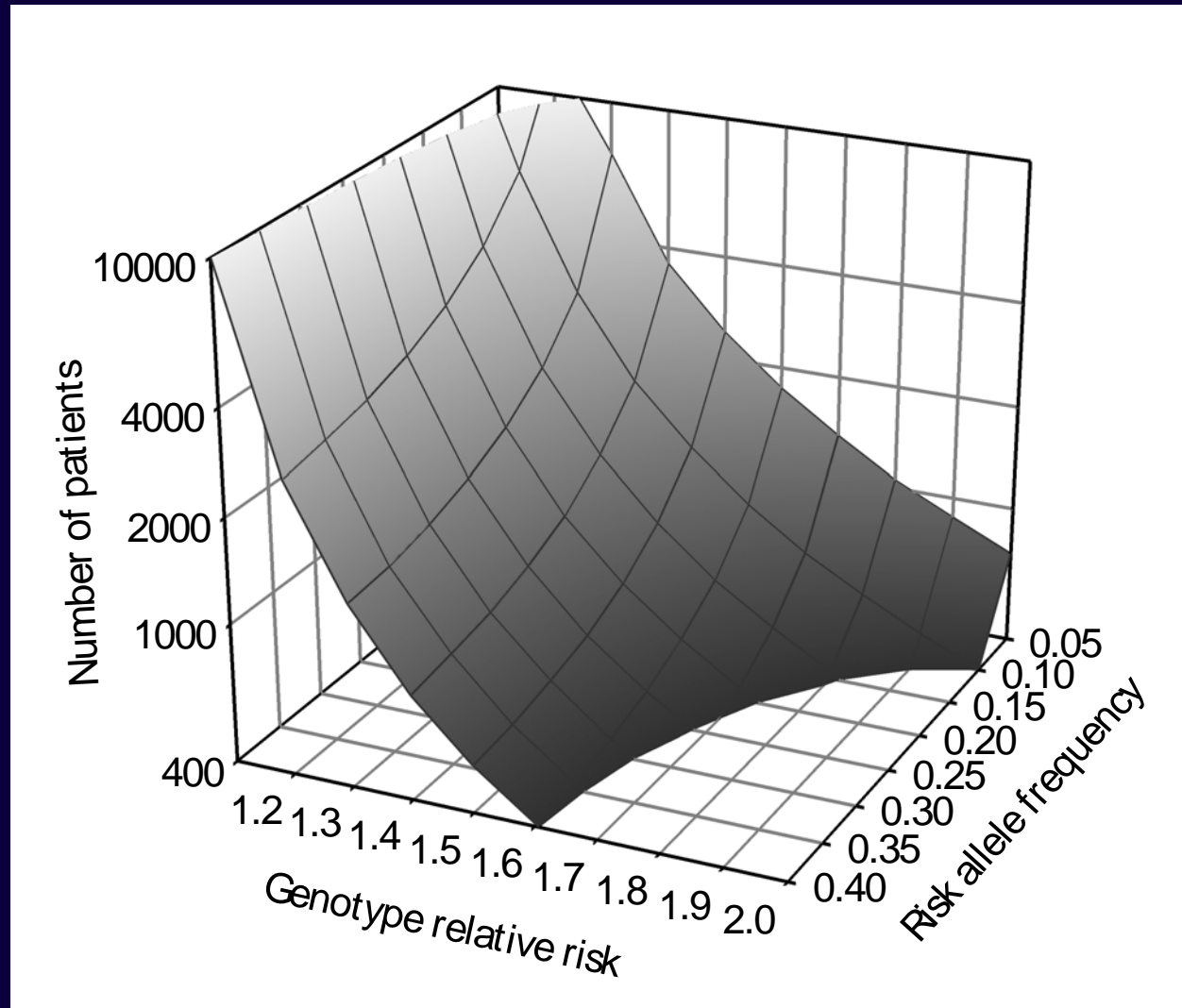
OR 7.03

$p=5.46 \times 10^{-8}$

Bonferroni

$p=0.028$

Number of patients needed to obtain 80% power



Andreassen and Alsner, R&O 2009

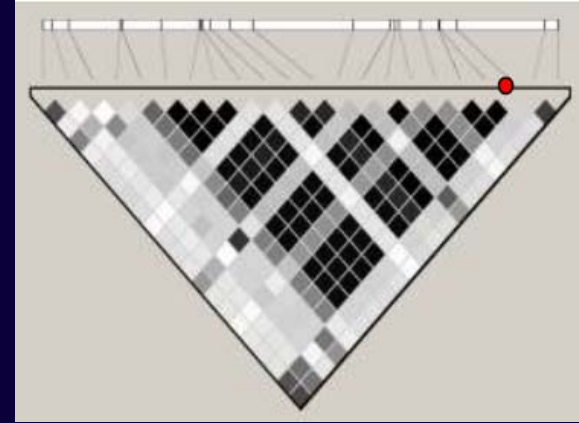


Genome-wide association studies



5,000 – 50,000 subjects

11 million SNPs



200,000 – 500,000 tag SNPs



Genome-wide association studies



11 million SNPs



500,000 tag SNPs

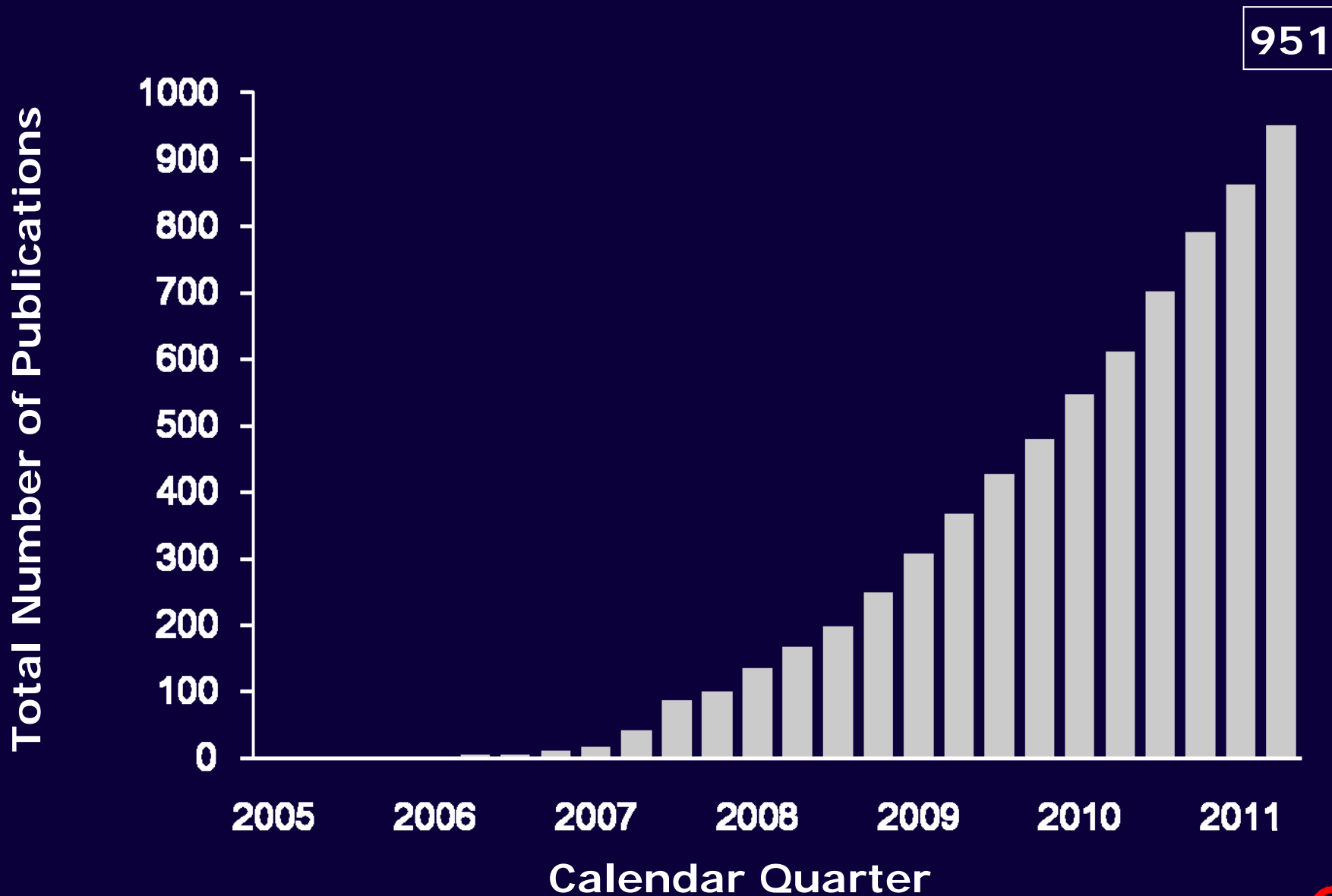


Around 1,000 studies published so far

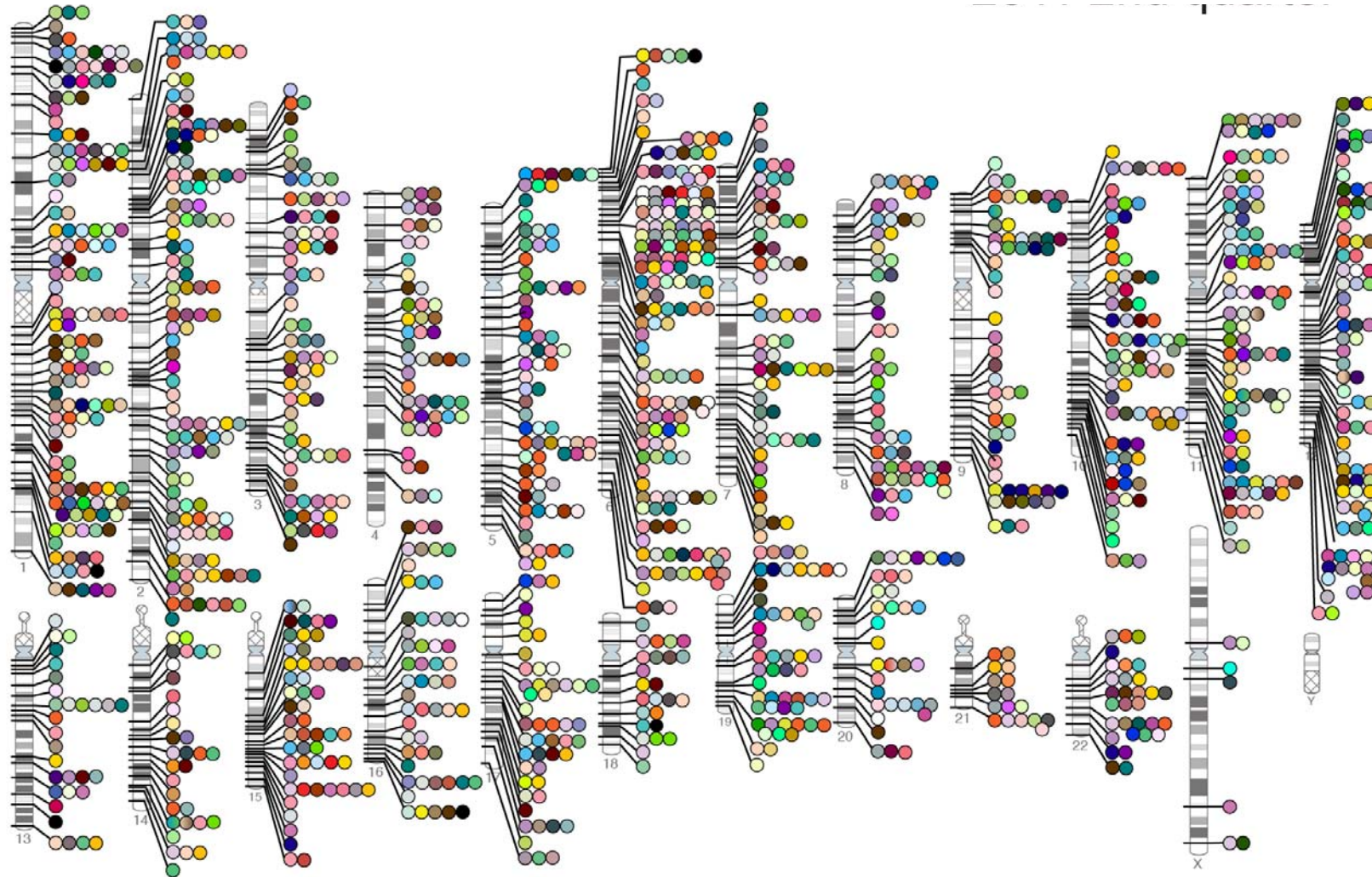
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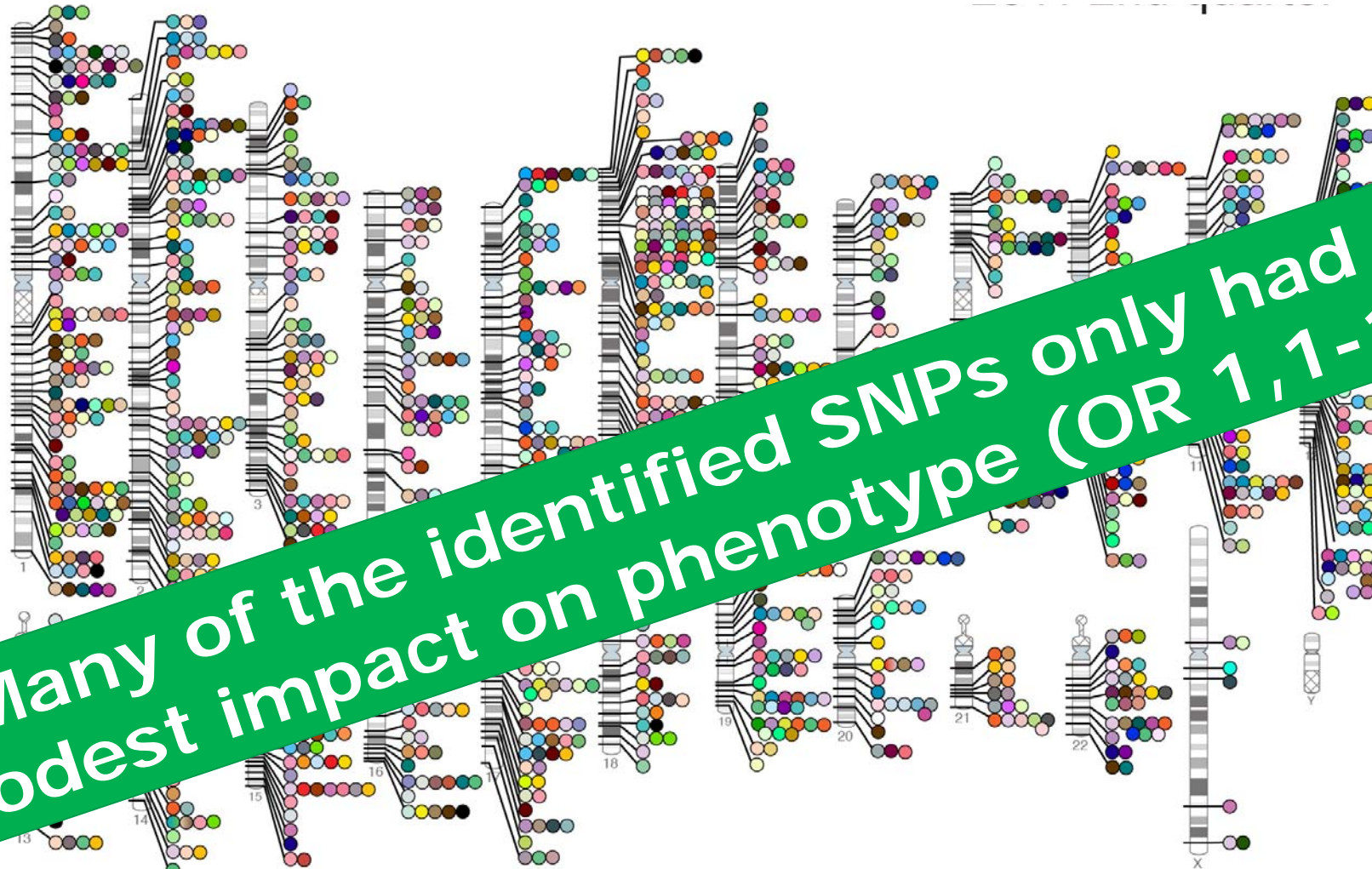
Published GWA Reports, 2005 – 6/2011



Published Genome-Wide Associations through 6/2011, 1,449 published GWA at $p \leq 5 \times 10^{-8}$ for 237 traits



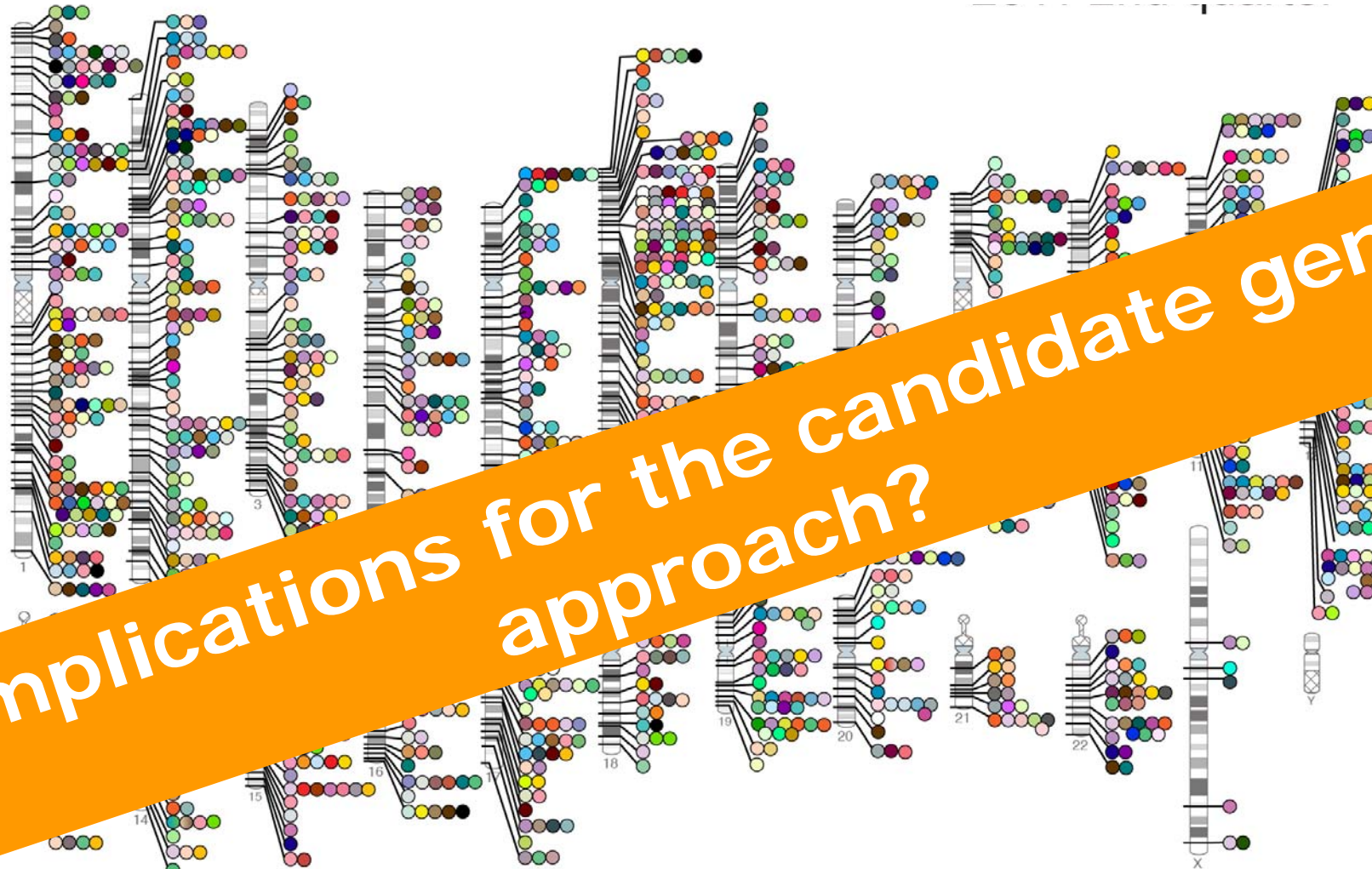
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Implications for the candidate gene approach?

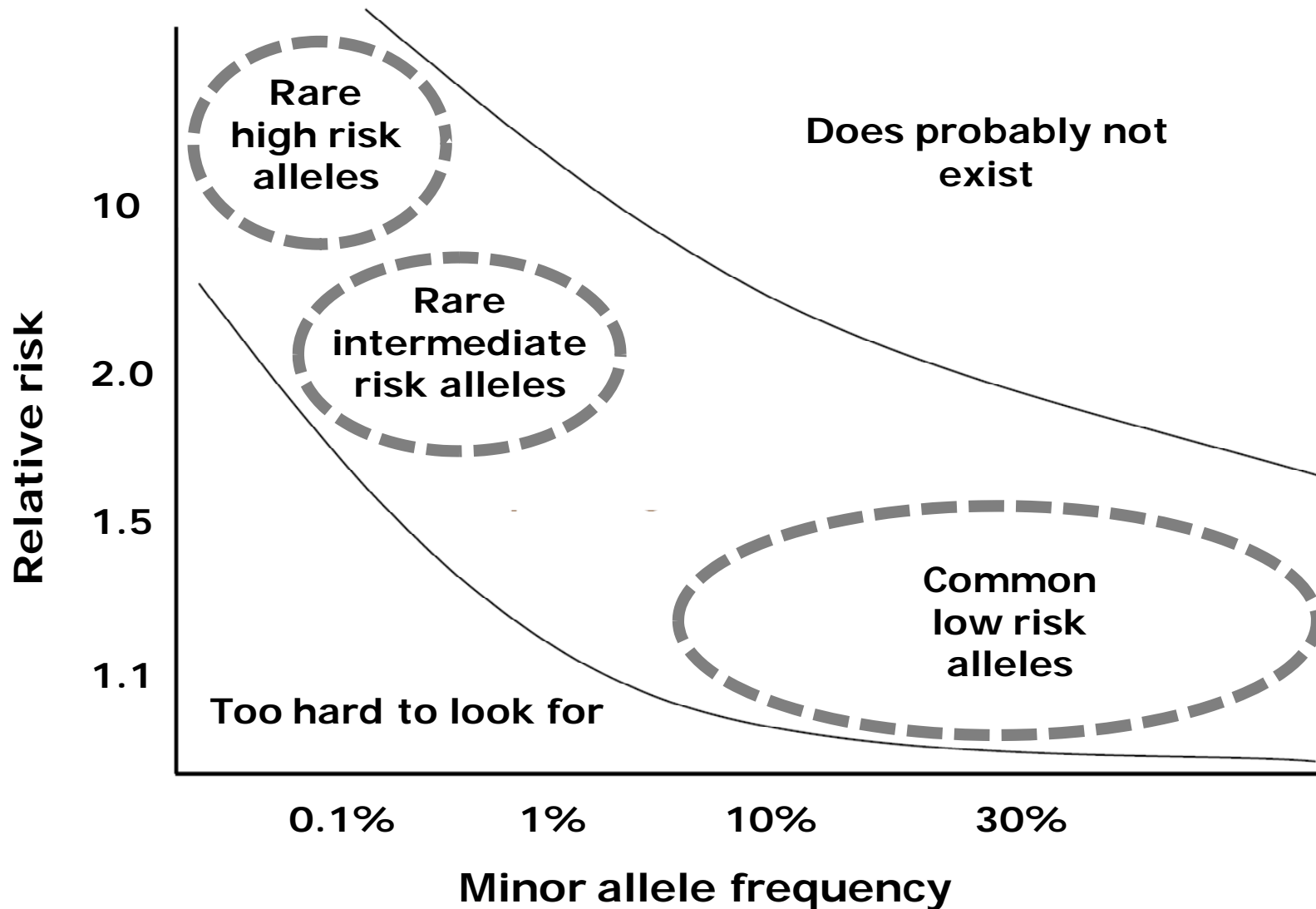


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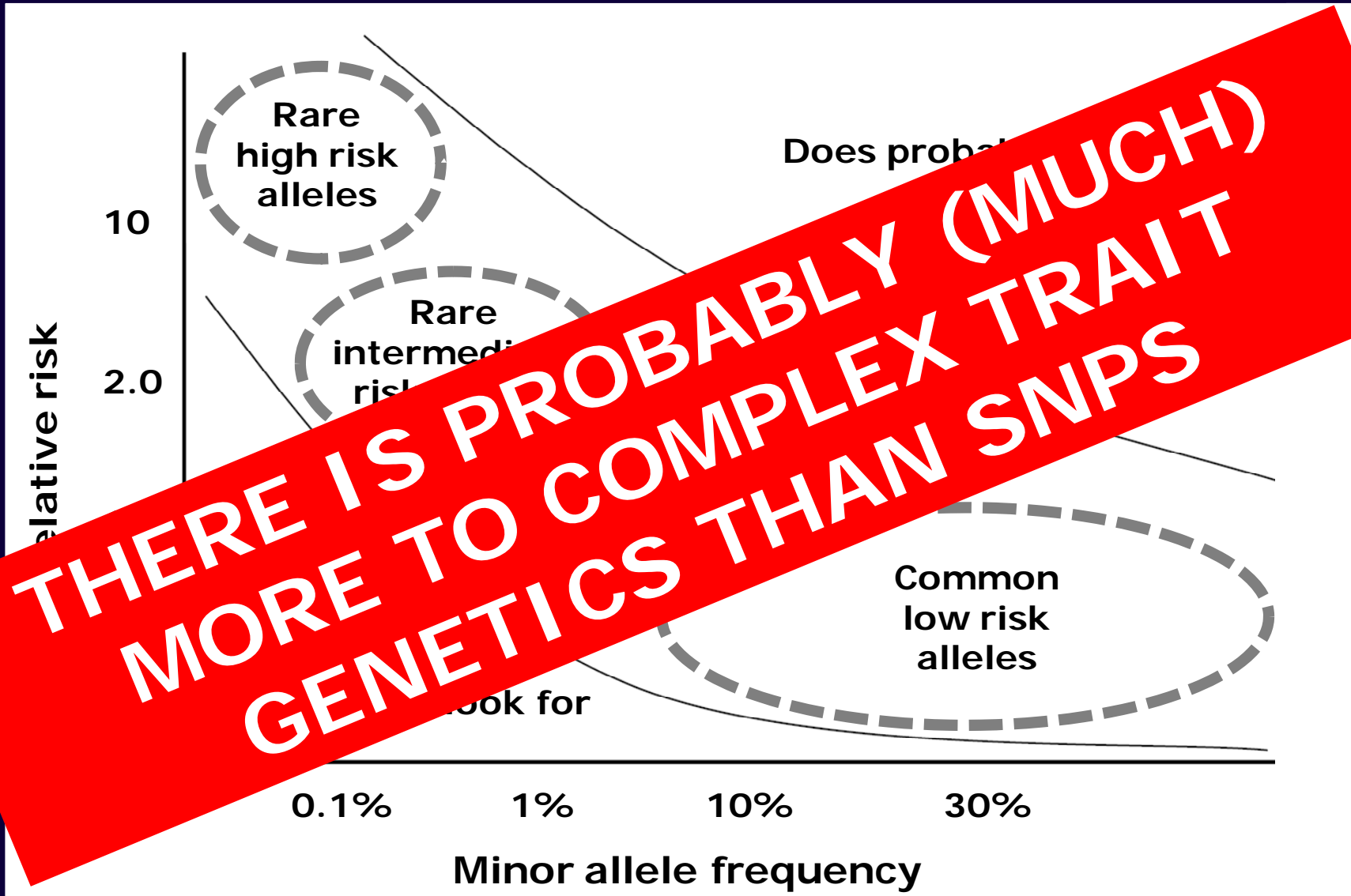


The identified SNPs usually only
accounted for 5-25% of the
expected heritability

A proposed model



A proposed model



Lessons learned

- 1. The typical impact on phenotype of common alterations (i.e. SNPs) is probably rather small**
- 2. Alterations with major impact on phenotype are presumably very rare**
- 3. The number of alterations in the genome is immense and the candidate gene approach has not been very successful**





A lot of needles need to be found before we can tailor treatment

HOW TO PROCEED?



HOW TO PROCEED?



HOW TO PROCEED?



HOW TO PROCEED?





Well-powered SNP studies
Large GWASs
Studies based on sequencing?





Think big
Establish international
cooperation



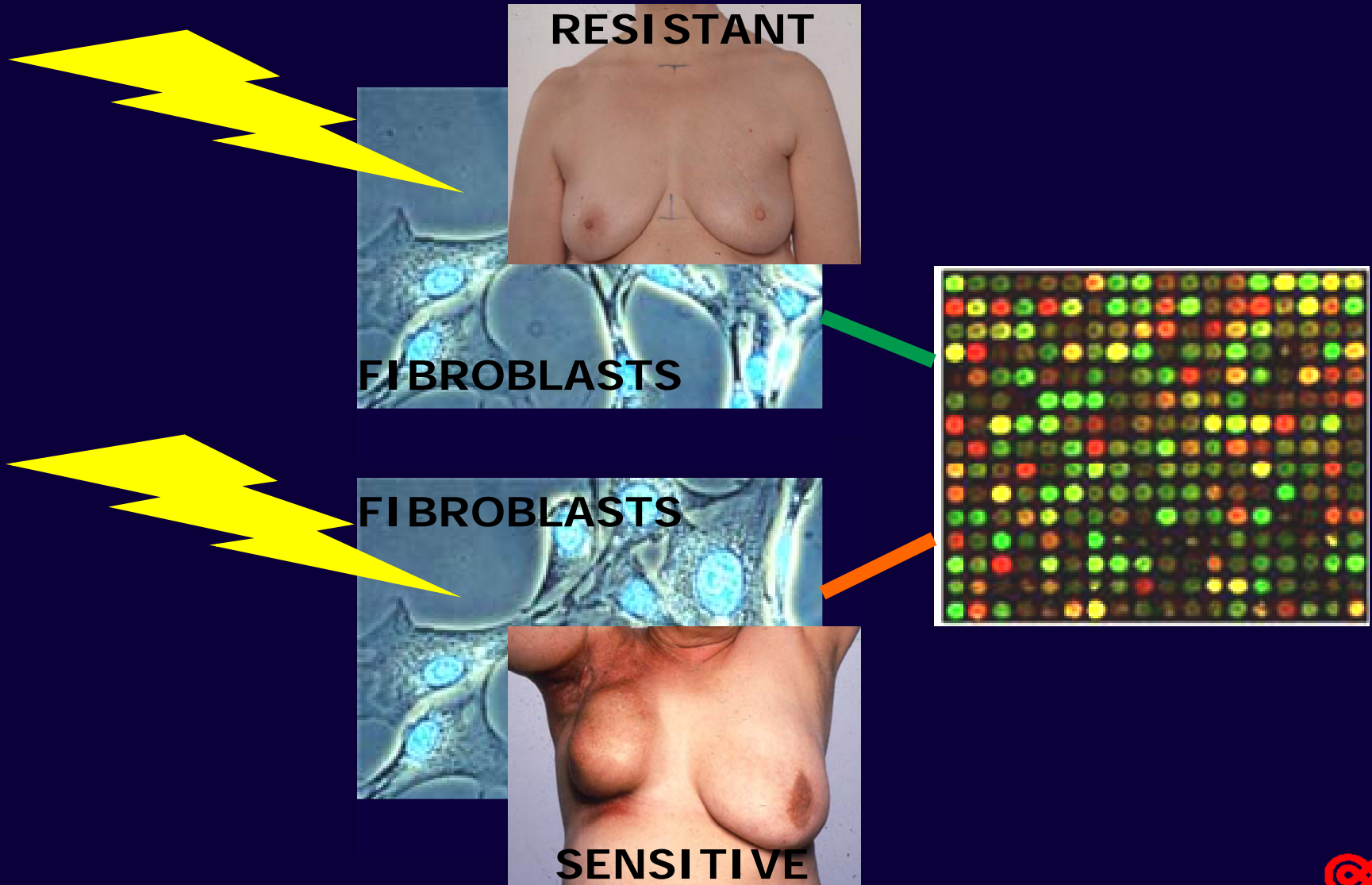
ESTRO GENEPI
RAPPER GENEPAR
RadGenomics
International Radiogenomic
Consortium



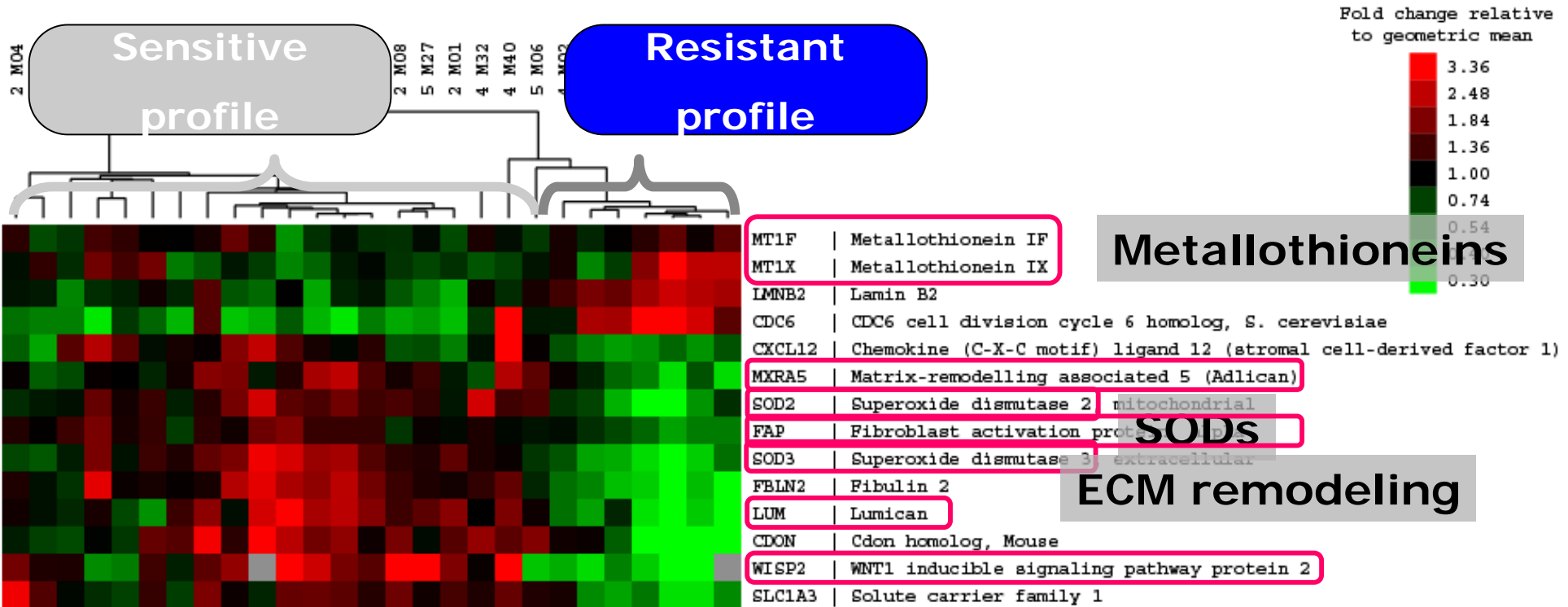
Alternative approaches??

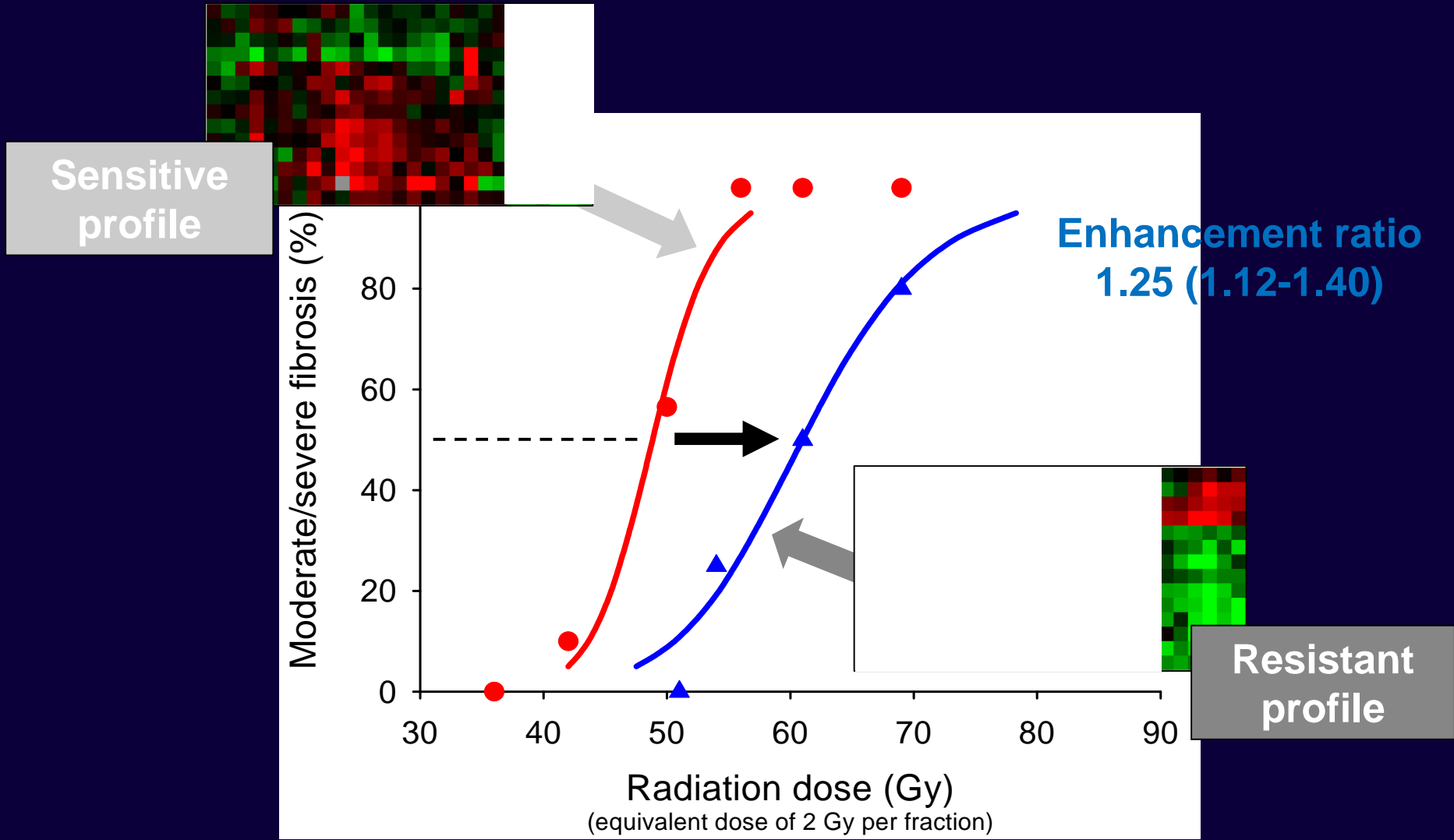


Gene expression profiling



- Extended cohort, qPCR





Thank you for your
attention!

nicolaj@oncology.dk

