

Molecular susceptibility for lung cancer and nicotine addiction

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● Institut national
de la santé et de la recherche médicale
UMR 1086 Cancers & Préventions

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COI disclosures

Investigator in phases I, II & III clinical trials from Lilly, GSK, Roche, MSD, Merck-Serrono, Pfizer, Astra-Zeneca, Sanofi-Aventis, Pierre Fabre, Borhinger, BMS, Novartis, [with no personal payment](#), all honoraria being perceived by my Institution (CHU de Caen, Research Clinical Center), or by the Association A.D.P, located at CHU de Caen, or by IFCT in accordance with their reglementary status and research goals...

SUBVENTIONS OR COLLECTIVE ADVANTAGES

Lilly, Roche, Pfizer, Astra-Zeneca, Sanofi-Aventis, GSK, BMS, Amgen, Chugai, Pierre Fabre, Borhinger-Ingelheim, Merck-Serono, Chugai, Novartis, Janssen-Cilag (**Subventions for IFCT**)

Lilly, Roche, Astra-Zeneca, Clovis Oncology, Pfizer, Gsk-bio, Merck, Pierre Fabre, BMS: Honoraria for advisory boards or talks payed to the **Association pour le Développement de la Pneumologie** (ADP, CHU Caen)

PERSONAL PAYMENTS OR ADVANTAGES

Lilly, Roche, Astra-Zeneca, Pfizer, Merck, Pierre Fabre, Borhinger, BMS
(**invitations at international congresses:** ASCO, ESMO, ERS, CPLF, ELCC, AACR)

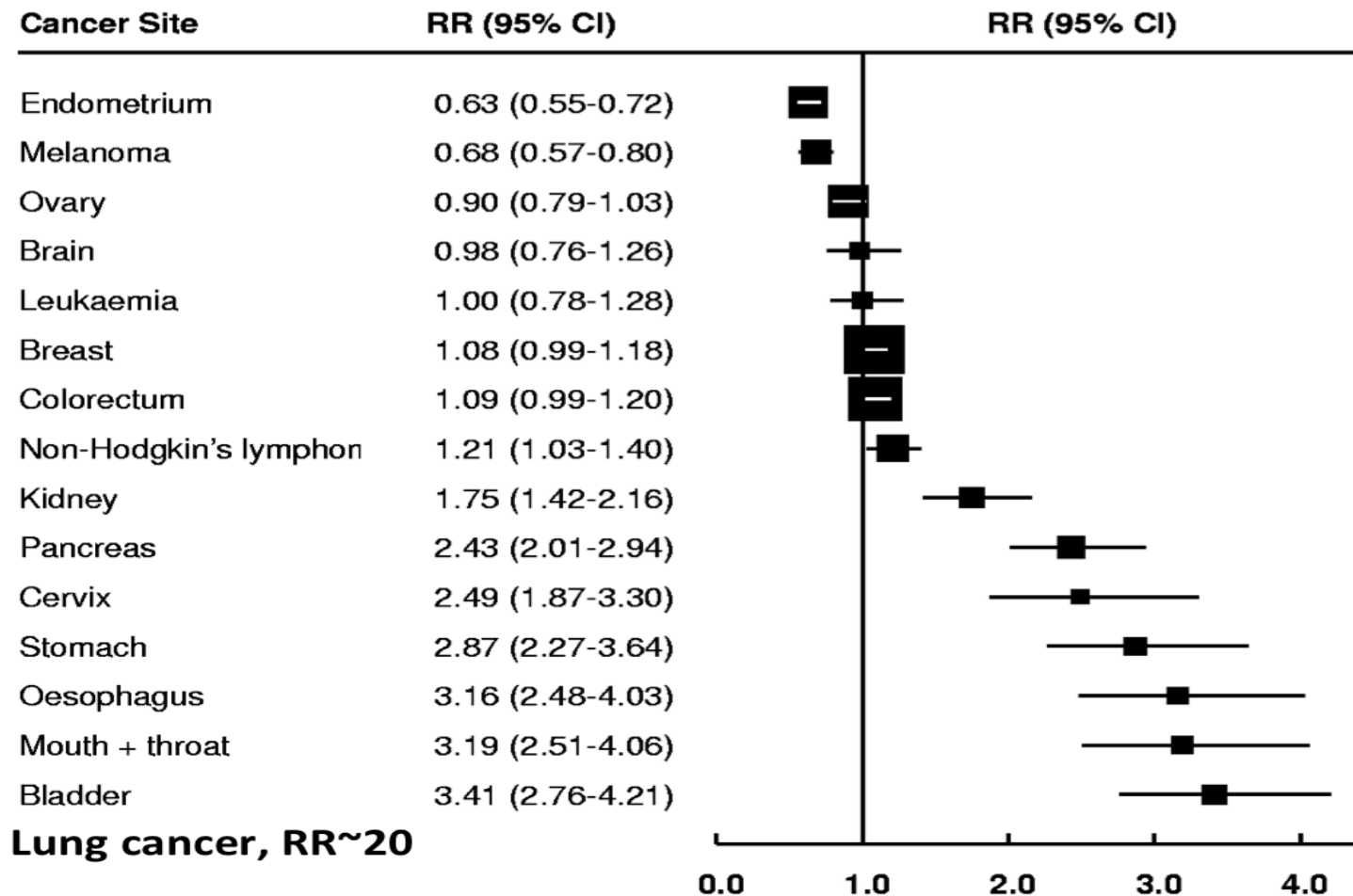
Honoraria for participation to scientific or advisory Boards, organized by Roche, Lilly, BMS, GSK-Bio, Aventis, Clovis Oncology, Pfizer, Borhinger-Ingelheim, Astra-Zeneca:

[The total amount of payments received does not exceed 10, 000 Euros in the last 10 years;](#)

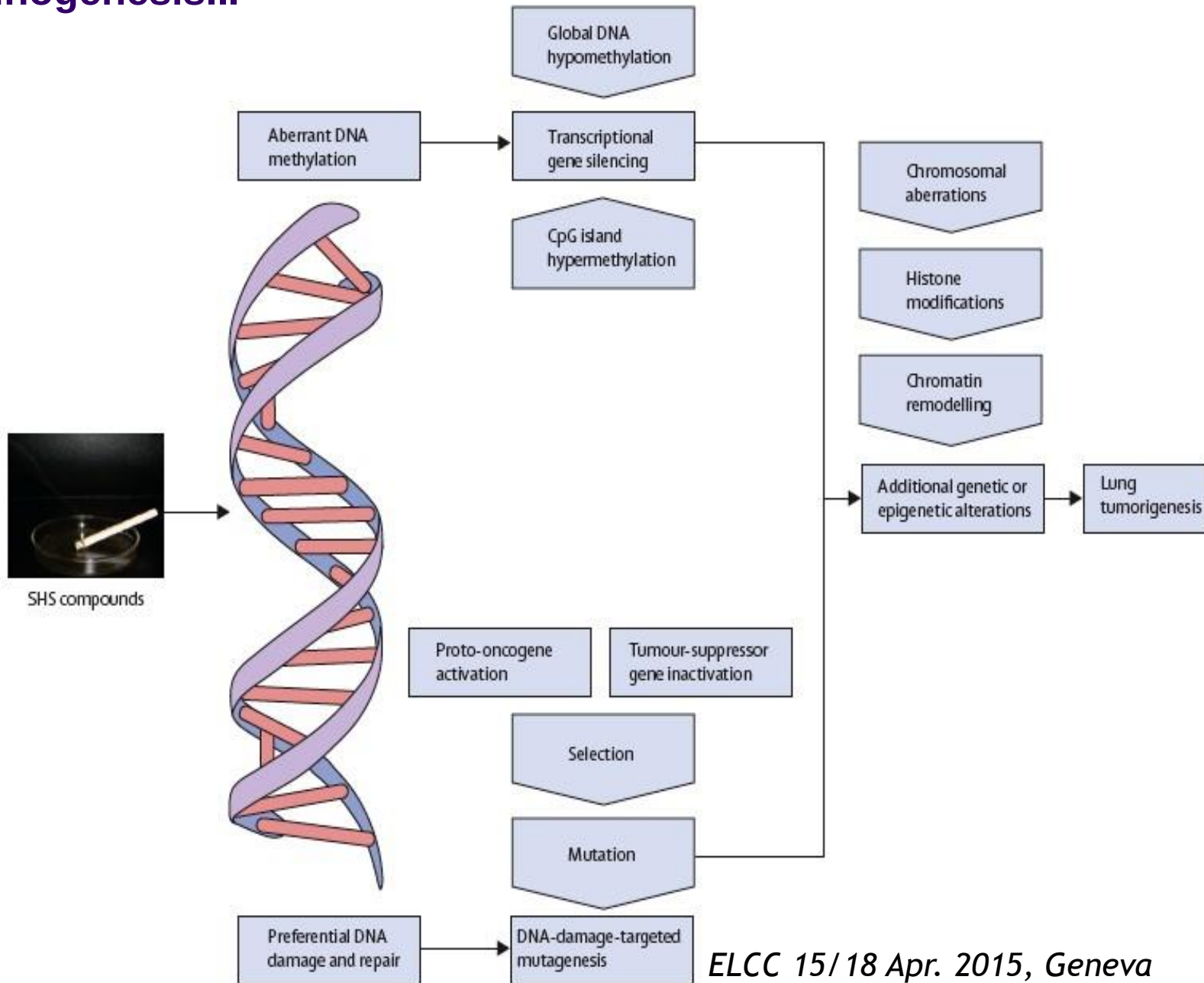
But the author, according to his personal ethic rules and those of the French Society of Pulmonology (SPLF) never had, and will never have any partnership with tobacco Industry

ELCC 15/18 Apr. 2015, Geneva

Smoking & Cancer Risk

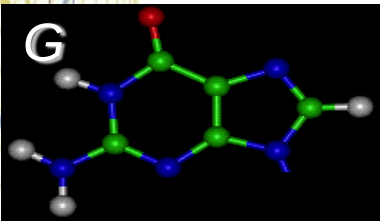
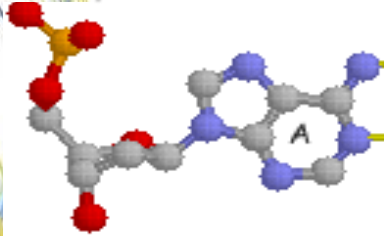


Role of tobacco carcinogens in broncho-pulmonary carcinogenesis...

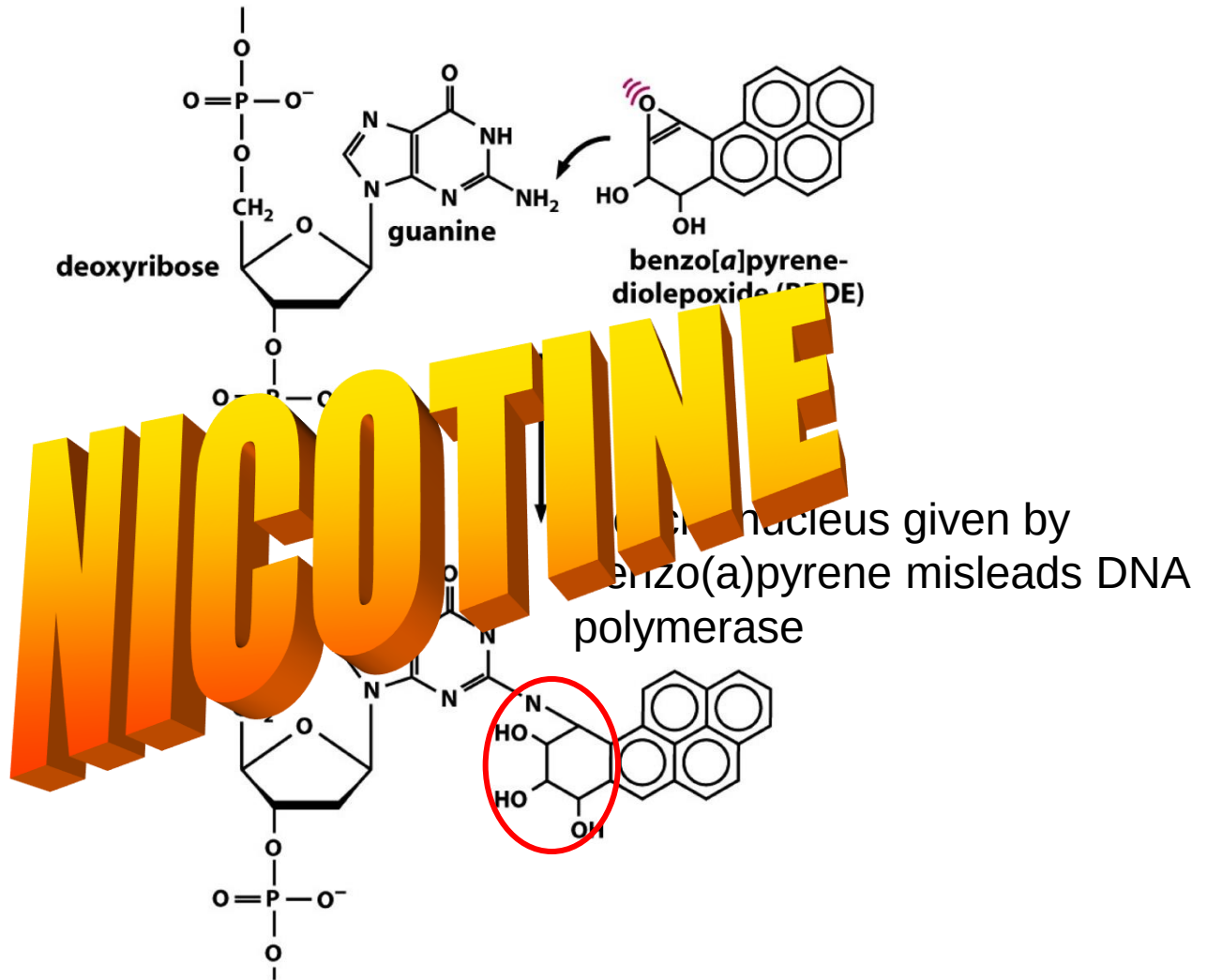
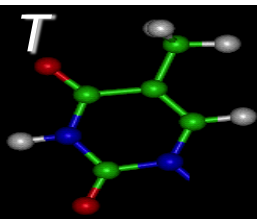
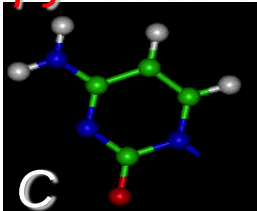


Transversions G->T are linked to direct interaction DNA/ tobacco Benzo(a)pyrene

purins

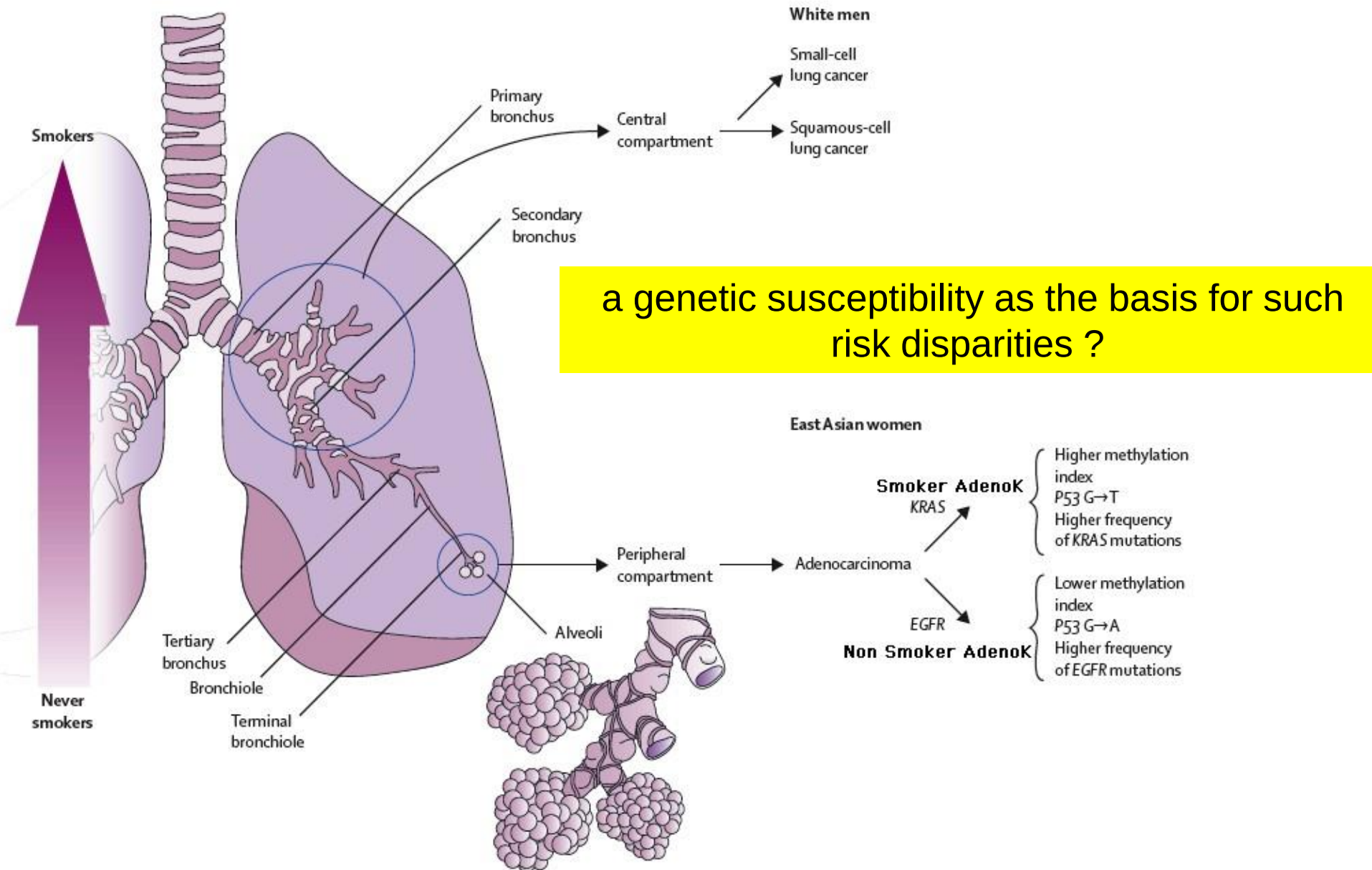


pyrimidins



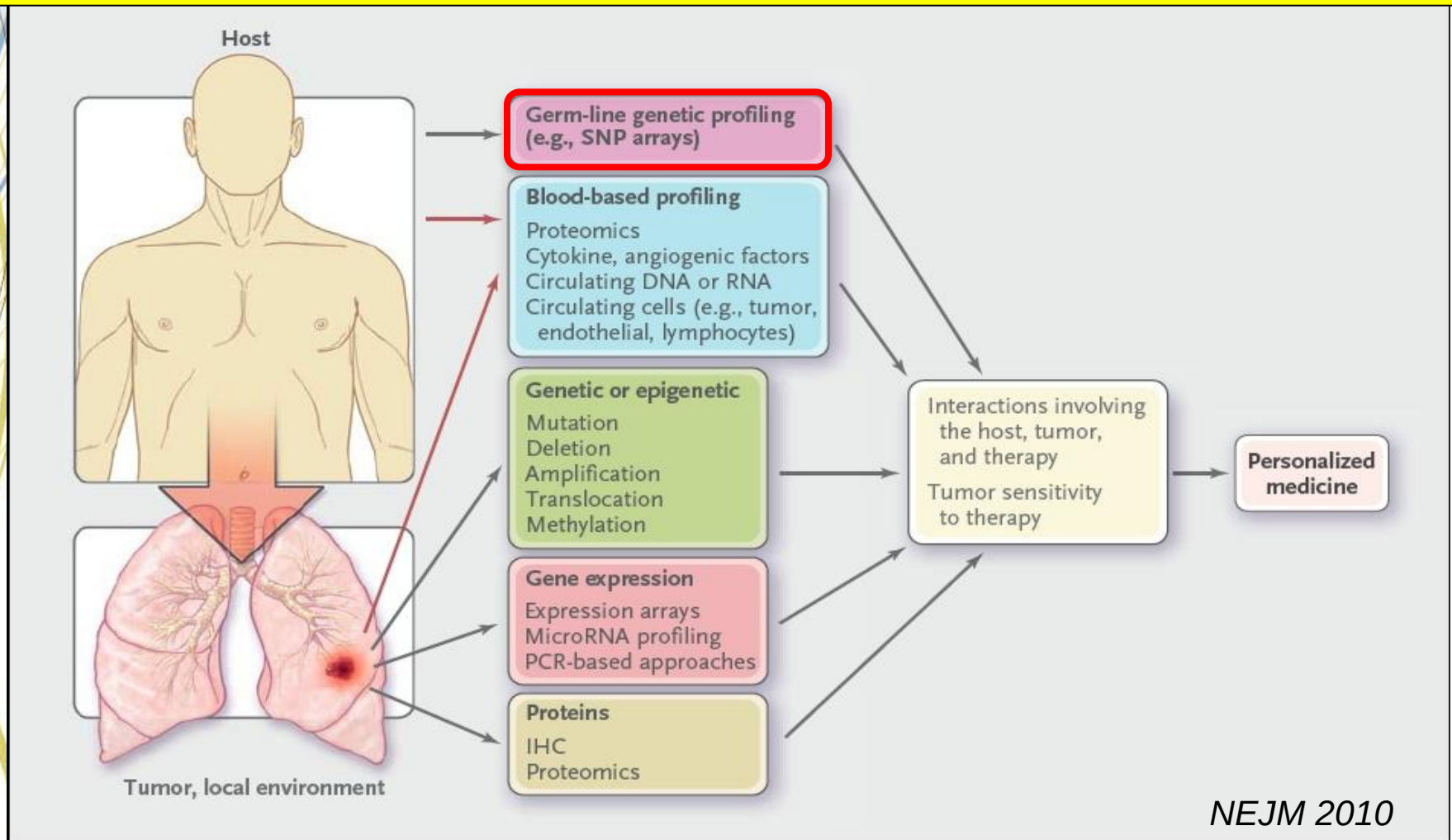
2 genes uppermost altered c/o smokers: p53 et K-Ras

But not all Smokers will suffer from Lung cancer



Personalized Medicine in Non Small Cell Lung Cancer (NSCLC): assessing genetic susceptibility

In which regard genetic susceptibility to lung cancer could influence clinical care or health interventions (favoring smoking cessation in high-risk patients ?) or lung cancer screening ?



"This book is readable and provides solid information." Joseph W. Yates, MD, National Vice President, Research, American Cancer Society

Genome Wide Association Studies

FOR DUMMIES®

Gérard Zalcman
MD PhD

Professor of Pulmonary
Medicine

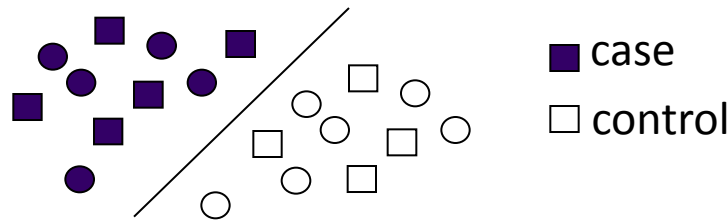
A Reference for the Rest of Us!



FREE eTips at
dummies.com

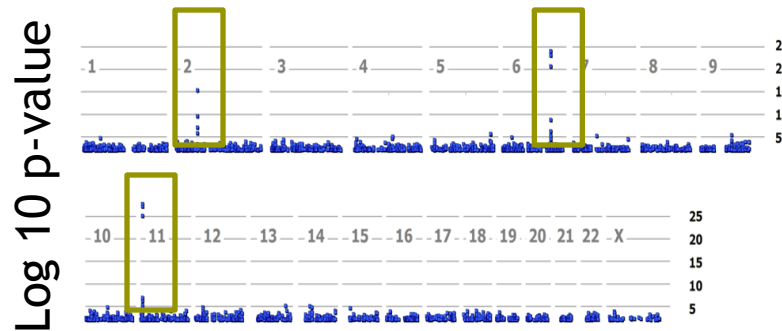
Detection of frequent disease-associated variants: **genome-wide association studies**

1.



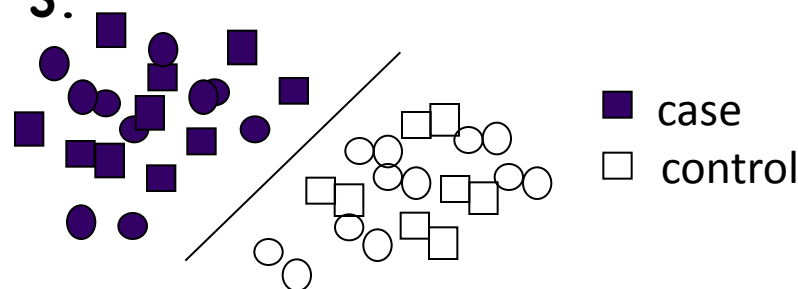
Scan genome for all in DNA collection with 317,000 variants

2.

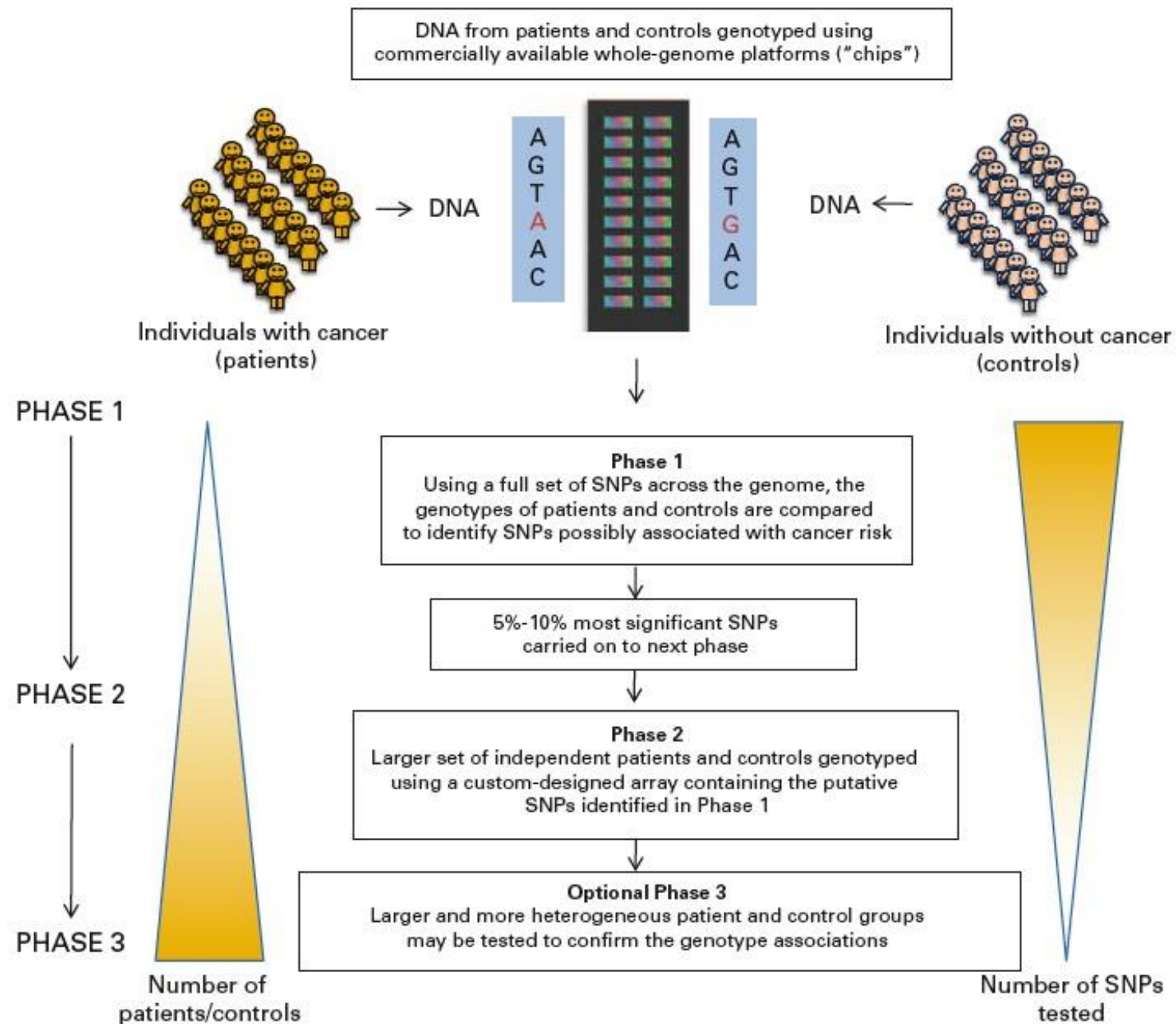


Identify regions of marked differences in frequencies of variants in cases/controls

3.



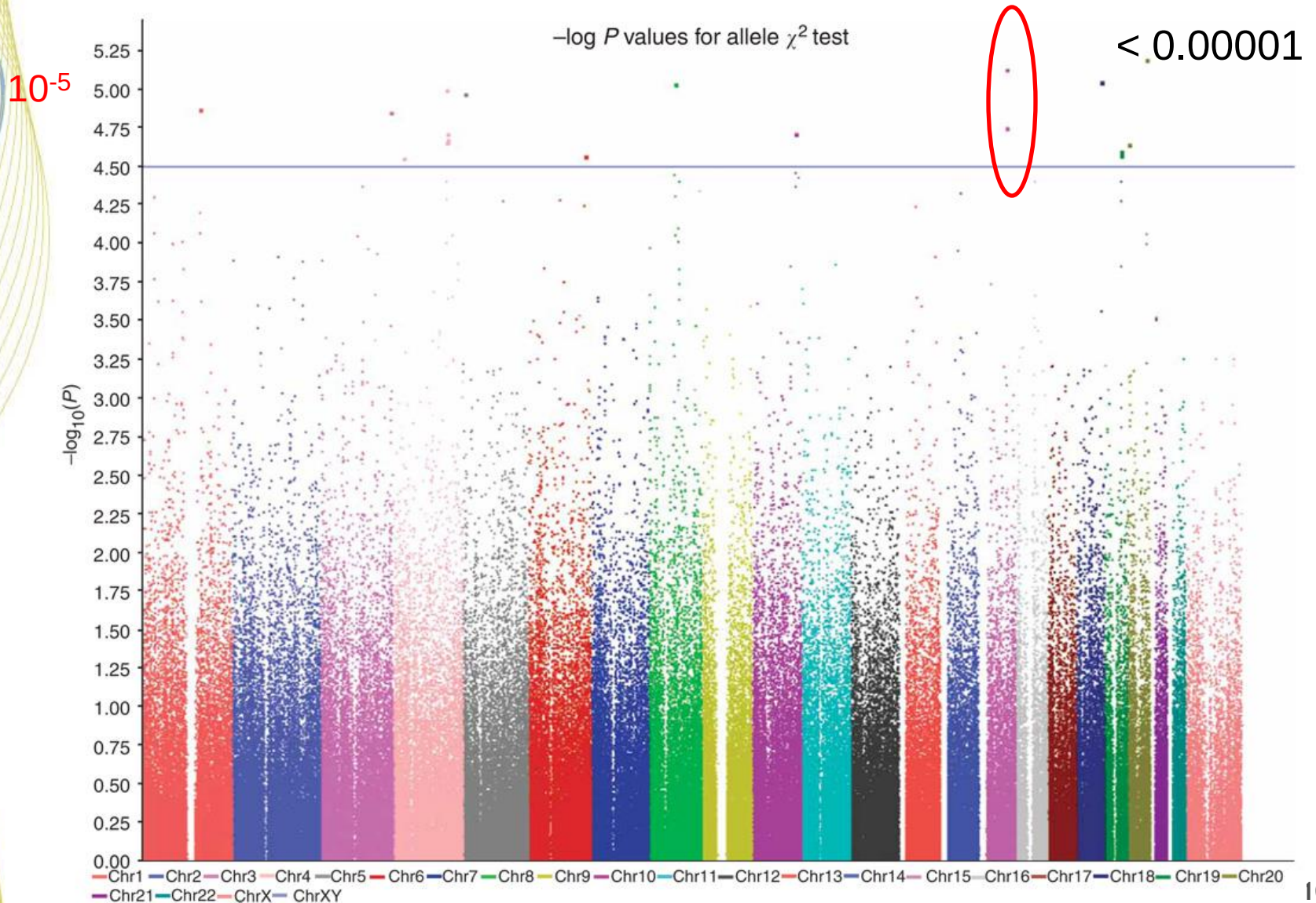
Confirm in additional larger collections



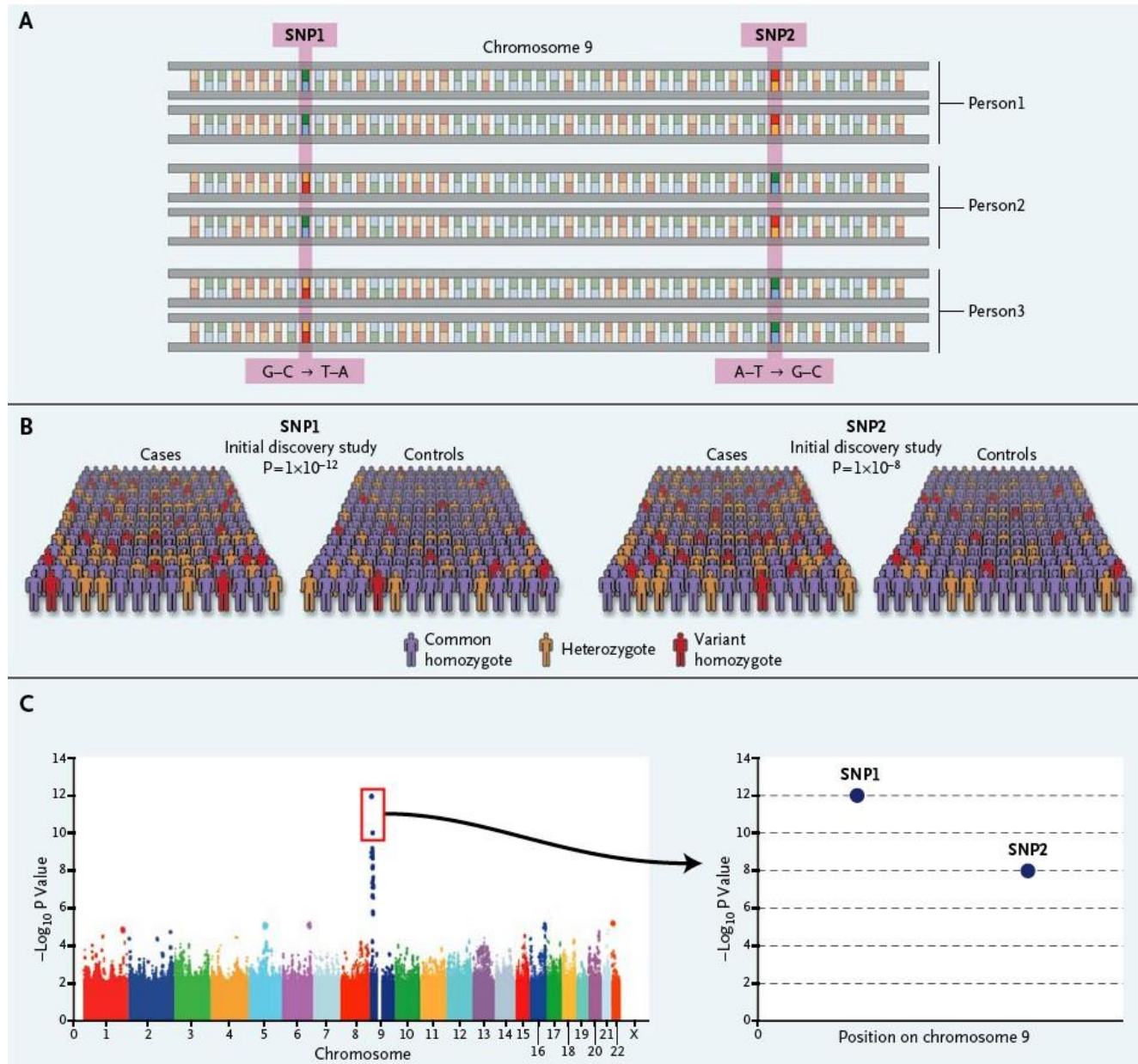
International consortiums in Cancer Genomics

- **Lung cancer:** Hung *et al.* Nature 452:633-7, 2008;
McKay *et al.* Nat Genet. 40:1404-6, 2008;
Lips *et al.* Int. J. of Epidemiol, 2009;
Landi *et al.* Am J Hum Genet 85:679-691,2009.
- **Head & neck cancers:** McKay *et al.* PLoS Genet.;7(3):e1001333, 2011
- **Kidney cancer:** Gudmunsson *et al.* Nat Commun;4:2776,2013
Henrion *et al.* Hum Mol Genet;22: 825-31, 2013
Perdue *et al.* Nat Genet;43(1):60-5,2011
- **Melanoma:** Bishop *et al.* Nat Genet. 41:920-5, 2009
- **Glioma:** Shete *et al.* Nat Genet. 41:899-904, 2009
- **Breast cancer:** Reeves *et al.* Jama 304:426-434, 2010
Travis *et al.* Lancet 375:2143-2151, 2010
- **Prostate cancer:** Al Olama *et al.* Nat Genet 2014,46: 1103-09
- **Pancreatic cancer:** Wolpim *et al.* Nat Genet 2014,46:994-1000

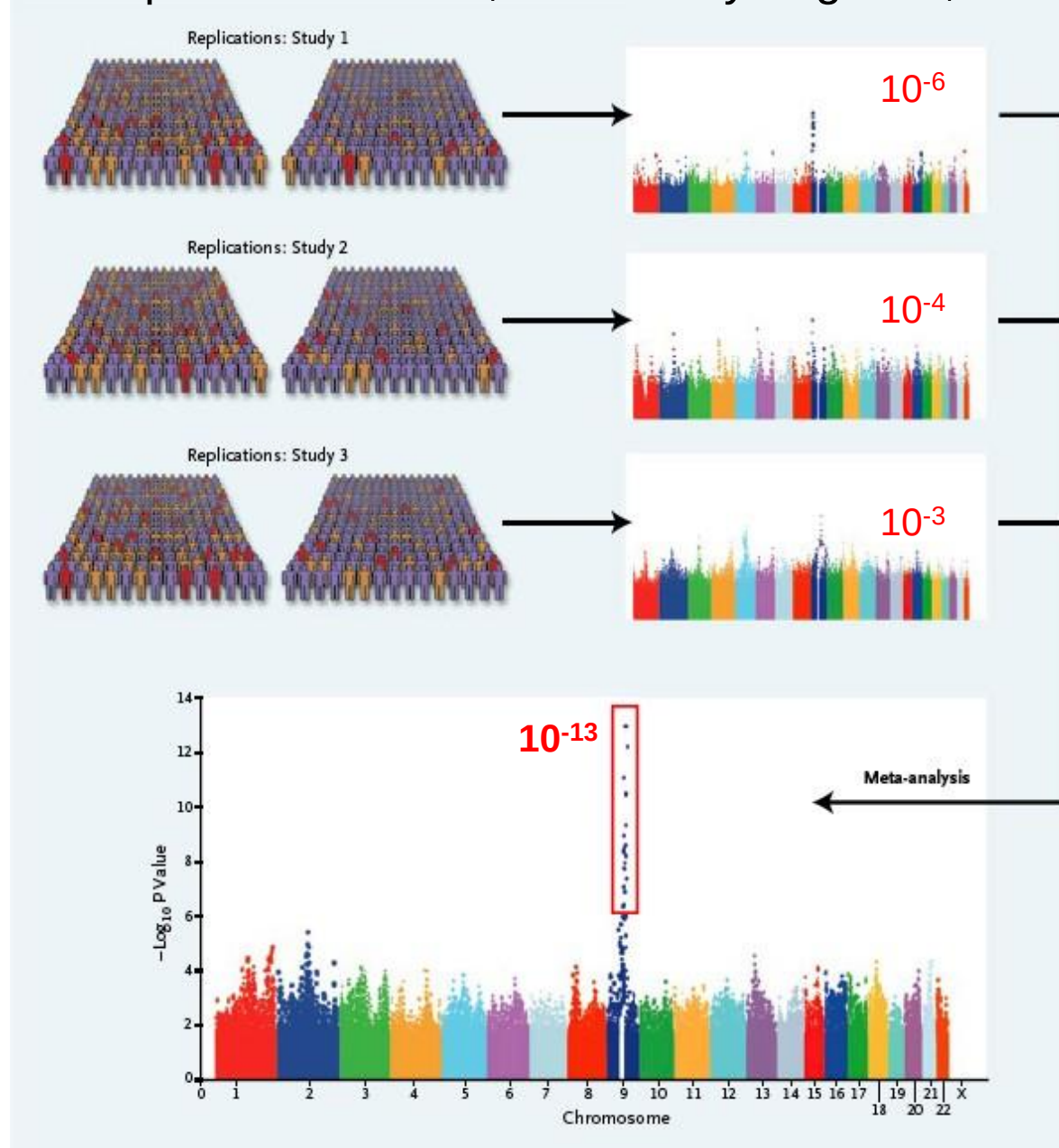
A SNP Identification



GWAS: n markers $\Rightarrow n \cdot 10^4$ subjects (patients & controls)



Meta-analysis of replication studies, individually negative, could be positive



Here the
powers are
added

Lung cancer samples: IARC & CEPH study

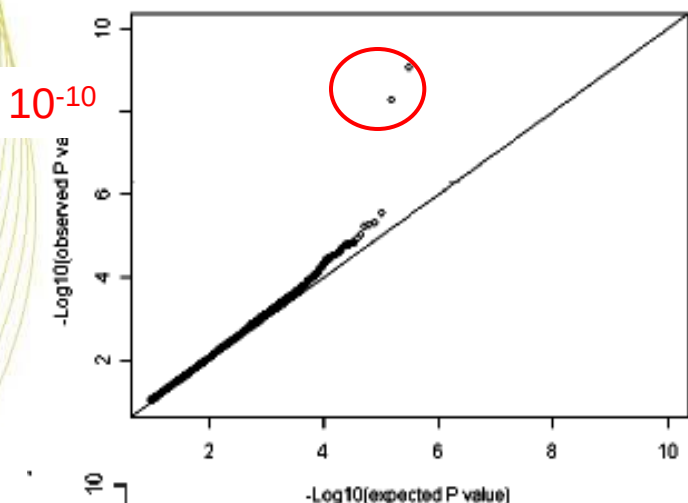


International Agency for Research on Cancer
Centre International de Recherche sur le Cancer

Study	Case	Controls	Countries of Origin
Genome- Wide Association¹			
Central Europe	1 841	2 441	6 Eastern European countries
Toronto	330	500	Canada
HUNT2/ Tromsø	403	412	Norway
CARET	397	392	USA
Total	2 971	3 745	2nd phase GWA
Replication			
EPI C	1 213	2 591	10 Western European countries
Szczecin	908	1 037	Poland
CARET2	363	1 128	USA
Liverpool	415	817	UK
Total	2 899	5 573	3rd phase GWA
Overall Total	5 870	9 318	

¹ After QC & PCA

Lung cancer 1st phase GWA scan



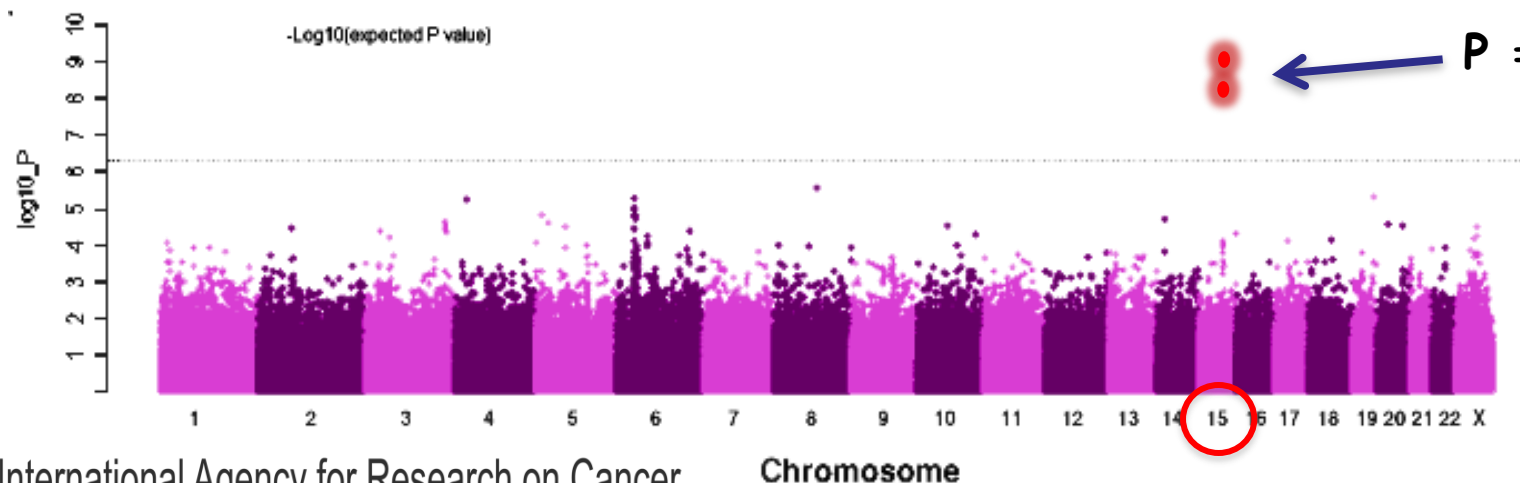
1989 lung cancer cases

2625 hospital matched controls

310,023 SNPs

Genome-wide significance

$p < 5 \times 10^{-7}$

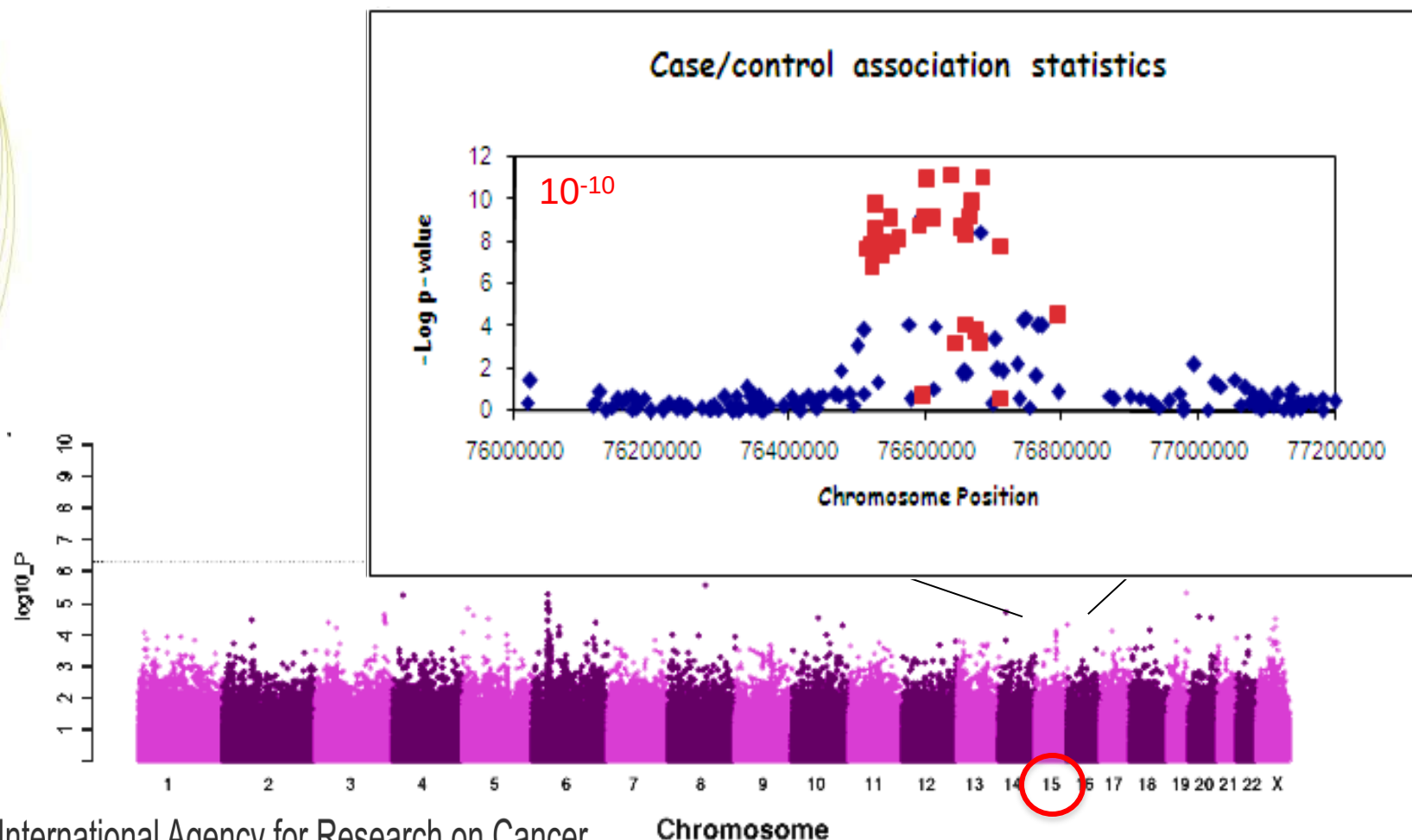


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Lung cancer 1st phase GWA scan

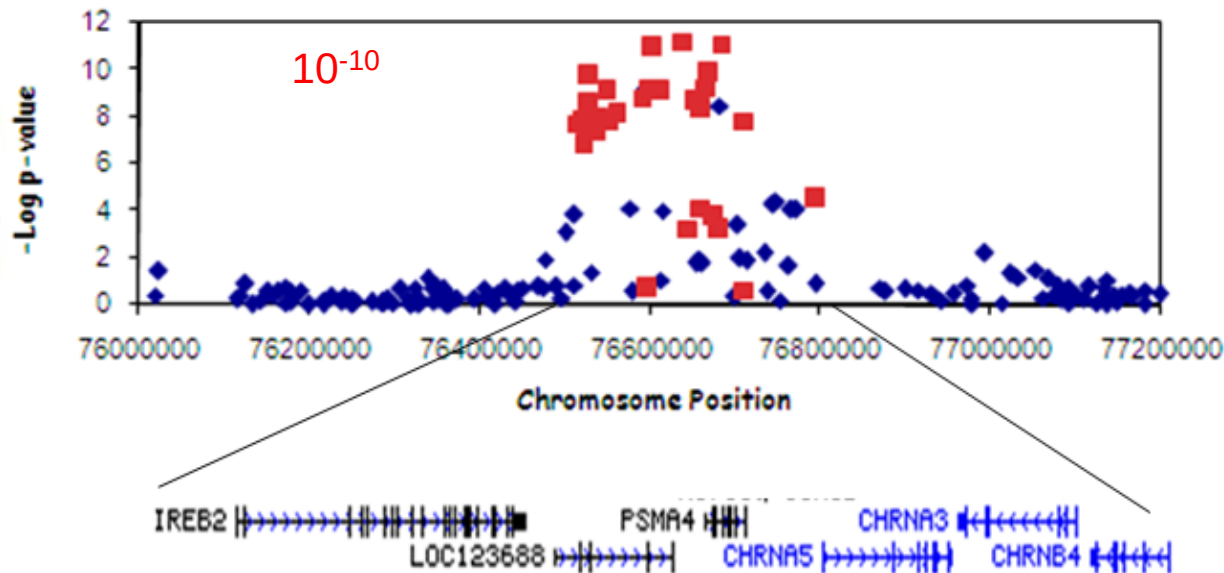


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Lung cancer association region



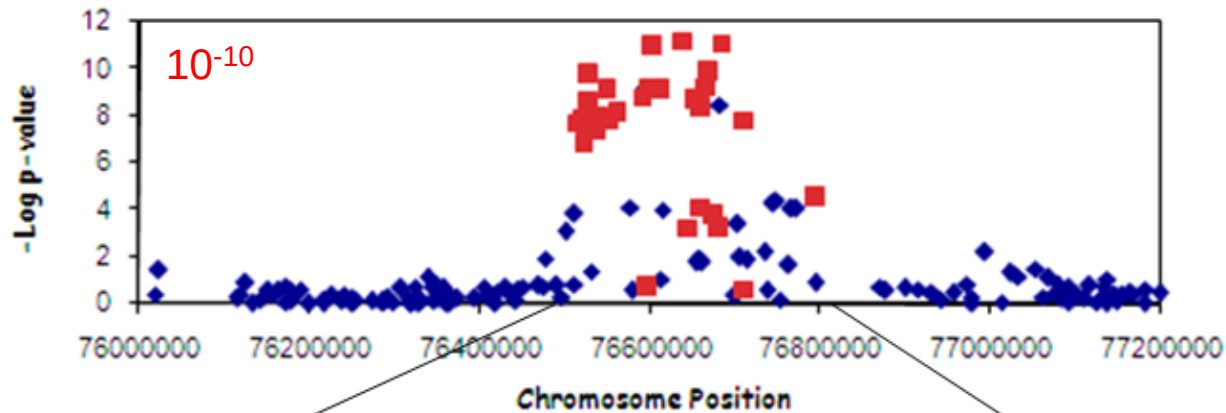
Identification of a DNA region encoding for 3 subunits composing the nicotinic acetylcholine receptor

CHRNA3, CHRNA5, CHRNB4

Nicotinic acetylcholine receptor subunits expressed in multiple cell types

Bind to nicotine and potent lung carcinogens

Lung cancer association region



rs17483548	rs17405217	rs2656052	rs2568494	rs7181486	rs17483929	rs2656065	rs2009746	rs17484235	rs1504550	rs17484524	rs9788721	rs8034191	rs10519203	rs8031948	rs931794	rs2036527	rs17486278	rs7180002	rs951266	rs16969968	rs1051730	rs1317286	rs17487223	European Cases	European Controls	Czech Cases	Czech Controls	Hungarian Cases	Hungarian Controls	Polish Cases	Polish Controls	Romanian Cases	Romanian Controls	Russian Cases	Russian Controls	Slovakian Cases	Slovakian Controls
G	C	A	G	T	G	G	A	C	A	A	T	T	A	G	A	G	A	A	G	G	G	A	C	0.51	0.57	0.49	0.57	0.52	0.58	0.53	0.57	0.48	0.52	0.53	0.57	0.48	0.59
A	T	C	A	C	A	A	G	G	G	G	C	C	G	T	G	A	C	T	A	A	A	G	T	0.34	0.28	0.37	0.27	0.32	0.29	0.33	0.28	0.36	0.29	0.35	0.28	0.35	0.28
G	C	A	G	T	G	G	A	C	A	A	C	C	G	T	G	A	C	T	A	A	A	G	T	0.04	0.03	0.03	0.04	0.05	0.03	0.04	0.03	0.04	0.03	0.02	0.03	0.06	0.02
G	C	A	G	T	G	G	A	C	A	A	T	T	A	G	A	G	A	A	G	G	G	A	T	0.03	0.03	0.03	0.03	0.04	0.03	0.02	0.04	0.02	0.03	0.02	0.03	0.02	0.02
A	T	C	A	C	A	A	G	G	G	G	T	T	A	G	A	G	A	A	G	G	G	A	C	0.02	0.02	0.01	0.02	0.01	0.02	0.02	0.02	0.01	0.03	0.03	0.02	0.03	0.02

Why ? How ?

nAChR - polymorphism

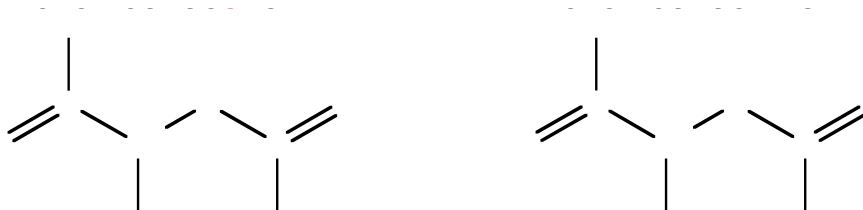
Polymorphism: rs 16969968

Chromosome region 15q25

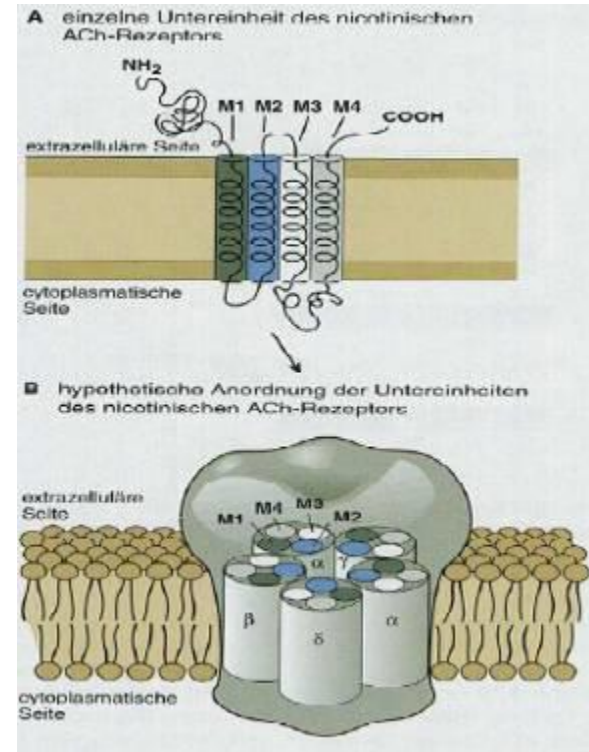
nAChR, **α5 subunit**

Exon 5, **Codon 398**

G (65%)	→	A (35%)
Asp (D)	→	Asn (N)



rs – Reference SNP
SNP – single nucleotide polymorphism



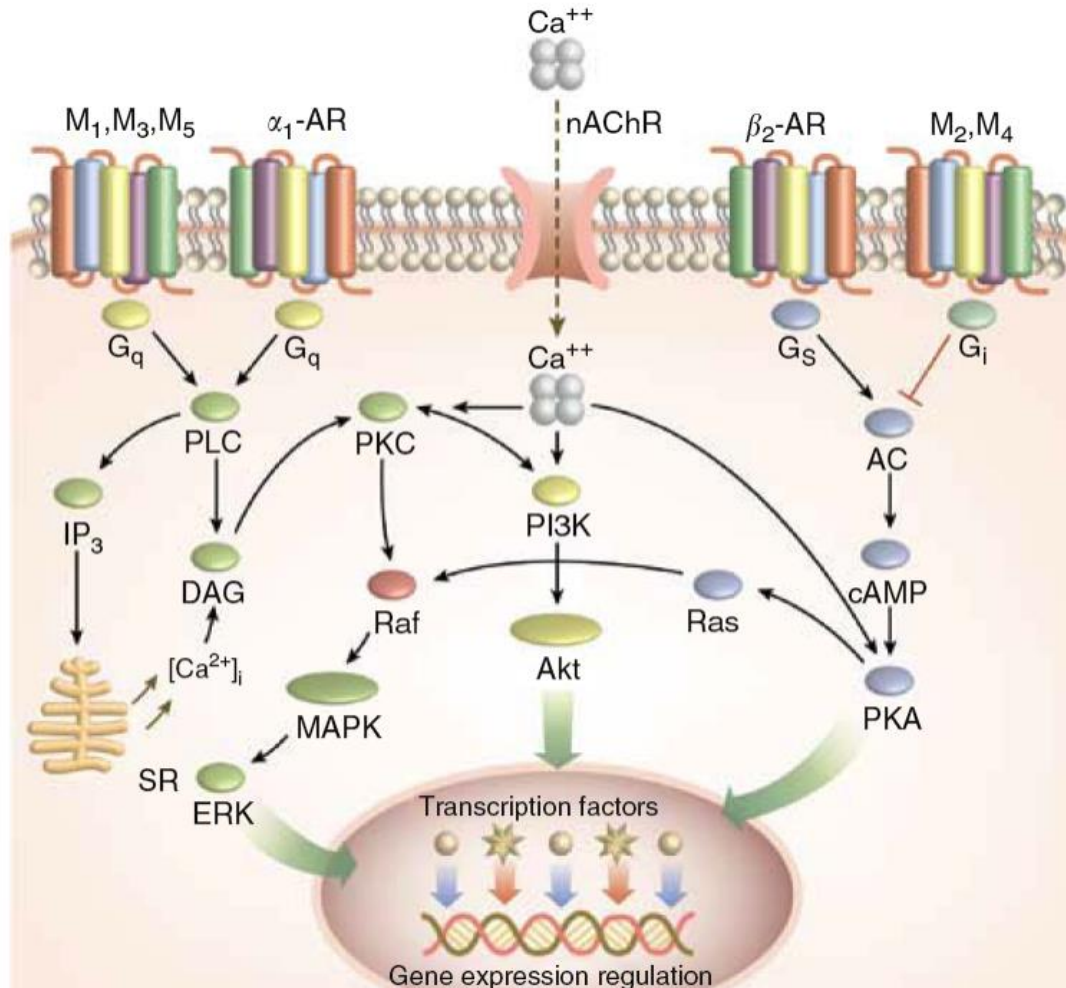
central part of the
2nd intracellular loop

Why ? How ?

nAChR and proliferation

NICOTINIC acetylcholine receptors...

...bind nicotine !



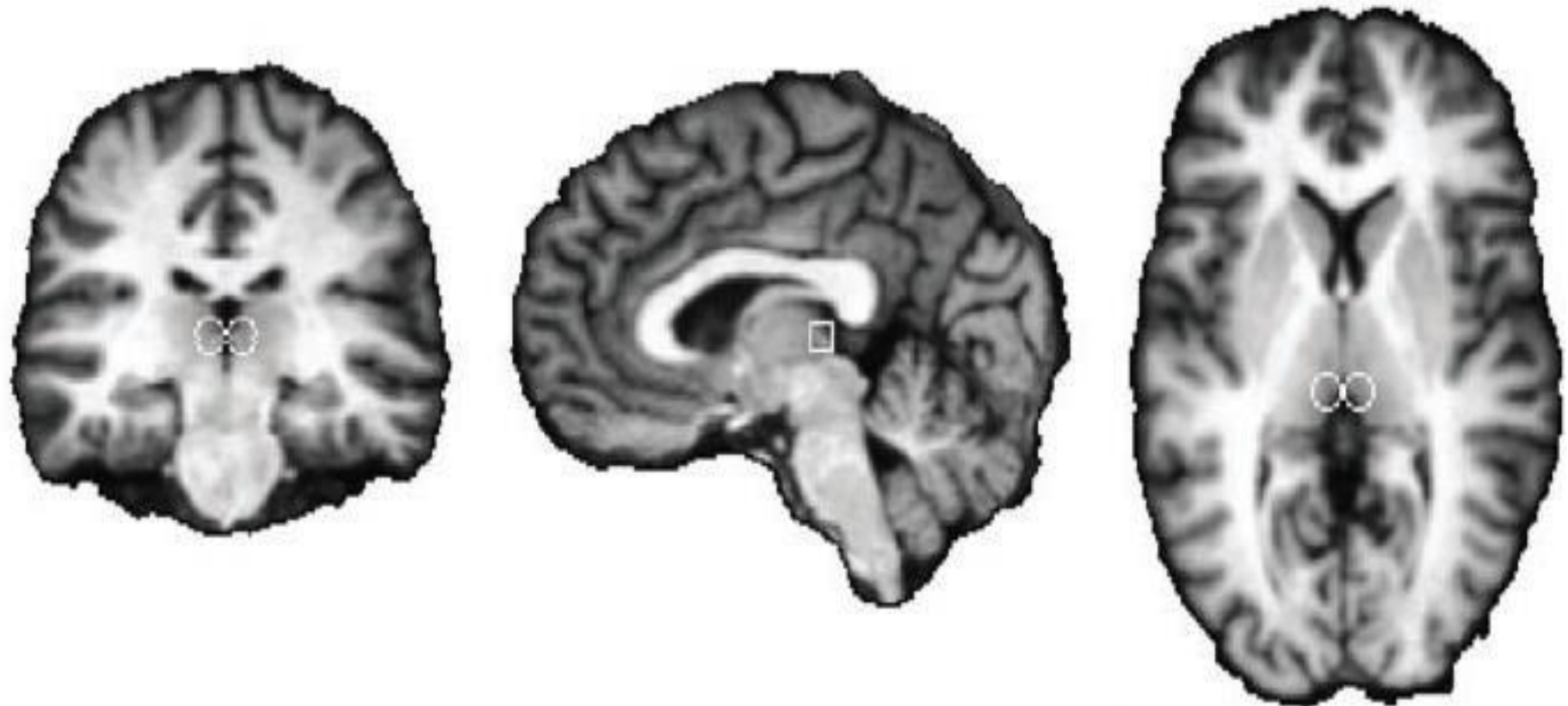
Aversion to Nicotine Is Regulated by the Balanced Activity of $\beta 4$ and $\alpha 5$ Nicotinic Receptor Subunits in the Medial Habenula

Neuron
Article

Silke Frahm,¹ Marta A. Slimak,¹ Leiron Ferrarese,¹ Julio Santos-Torres,¹ Beatriz Antolin-Fontes,¹ Sebastian Auer,¹ Sergey Filkin,³ Stéphanie Pons,⁵ Jean-Fred Fontaine,² Victor Tsetlin,³ Uwe Maskos,^{4,5} and Inés Ibañez-Tallón^{1,*}

Institut Pasteur, Unité Neurobiologie Intégrative des Systèmes Cholinergiques, Département de Neurosciences, F-75724 Paris cedex 15, France

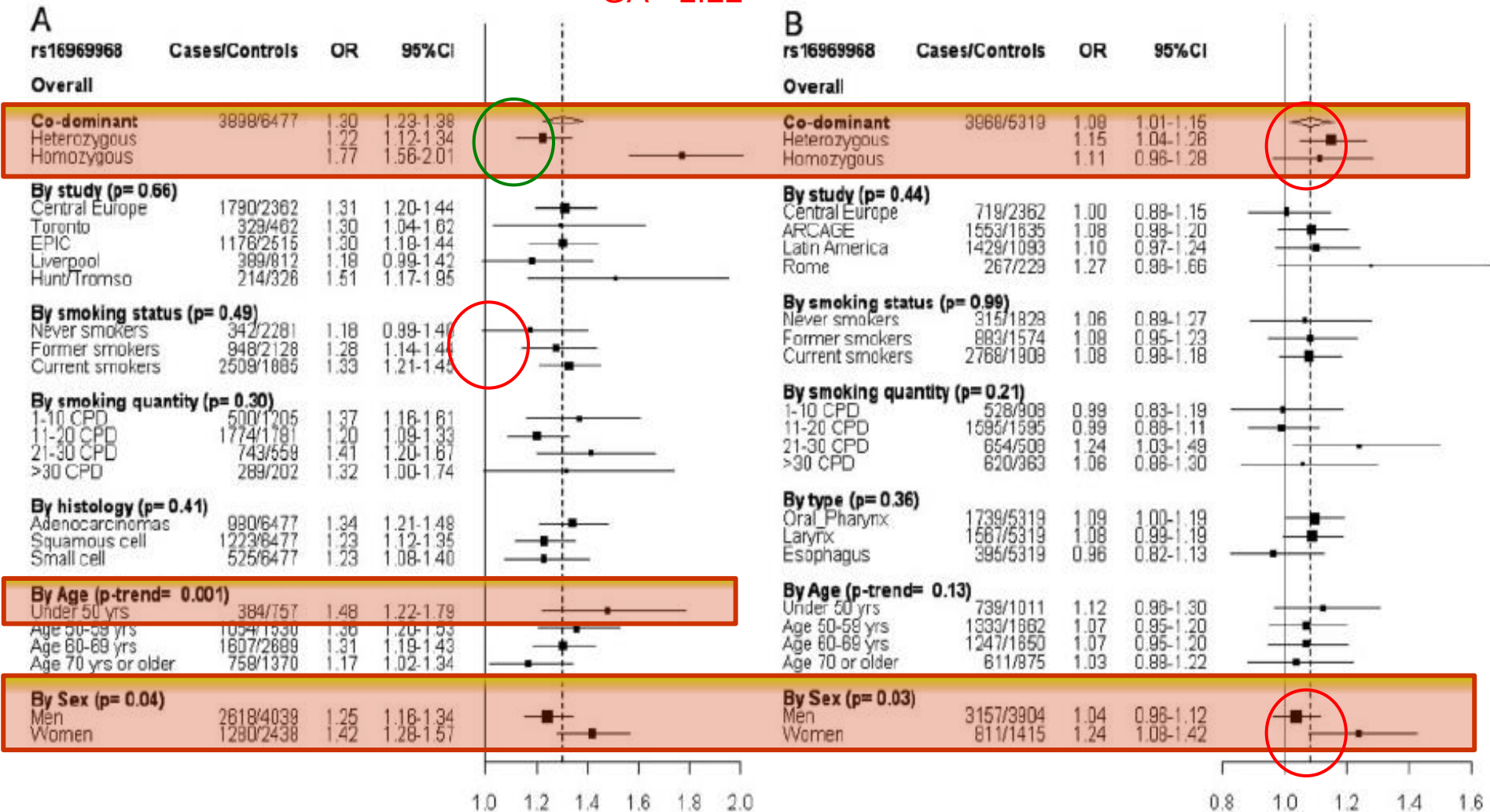
Neuron 70, 522–535, May 12, 2011



The 398 SNP, rs16969968, (15q25) and risk of smoking related lung (but not UADT) cancer

Lung cancer OR for Lung Cancer: AA=1.77
GA= 1.22

UADT cancer



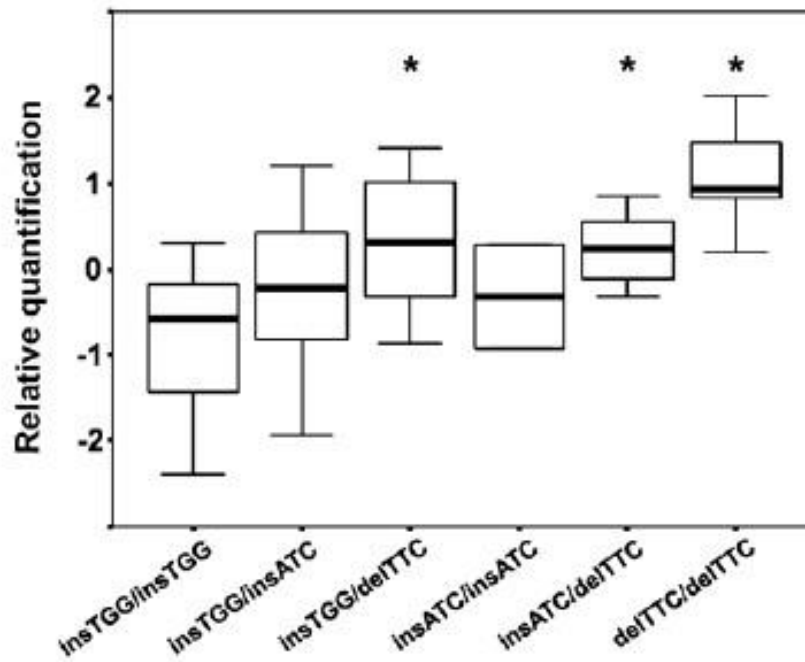
Why ? How ?

Promoter Polymorphisms and Transcript Levels of Nicotinic Receptor CHRNA5

Felicia S. Falvella, Antonella Galvan, Francesca Colombo, Elisa Frullanti,
Ugo Pastorino, Tommaso A. Dragani

JNCI 2010, 102: 1366-70

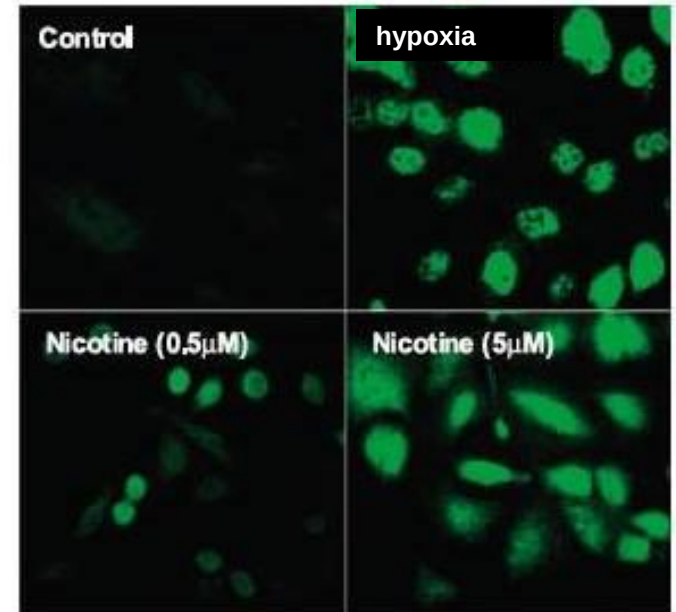
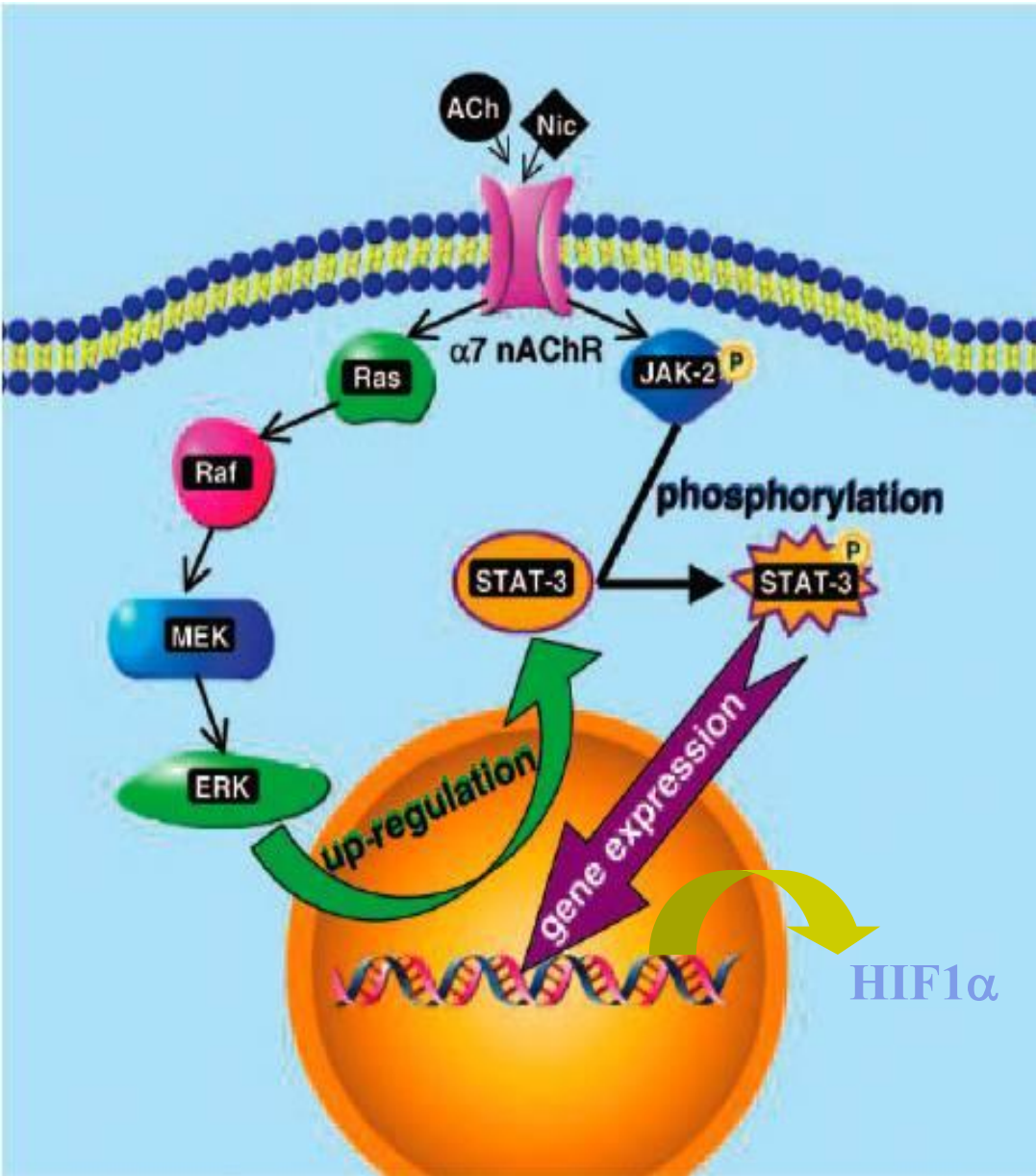
SNP in codon 398 (SNP 968) : linkage disequilibrium with another SNP in gene promoter



**Overexpression of CHRNA5
receptor**

Oncogenic role for Nicotine & AChRs ?

Nicotine induces HIF1 α & VEGF expression

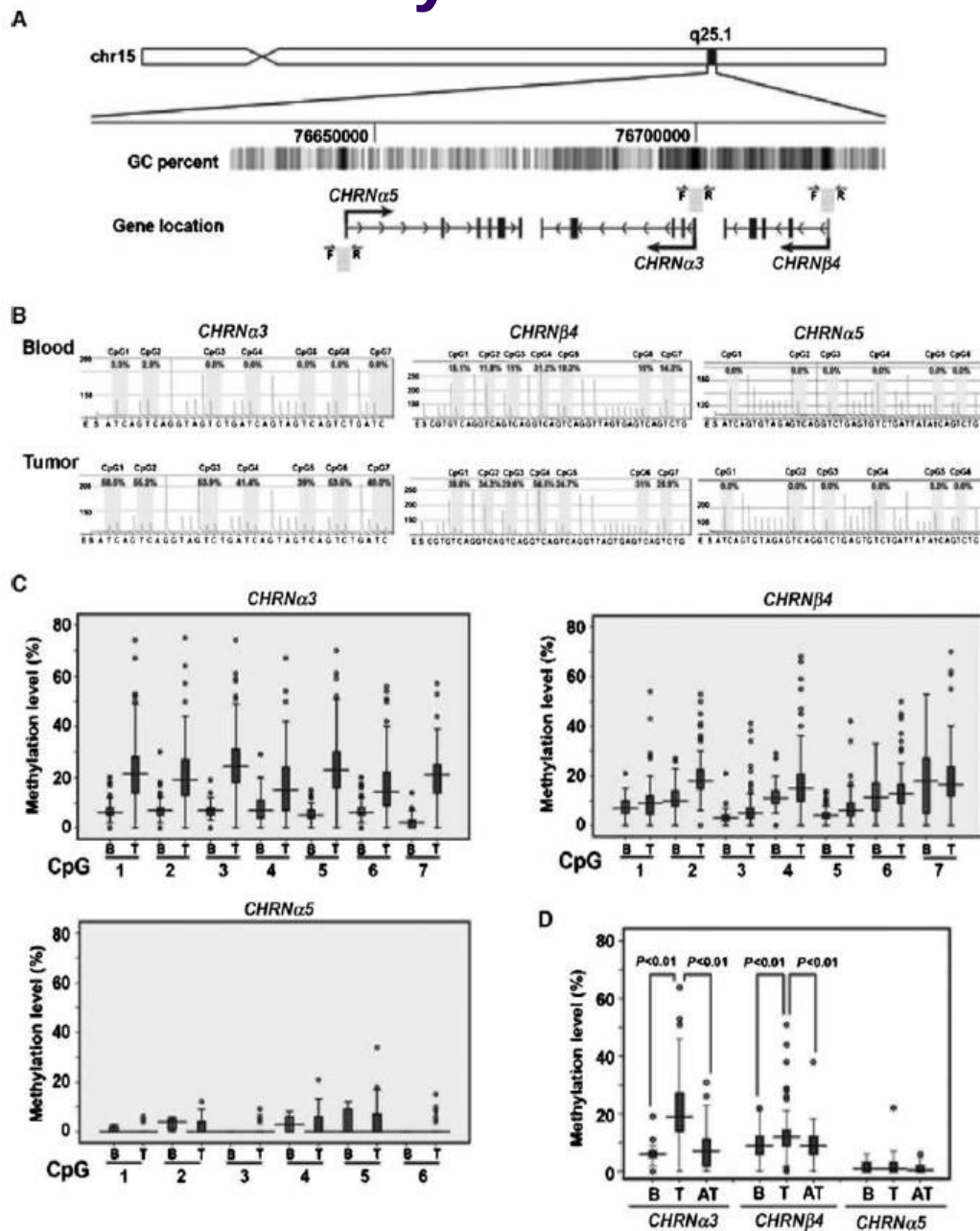


... anti-VEGF therapies
could be impaired in
active smokers

Zhang Q et al. CCR 2007

ELCC 15/18 Apr. 2015, Geneva

Why? How ?



CHRNA3 et CHRNβ4 are methylated in NSCLC (36 to 24%) and not expressed:

CHRNA3 is pro-apoptotic in bronchial cells

CHRNA5 is never methylated and highly expressed in NSCLC: stimulation of cell proliferation and migration

Link with polymorphisms ?

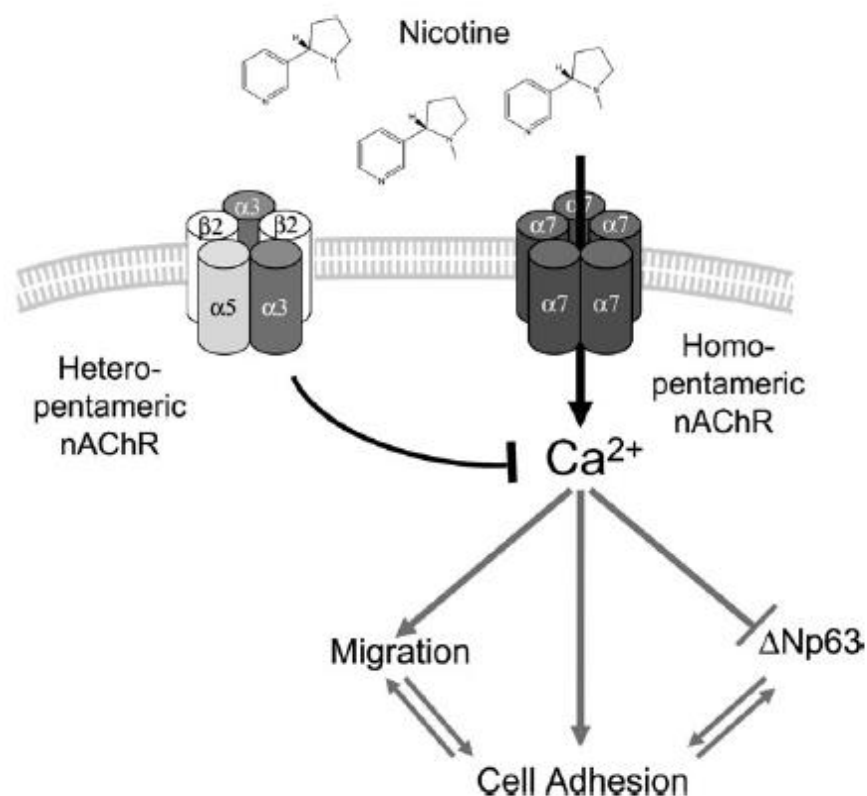
Paliwal A. et al. Cancer Res 2010, 70:2779-88

ELCC 15/18 Apr. 2015, Geneva

CHRNA5 as negative regulator of nicotine signaling in normal and cancer bronchial cells: effects on motility, migration and p63 expression

Annette M.Krais¹, Agnès H.Hautefeuille¹, Marie-Pierre Cros², Vladimir Krutovskikh², Jean-Marie Tournier³, Philippe Birembaut³, Amélie Thépot¹, Anupam Paliwal², Zdenko Herceg², **Paolo Boffetta⁴**, Paul Brennan⁵ and Pierre L.Hainaut^{1,*}

“This model suggests that the high-risk $\alpha 5$ protein variant (398N) may exert a less potent negative regulatory effect on nicotine signaling than the low-risk protein variant (398D), thus making cells more susceptible.... to proliferation or migration”



CHRNA5 SNP968 (398N)

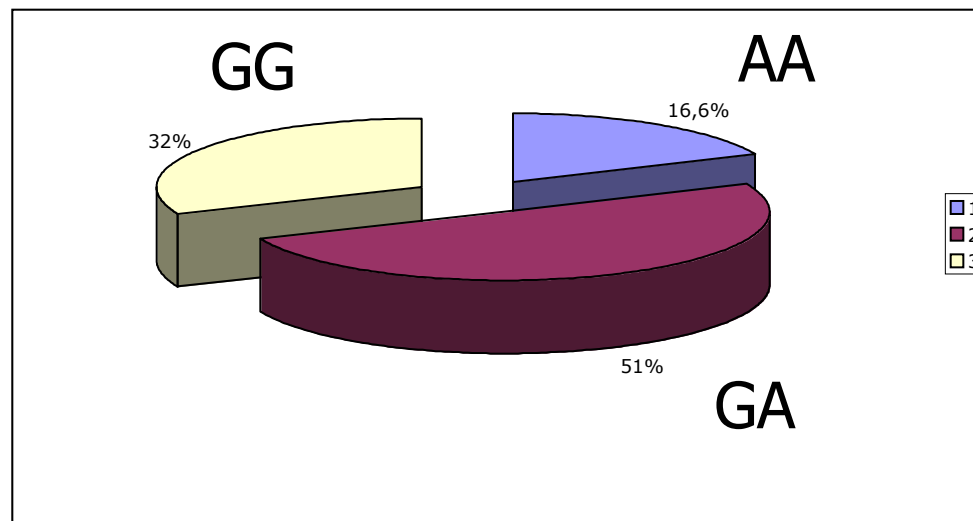
208 early stage NSCLC cancer patients of bio-IFCT 0002 cohort (phase 3 trial) :

(RFLP & sequencing)

"AA": 16,6% « allele A » variant homozygous

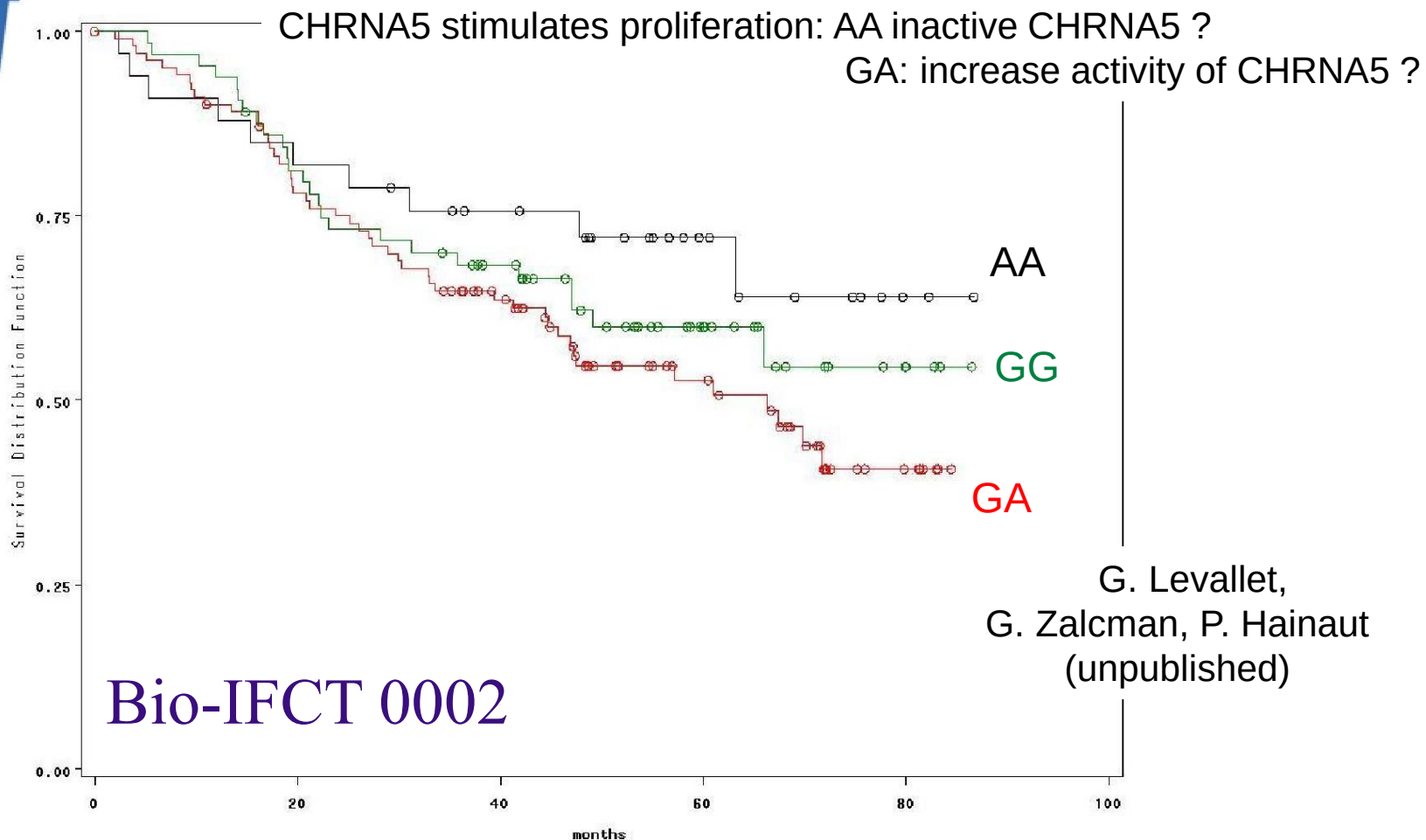
"GA": 51,1% variant heterozygous

"GG": 32,2% « allele G wild-type » coding pour Asp



G. Levallet,
G. Zalcman, P. Hainaut
(unpublished)

(GG=35,7%, AA=17%, GA=47% in NSCLC cases, in the series by Lips EH et al., Int J Epidemiol 2009, on 3306 cas)



Variable	Modalités	HR	Borne inf IC95%	Borne sup IC95%	p
CHRNA5	AA	0.569	0.288	1.124	0.1047
	GG	0.794	0.490	1.286	0.3482
	AG	1	-	-	

The other SNP rs8034191 (15q25.1) is only associated to an increase of lung cancer risk in current or former smokers

No effect in “never smokers”

Cancer risk or ou addiction risk?

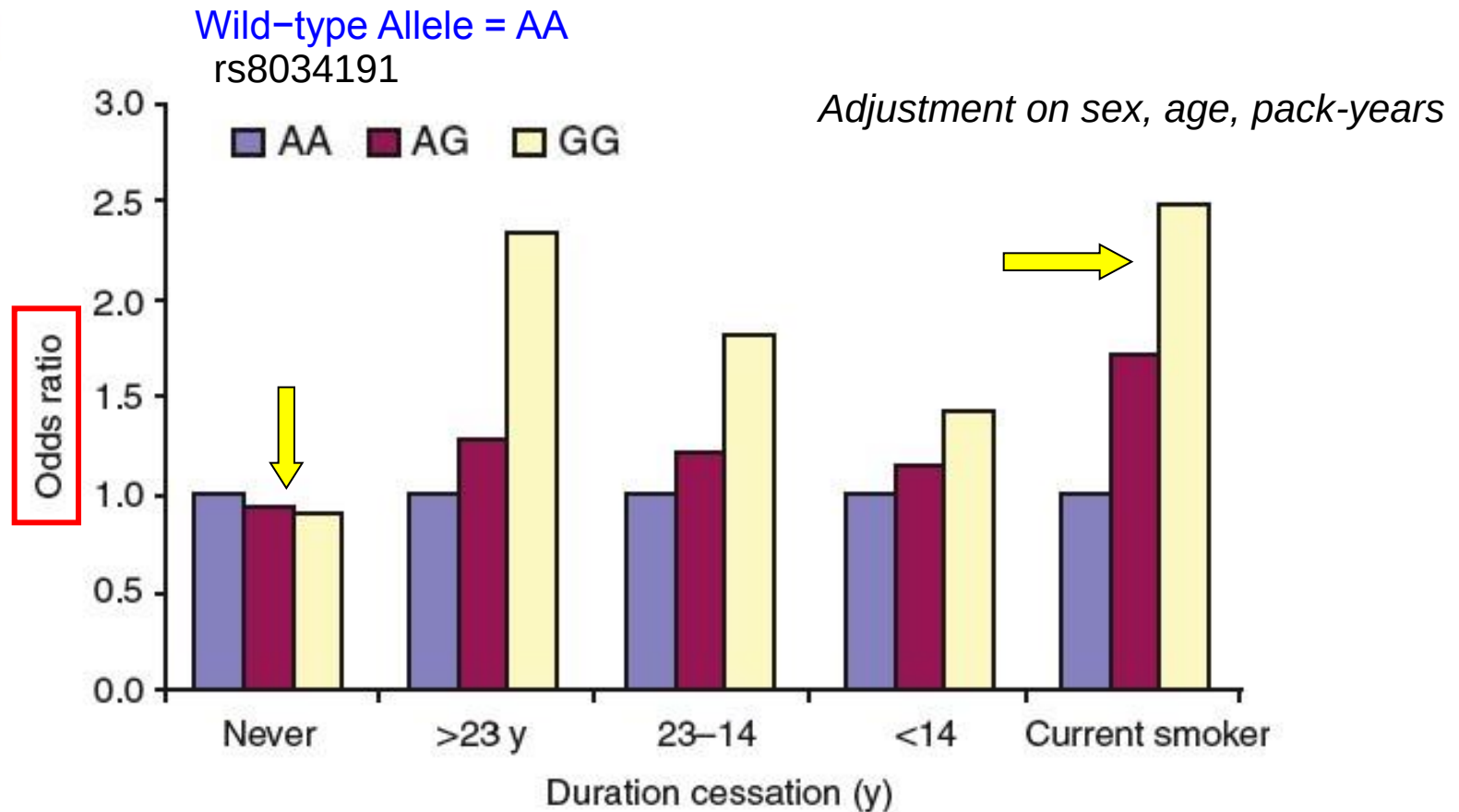




Table 1. GWAS of lung cancer vs. controls.

Study	Lung cancer cases (discovery set)*	Controls (discovery set)	Arrays [nos. of SNPs]	Chromosomal regions and main associated genes
Spinola 2007 (24)	335 smokers	338 smokers	Affymetrix [116,204]	10p KLF6
Amos 2008 (25)	1,154 smokers	1,137 smokers	Illumina [317,498]	15q CHRNA3
Hung 2008 (26)	1,989 smokers	2,625 smokers	Illumina [317,139]	15q CHRNA3, CHRNA5
Liu 2008 (27)	194 with familial lung cancer	219 smokers and non-smokers	Affymetrix [500,568 and 906,703]	15q various genes
Thorgerirsson 2008 (28)	1,024 smokers	32,244 controls	Illumina [306,207]	15q CHRNA3
McKay 2008 (29)	3,259 smokers	4,159 smokers	Illumina [315,194]	5p TERT-CLPTMIL , 15q CHRNA3
Wang 2008 (30)	1,952 smokers	1,438 smokers	Illumina [511,919]	5p CLPTMIL , 6p BAT3-MSH5, 15q CHRNA3
Broderick 2009 (31)	1,978 smokers, and meta-analysis	1,438 smokers, and meta-analysis	Meta-analysis	5p TERT-CLPTMIL , 6p BAT3-MSH5, TNXB, 15q CHRNA3
Landi 2009 (32)	5,739 smokers	5,848 smokers	Illumina [515,922]	5p TERT-CLPTMIL , 15q CHRNA3
Hsiung 2010 (33)	584 cases (never smoking females with lung adenocarcinoma)	585 controls (never smoking females)	Illumina [610,901]	5p15 TERT-CLPTMIL
Li 2010 (34)	377 never smokers	377 never smokers	Illumina [373,397 and 592,532]	13q31.3 GPC5
Miki 2010 (35)	1,004 with lung adenocarcinoma	1,900 controls	Illumina [610,901]	3q28 TP63 , 5p15 TERT
Yoon 2010 (36)	621 cases (smokers and never smokers)	1,541 controls (smokers and never smokers)	Affymetrix [500,568]	3q29 C3orf21, 5p TERT-CLPTMIL
Hu 2011 (37)	2,331 cases (smokers and never smokers)	3,077 controls (smokers and never smokers)	Affymetrix [906,703]	3q28 TP63 , 5p15 TERT-CLPTMIL , 13q12 MIPER-TNFRSF19, 22q12 MTMR3-HORMAD2-LIF
Ahn 2012 (38)	446 never smokers	497 healthy controls	Affymetrix [906,703]	18p11 FAM38B
Dong 2012 (39)	833 cases with SCC	3,094 controls	Affymetrix [906,703]	12q23 SLC17A8-NR1H4
Lan 2012 (40)	5,510 never-smoking female lung cancer cases	4,544 controls	Various	3q28 TP63 , 5p15, 6p21 HLA, 6q22 ROS1, DCBLD1, 10q25 VTI1A, 17q24 BPTF
Shiraishi 2012 (41)	1,722 cases (smokers and never smokers)	5,846 controls (smokers and never smokers)	Illumina [709,857]	3q28 TP63 , 5p15 TERT , 6p21 BTNL2, 17q24 BPTF
Timofeeva 2012 (42)	Meta-analysis: 14,900 cases (smokers and never smokers)	29,485 controls (smokers and never smokers)	Various	5p15, 6p21, 15q25 for NSCLC, 9p21 for SCC
Kim 2013 (43)	285 female never smokers with lung cancer	1,455 controls	Affymetrix [440,794]	2p16 NRXN1

* In Tables 1 to 3, discovery study details have been included, and replication study samples sizes have not been included. For details, see <http://www.genome.gov/gwastudies>.

A Candidate Gene Approach Identifies the *CHRNA5-A3-B4* Region as a Risk Factor for Age-Dependent Nicotine Addiction

SNP	Gene	Location	Chr. 15 Position ^a	Minor allele	Early onset of daily smoking by 16			Late onset of daily smoking after 16		
					Frequency: Low, High ^b	p-Value	Allelic OR (95% CI)	Frequency: Low, High ^c	p-Value	Allelic OR (95% CI)
rs17486278	CHRNA5	intron 1	76654537	C	0.279, 0.417	0.0005	1.85 (1.31–2.62)	0.403, 0.395	0.81	0.96 (0.72–1.29)
rs680244	CHRNA5	intron 1	76658343	A	0.461, 0.419	0.32	0.84 (0.61–1.17)	0.406, 0.401	0.88	0.98 (0.73–1.31)
rs569207	CHRNA5	intron 1	76660174	A	0.260, 0.167	0.0042	0.57 (0.39–0.84)	0.191, 0.203	0.68	1.08 (0.75–1.54)
rs555018	CHRNA5	intron 3	76666297	C	0.462, 0.415	0.25	0.83 (0.60–1.15)	0.407, 0.400	0.85	0.97 (0.73–1.30)
rs16969968	CHRNA5	Asn348Asp	76669980	A	0.284, 0.415	0.0009	1.79 (1.27–2.54)	0.401, 0.400	0.97	1.00 (0.75–1.33)
rs578776	CHRNA3	3' UTR	76675455	T	0.317, 0.218	0.0048	0.60 (0.42–0.86)	0.218, 0.223	0.87	1.03 (0.73–1.45)
rs1051730	CHRNA3	Gly394Gly	76681394	T	0.284, 0.415	0.0009	1.79 (1.27–2.54)	0.398, 0.401	0.93	1.01 (0.76–1.35)
rs2869546	CHRNA3	intron 4	76694400	C	0.394, 0.374	0.61	0.92 (0.66–1.28)	0.384, 0.374	0.77	0.96 (0.72–1.28)
rs7177514	CHRNA3	intron 4	76694461	G	0.341, 0.235	0.0032	0.59 (0.42–0.84)	0.229, 0.245	0.59	1.10 (0.78–1.53)
rs12443170	CHRNA3	intron 4	76694791	A	0.149, 0.096	0.038	0.61 (0.38–0.98)	0.111, 0.148	0.13	1.39 (0.91–2.13)
rs11636605	CHRNA3	intron 1	76715933	A	0.212, 0.156	0.072	0.69 (0.46–1.04)	0.159, 0.182	0.40	1.18 (0.80–1.72)
rs11633223	CHRNA3	5' flank	76722531	C	0.414, 0.378	0.37	0.86 (0.62–1.20)	0.384, 0.376	0.81	0.96 (0.72–1.29)
rs3971872	CHRNA3	5' flank	76729090	T	0.096, 0.092	0.86	0.95 (0.55–1.65)	0.068, 0.079	0.57	1.17 (0.68–2.02)

The *CHRNA5-A3* Region on Chromosome 15q24-25.1 Is a Risk Factor Both for Nicotine Dependence and for Lung Cancer

Margaret R. Spitz, Christopher I. Amos, Qiong Dong, Jie Lin, Xifeng Wu

Smoking intensity and dependence by rs1051730 genotype*

Genotype	Case subjects						Control subjects					
	n	Cig/d	P value†	n	FTND score	P value†	n	Cig/d	P value†	n	FTND score	P value†
GG	685	27.16		583	4.68		764	25.06		729	4.13	
AG	869	27.09		733	4.63		770	25.99		733	4.27	
AA	300	29.80	.004	259	5.13	.012	193	29.36	<.001	187	5.10	<.001

* N=number of case or control subjects used to analyze a given smoking behavior; Cig=cigarettes; FTND=Fagerstrom Test for Nicotine Dependence.

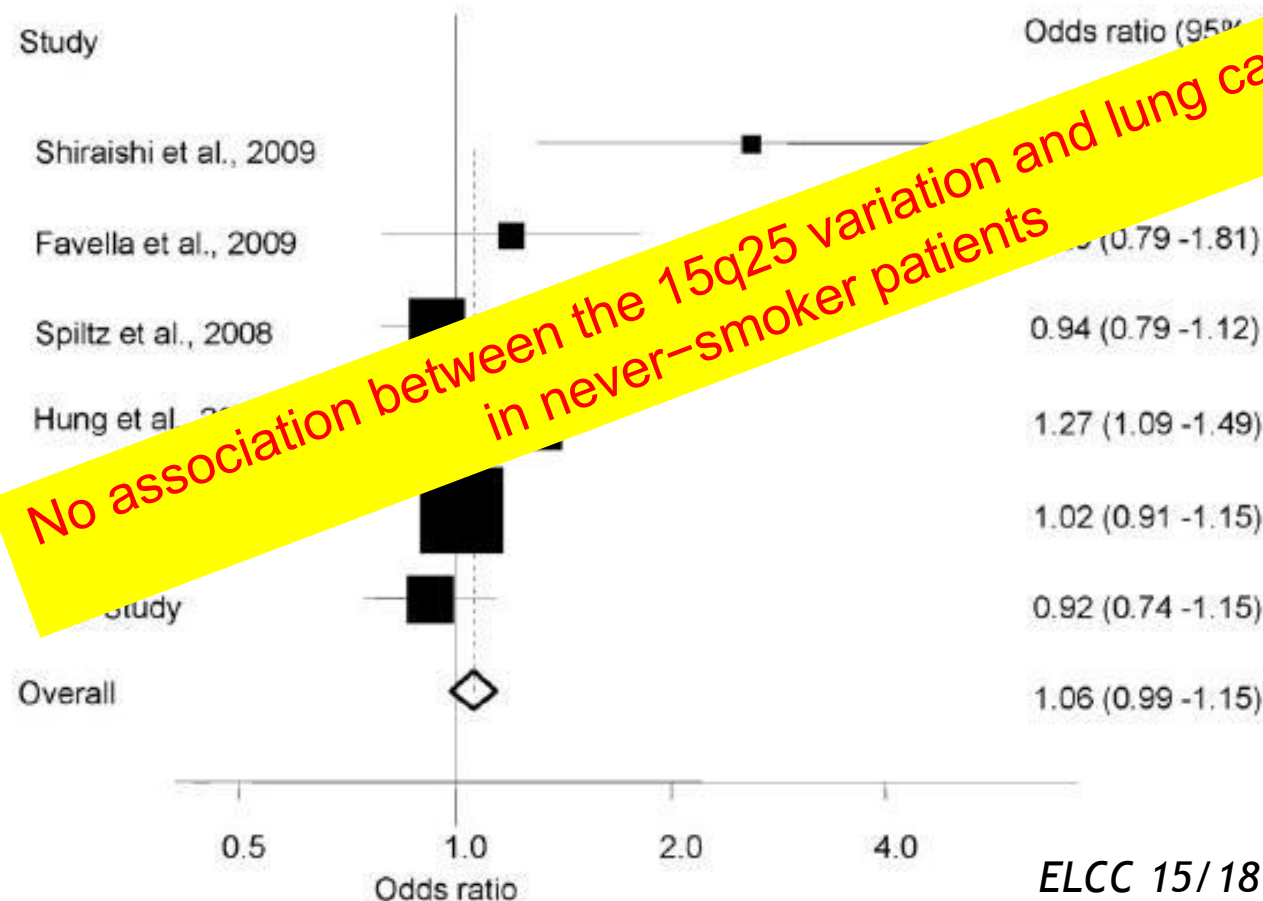
† From Kruskal-Wallis test.

Chromosome 15q25 (*CHRNA3-CHRNA5*) Variation Impacts Indirectly on Lung Cancer Risk

Yufei Wang¹, Peter Broderick¹, Athena Matakidou², Timothy Eisen², Richard S. Houlston^{1*}

¹ Section of Cancer Genetics, Institute of Cancer Research, Sutton, Surrey, United Kingdom, ² Department of Oncology, Cambridge University, Cambridge, United Kingdom

2,405 never-smoker lung cancer cases vs. 7,622 controls



Genetic Variants in Nicotine Addiction and Alcohol Metabolism Genes, Oral Cancer Risk and the Propensity to Smoke and Drink Alcohol: A Replication Study in India

Devasena Anantharaman¹, Amélie Chabrier², Valérie Gaborieau¹, Silvia Franceschi³, Rolando Herrero⁴, Thangarajan Rajkumar⁵, Tanuja Samant⁶, Manoj B. Mahimkar⁶, Paul Brennan¹, James D. McKay^{2*}

Propensity to chew tobacco* (Chewing events/day)

rs16969968 (CHRNA5)

Base model [§]	622	1.13 (1.01– 1.25)	0.03
Base+ smoking frequency	622	1.13 (1.01– 1.26)	0.03
Base, among never smokers only	446	1.19 (1.06– 1.34)	0.003

rs578776 (CHRNA3)

Base model [§]	613	0.91 (0.84– 0.99)	0.04
Base+ smoking frequency	613	0.91 (0.84– 0.99)	0.04
Base, among never smokers only	435	0.88 (0.81– 0.97)	0.01

And tobacco chewing is associated with oral cancer risk : OR= 8.30 95%CI (5.78–11.93)

Susceptibility to tobacco addiction... CHRNA 3 & 5

Table 2. GWAS of smoking behaviour (selected studies).

Study	Subjects	Arrays (nos. of SNPs)	Chromosomal regions and main associated genes
Liu 2010 (60)	41,150 from 20 cohorts	Various (>500,000)	15q CHRNA3 and CHRNA5 with smoking quantity
Tobacco and Genetics Consortium 2010 (61)	74,053 from 16 cohorts	Various (>500,000)	10q25 various genes, 15q CHRNA3 , 19q <i>EGLN2</i> with smoking quantity; 11p <i>BDNF</i> with smoking initiation; 9q <i>DBH</i> with smoking cessation
Thorgeirsson 2010 (62)	31,266 and 46,481 subjects from cohorts	Various (>500,000)	8p <i>CHRNA3</i> , 15q CHRNA3 and CHRNA5 , 19q CYP2A6 with smoking quantity
Siedlinski 2011 (63)	3,441 ever-smoking patients with COPD	Illumina (>500,000)	15q CHRNA3 and CHRNA5 , 19q CYP2A6 with smoking quantity; 2q21 intergenic region, 6p21 <i>HLA</i> with smoking initiation; 9q <i>DBH</i> with smoking cessation

Yang IA et al. *Journal of Thoracic Disease*, Vol 5, Suppl 5 October 2013

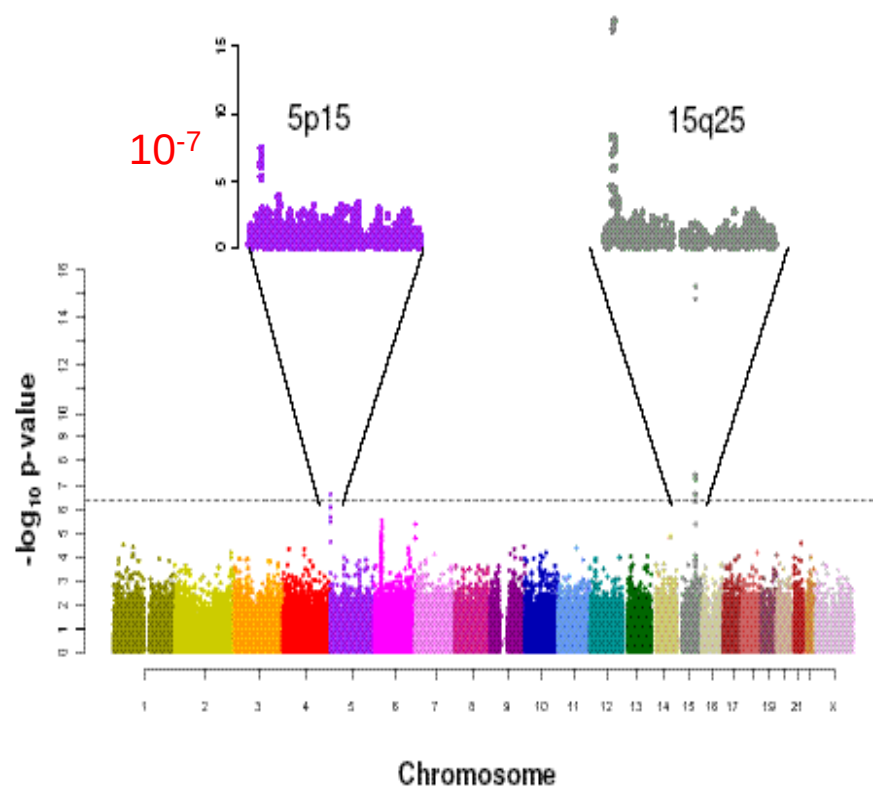
Susceptibility to COPD...again CHRNA 3 & 5

GWAS of COPD vs. controls.

Study	COPD cases (discovery)	Controls (discovery)	Arrays (nos. of SNPs)	Chromosomal regions and main associated genes
Wilk 2009 (71)	7,691 Framingham study participants, plus replication cohort		Affymetrix (550,000)	4q31 <i>HHIP</i>
Pillai 2009 (72)	823 COPD	810 smokers	Illumina (561,466)	4q31 <i>HHIP</i> , 15q <i>CHRNA3</i> , <i>CHRNA5</i>
Cho 2010 (73)	2,940 COPD	1,380 smokers	Various (>500,000)	4q24 <i>FAM13A</i>
Cho 2012 (74)	3,499 COPD	1,922 controls	Illumina (>500,000)	4q24 <i>FAM13A</i> , 19q13 <i>RAB4B</i> , <i>EGLN2</i> , <i>CYP2A6</i>
Wilk 2012 (75)	3,368 COPD, plus replication cohort	29,507 controls	Various (>500,000)–meta-analysis	5q <i>HTR4</i> ; 15q <i>AGPHD1</i> , <i>IREB2</i> , <i>CHRNA5</i> , <i>CHRNA3</i>

via their role in tobacco addiction ?

Lung cancer 2nd phase GWA scan

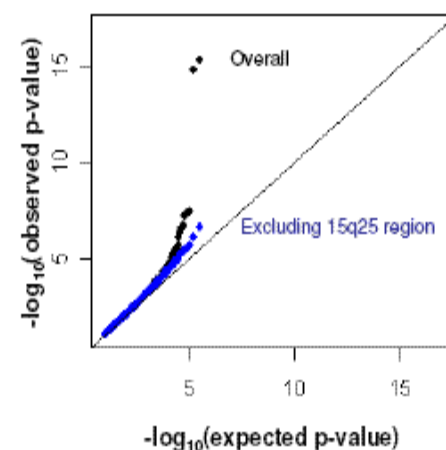


3259 Caucasian lung cancer cases (1st phase + Toronto, HUNT2 + CARET)

4159 matched controls

315,194 SNPs

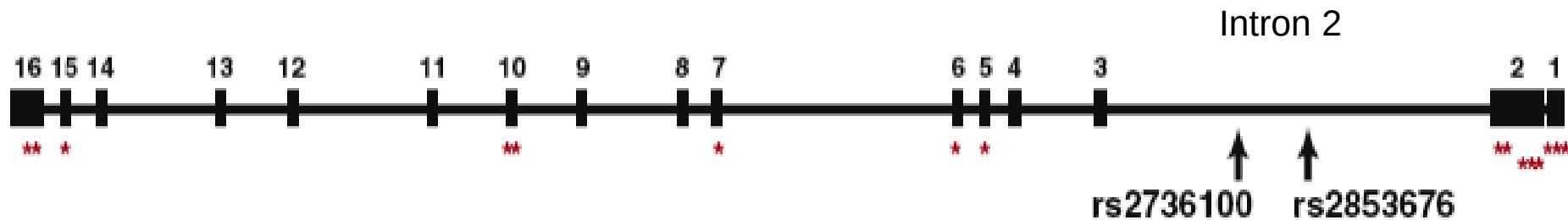
Genome-wide significance
 $p < 5 \times 10^{-7}$



Location of SNPs and mutations in TERT gene

Higher risk for adenocarcinoma

chr5: 1306282-1348162



* Known mutations that compromise telomerase activity

rs2736100 is also associated with susceptibility to sporadic idiopathic pulmonary fibrosis (IPF)and IPF patients develop lung cancer... but IPF is also linked to tobacco smoking

rs2736100 is also associated with an increased risk of glioma and pancreas K

Detection & replication of lung cancer association on chromosome 5p

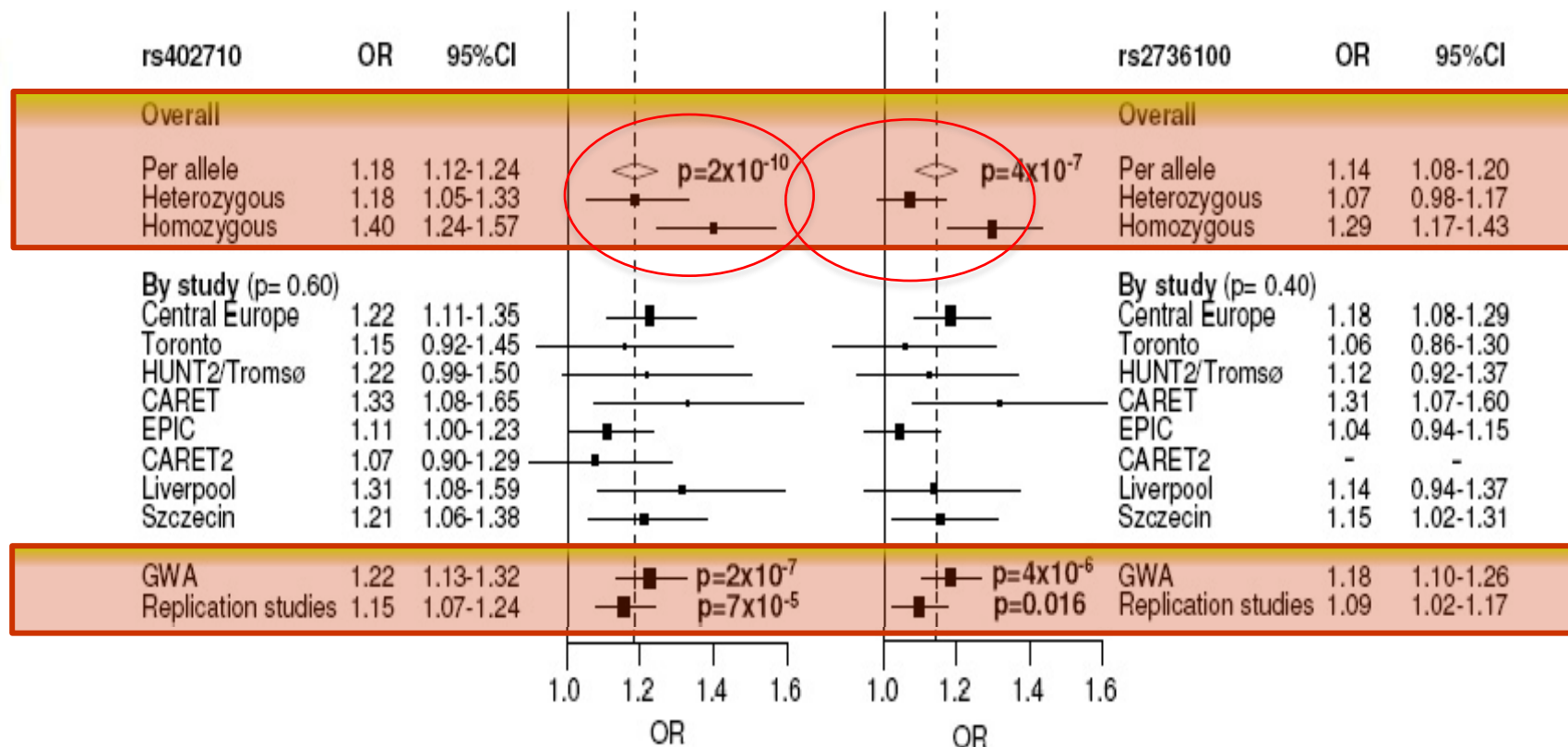


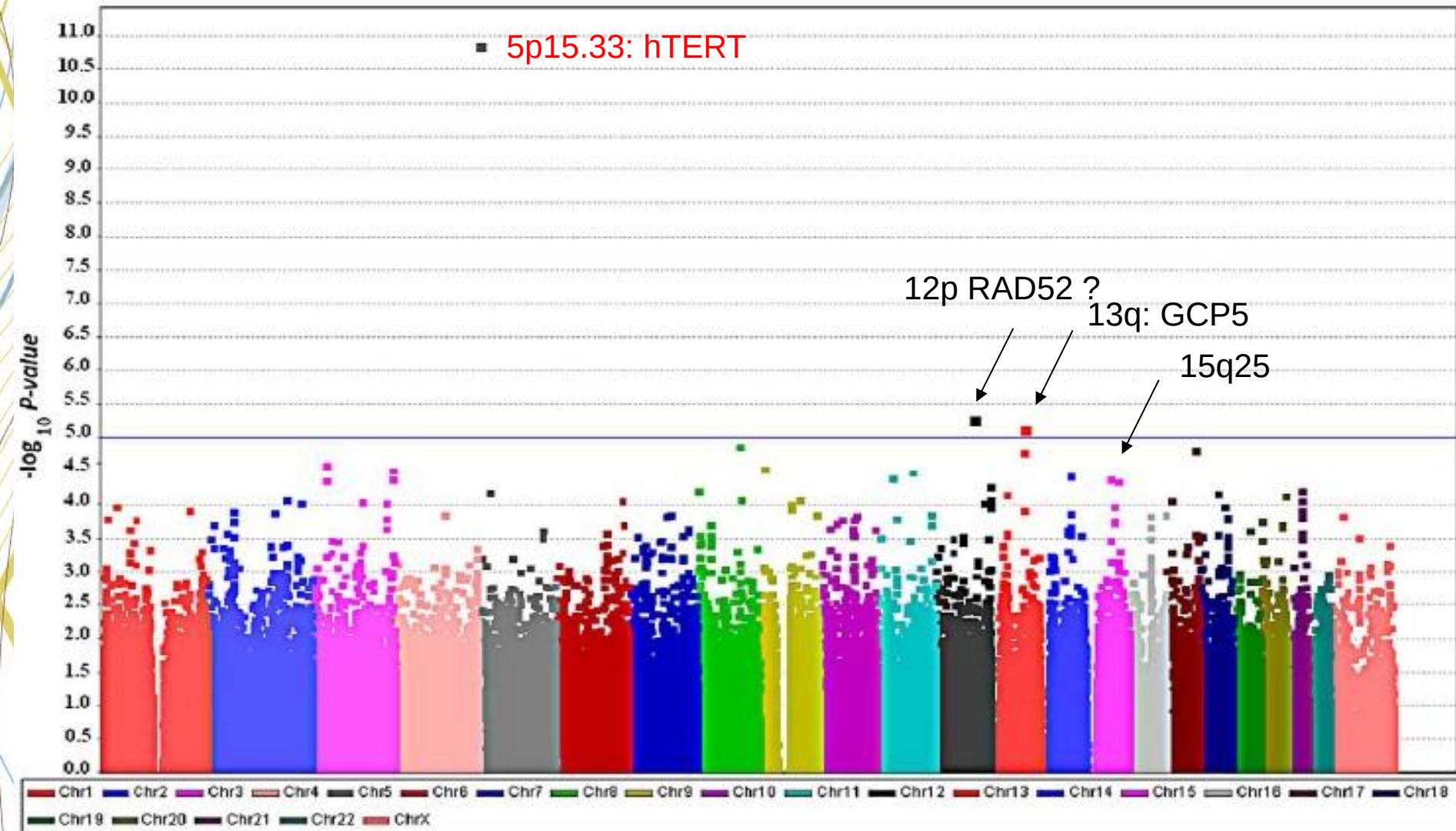


Table 1. GWAS of lung cancer vs. controls.

Study	Lung cancer cases (discovery set)*	Controls (discovery set)	Arrays [nos. of SNPs]	Chromosomal regions and main associated genes
Spinola 2007 (24)	335 smokers	338 smokers	Affymetrix [116,204]	10p KLF6
Amos 2008 (25)	1,154 smokers	1,137 smokers	Illumina [317,498]	15q CHRNA3
Hung 2008 (26)	1,989 smokers	2,625 smokers	Illumina [317,139]	15q CHRNA3, CHRNA5
Liu 2008 (27)	194 with familial lung cancer	219 smokers and non-smokers	Affymetrix [500,568 and 906,703]	5q various genes
Thorgeirsson 2008 (28)	1,024 smokers	32,244 controls	Illumina [306,207]	5q CHRNA3
McKay 2008 (29)	3,259 smokers	4,159 smokers	Illumina [315,194]	5p TERT-CLPTMIL , 15q CHRNA3
Wang 2008 (30)	1,952 smokers	1,438 smokers	Illumina [511,919]	5p CLPTMIL , 6p BAT3-MSH5, 15q CHRNA3
Broderick 2009 (31)	1,978 smokers, and meta-analysis	1,438 smokers, and meta-analysis	Meta-analysis	5p TERT-CLPTMIL , 6p BAT3-MSH5, TNXB, 15q CHRNA3
Landi 2009 (32)	5,739 smokers	5,848 smokers	Illumina [515,922]	5p TERT-CLPTMIL , 15q CHRNA3
Hsiung 2010 (33)	584 cases (never smoking females with lung adenocarcinoma)	585 controls (never smoking females)	Illumina [610,901]	5p15 TERT-CLPTMIL
Li 2010 (34)	377 never smokers	377 never smokers	Illumina [373,397 and 592,532]	13q31.3 GPC5
Miki 2010 (35)	1,004 with lung adenocarcinoma	1,900 controls	Illumina [610,901]	3q28 TP63 , 5p15 TERT
Yoon 2010 (36)	621 cases (smokers and never smokers)	1,541 controls (smokers and never smokers)	Affymetrix [500,568]	3q29 C3orf21, 5p TERT-CLPTMIL
Hu 2011 (37)	2,331 cases (smokers and never smokers)	3,077 controls (smokers and never smokers)	Affymetrix [906,703]	3q28 TP63 , 5p15 TERT-CLPTMIL , 13q12 MIPER-TNFRSF19, 22q12 MTMR3-HORMAD2-LIF
Ahn 2012 (38)	446 never smokers	497 healthy controls	Affymetrix [906,703]	18p11 FAM38B
Dong 2012 (39)	833 cases with SCC	3,094 controls	Affymetrix [906,703]	12q23 SLC17A8-NR1H4
Lan 2012 (40)	5,510 never-smoking female lung cancer cases	4,544 controls	Various	3q28 TP63 , 5p15, 6p21 HLA, 6q22 ROS1, DCBLD1, 10q25 VTI1A, 17q24 BPTF
Shiraishi 2012 (41)	1,722 cases (smokers and never smokers)	5,846 controls (smokers and never smokers)	Illumina [709,857]	3q28 TP63 , 5p15 TERT , 6p21 BTNL2, 17q24 BPTF
Timofeeva 2012 (42)	Meta-analysis: 14,900 cases (smokers and never smokers)	29,485 controls (smokers and never smokers)	Various	5p15, 6p21, 15q25 for NSCLC, 9p21 for SCC
Kim 2013 (43)	285 female never smokers with lung cancer	1,455 controls	Affymetrix [440,794]	2p16 NRXN1

* In Tables 1 to 3, discovery study details have been included, and replication study samples sizes have not been included. For details, see <http://www.genome.gov/gwastudies>.

The 5p15.33 Locus Is Associated with Risk of Lung Adenocarcinoma in Never-Smoking Females in Asia



Large validation studies: time to large meta-analyses

Timofeeva MN *et al.*
...63 co-authors

Human Molecular Genetics, 2012, Vol. 21, No. 22 4980–4995
doi:10.1093/hmg/dd334
Advance Access published on August 16, 2012

Influence of common genetic variation on lung cancer risk: meta-analysis of 14 900 cases and 29 485 controls

Confirms 5p 15.33 region: **hTERT** impacting lung adenocarcinoma risk
but also **CLPTM1L** gene: impacting SCC and large cell carcinoma risk

Confirms 15q25 region: **CHRNA5/CHRNA3/CHRNA4** nicotinic subunits genes
non specific histological effect

Confirms 6p21–6p22 region: **BAT3** p53 acetylase
MSH5 DNA mismatch repair gene

Suggests 9p21 region: **p16/p14^{ARF} (CDKN2 A & B)** cell cycle or p53 regulators
ANRIL intronic lncRNA

Suggests 12p13.33 region: **RAD52** DNA double-strand repair & homologous recombination

Genetic susceptibility 2015

- 15q25.1 CHRNA3, CHRNA5, CHRNA4 : **not for never smokers**
- 5p15.33 hTERT: telomerase catalytic sub-unit:

**smokers & Asian never smoker
adenocarcinomas**

CLPTM1L: replicated, anti-apoptotic/cytokinesis ?

- 6p21.33 BAT3 : regulation of p53 acetylation: replicated
MSH5: DNA reparation: replicated
- 13q31.3 GPC5, **not replicated (never smokers)**
- 12p13 RAD52, DNA reparation, replicated
SCC and confined to smokers
- 9p21 p16 / p14ARF & ANRIL : replicated in Han Chinese SCC

Wang Y, Carcinogenesis 2010;31:234-8 - Baird DM, Expert Rev Mol med 2010; 18:12:e16 - Truong T, J Natl cancer Inst 2010;102:959-71 - Liu P, Cancer Epidemiol Biomarkers Prev 2010;19:517-24 - Li Y, Lancet Oncol 2010;11:321-30; Timofeeva MN, Hum Mol Gen 2012;21: 4981-94; Shi J et al. Cancer Discov. 2012, 2: 131-39

ELCC 15/18 Apr. 2015, Geneva

Molecular Genetics of Successful Smoking Cessation

European American smokers who successfully vs. unsuccessfully abstain from smoking with biochemical confirmation, in a smoking cessation trial using NRT, bupropion, or placebo (n=550).

➔ No overlap susceptibility genes to nicotine addiction ...

CDH13: inhibits neurite extension

Neurexin 3: presynaptic protein linked to dependence to illegal substance

DSCAM: required for appropriate neuronal connections for memory associated circuit

PRKG1: cGMP-dependent protein kinase 1

.....

Still to be replicated....

In addition to 9q region previously reported:

including **DBH (Dopamine Beta Hydroxylase)**



HAMBONE by Mike Flanagan

I CAN'T SLEEP!

I ADVISE YOU TO EAT SOMETHING BEFORE YOU GO TO BED

BUT A MONTH AGO YOU ADVISED ME NOT TO EAT BEFORE GOING TO BED!

MEDICAL SCIENCE HAS TAKEN GREAT LEAPS FORWARD IN THE LAST FOUR WEEKS!

