

Molecular epidemiology of lung cancer and geographic variations

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Organisers



International Association for the Study of Lung Cancer



European Society for Medical Oncology

GOOD SCIENCE
BETTER MEDICINE
BEST PRACTICE

300 SCIENCE
BETTER MEDICINE
BEST PRACTICE

Disclosure slide

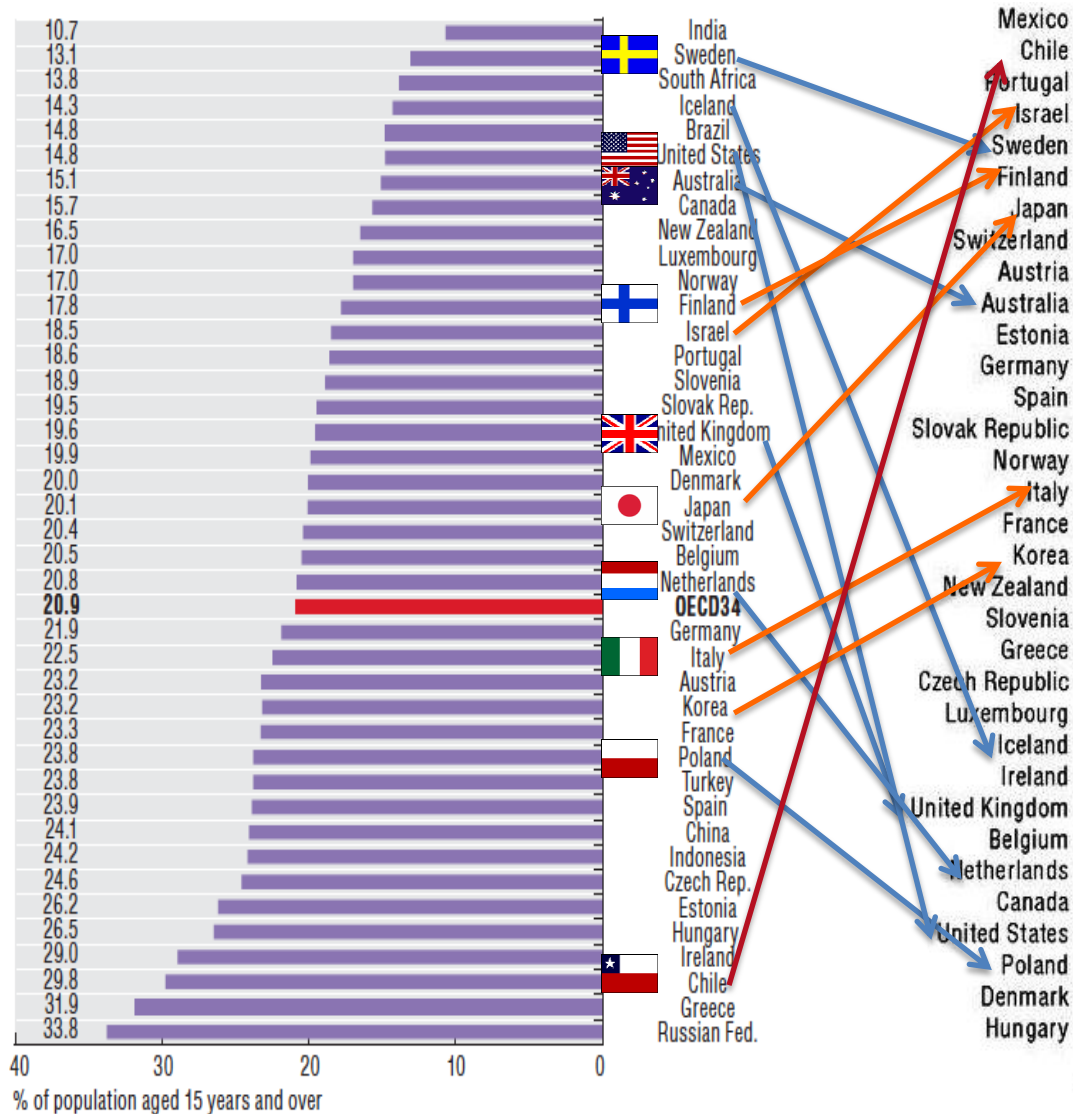
- **Advisor to company:** AstraZeneca, Novartis, Chugai, Boehringer-Ingelheim, Pfizer, Roche, Synta, Clovis, MSD
- **Lecture fees:** AstraZeneca, Chugai, Boehringer-Ingelheim, Pfizer, Taiho, Eli-Lilly, Daiichi-Sankyo
- **Research expenses:** AstraZeneca, Chugai, Boehringer-Ingelheim, Pfizer, Taiho, Ono, Daiichi-Sankyo, Eli-Lilly

Molecular epidemiology of lung cancer

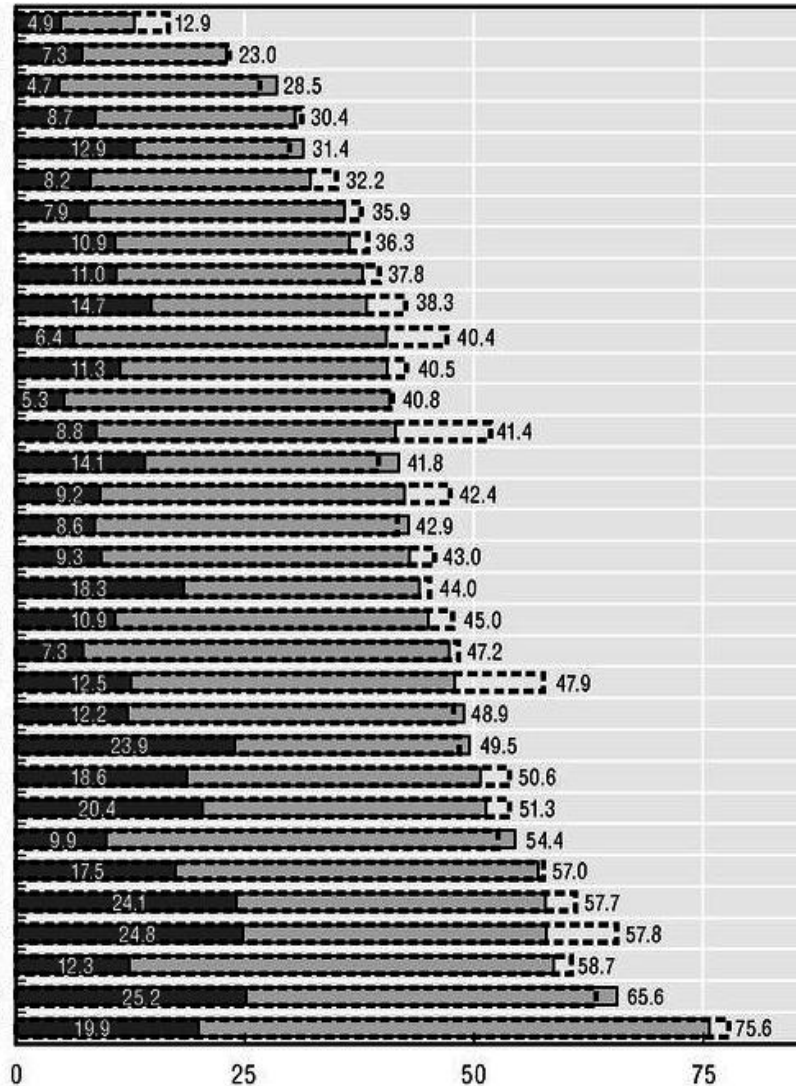
- Difference in incidence/mortality
- Difference in histologic distribution
- Difference in EGFR/KRAS mutation
- Difference in smoking habit
- Difference in susceptibility to different carcinogens based on SNPs

Lung cancer mortality and smoking rate
do not parallel

Adult population smoking daily, 2011



Lung cancer mortality 2011



Higher susceptibility to lung cancer
in Caucasian smokers than in Asian smokers

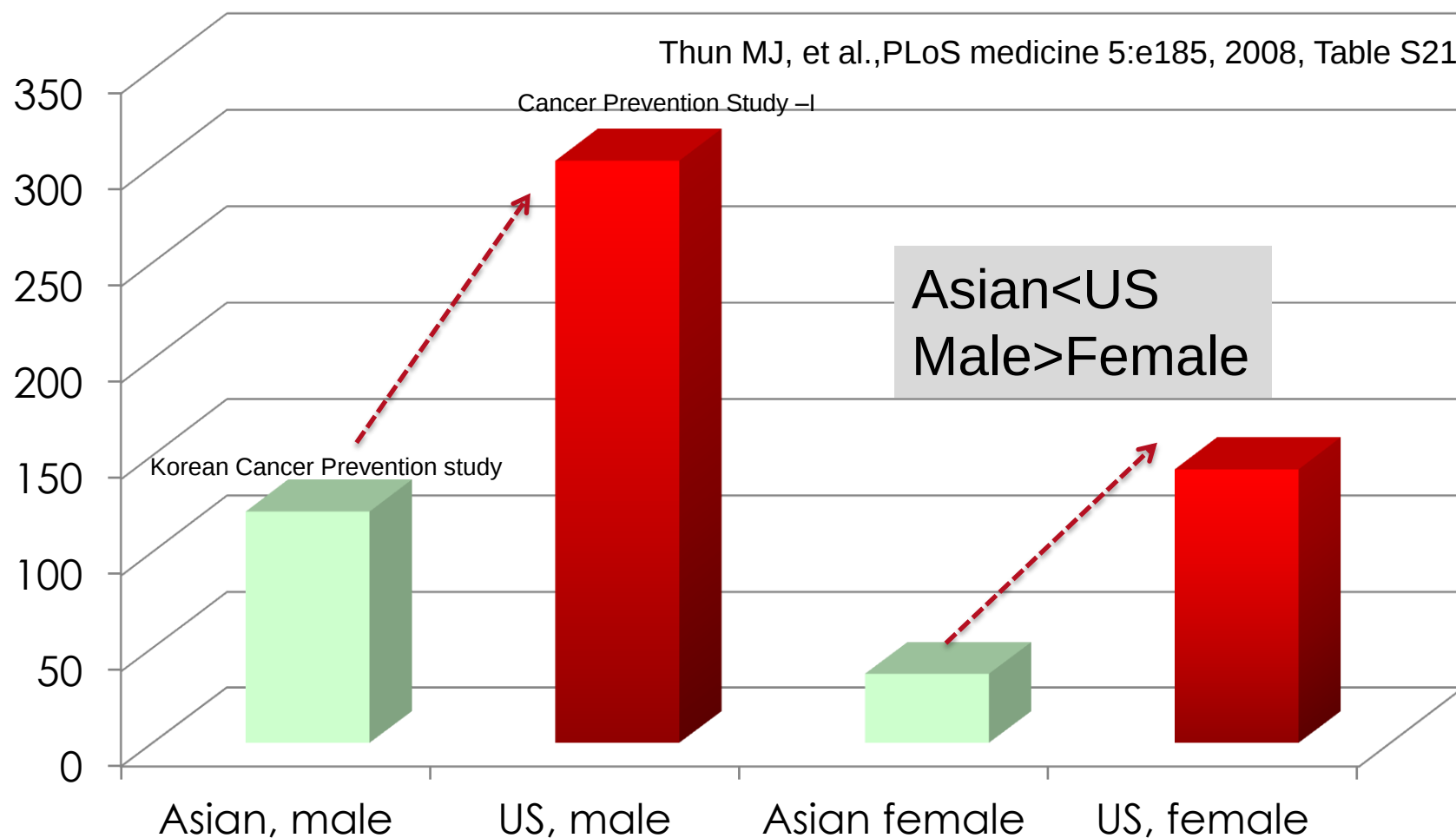
Lung Cancer Occurrence in Never-Smokers: An Analysis of 13 Cohorts and 22 Cancer Registry Studies

Michael J. Thun^{1*}, Lindsay M. Hannan¹, Lucile L. Adams-Campbell², Paolo Boffetta³, Julie E. Buring⁴, Diane Feskanich⁴, W. Dana Flanders^{1,5}, Sun Ha Jee⁶, Kota Katanoda⁷, Laurence N. Kolonel⁸, I-Min Lee⁴, Tomomi Marugame⁷, Julie R. Palmer⁹, Elio Riboli³, Tomotaka Sobue⁷, Erika Avila-Tang¹⁰, Lynne R. Wilkens⁸, Jon M. Samet¹⁰

- Men had higher death rates from lung cancer than women in all age and racial groups studied
- Male and female incidence rates were similar when standardized across all ages 40+ y, albeit with some variation by age
- African Americans and Asians living in Korea and Japan (but not in the US) had higher death rates from lung cancer than individuals of European descent
- No temporal trends were seen when comparing incidence and death rates among US women age 40–69 y during the 1930s to contemporary populations where few women smoke, or in temporal comparisons of never-smokers in two large American Cancer Society cohorts from 1959 to 2004
- Lung cancer incidence rates were higher and more variable among women in East Asia than in other geographic areas with low female smoking.

Thun et al., PLoS Med., 5(9) e185, 2008

Lung cancer mortality (per 100,000) among current smokers in two cohorts



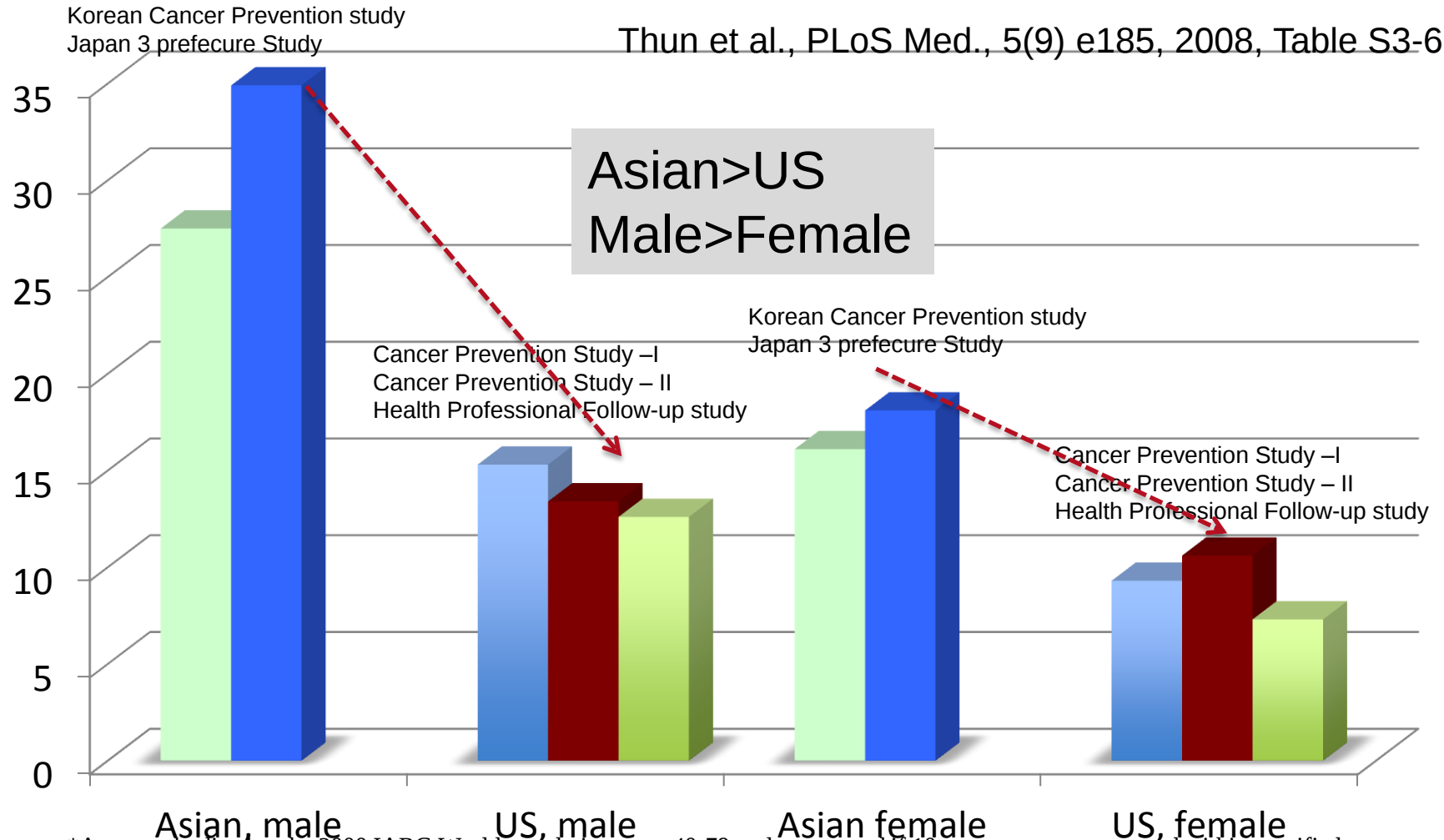
*Age-standardized to the 2000 IARC World population, ages 40-79, only presented if 10 or more events occurred within specified age-range

EGFR-mutated lung cancer typical of
lung cancer in non-smokers occurs
independent of smoking

Lung cancer mortality (per 100,000) among life-long never smokers in individual 6 cohort studies

(Korea x 1, Japan x 1, US x 3)

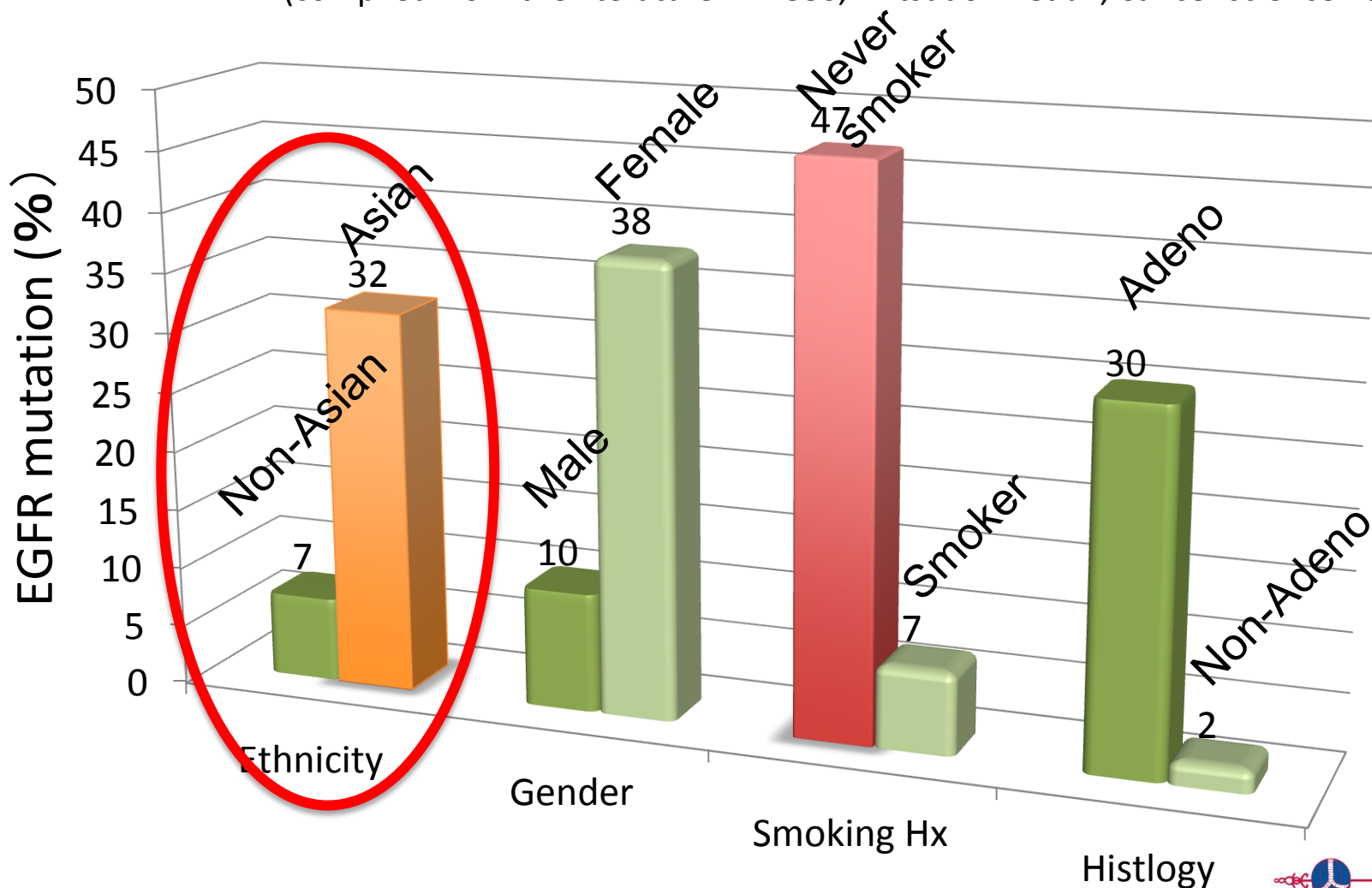
Thun et al., PLoS Med., 5(9) e185, 2008, Table S3-6



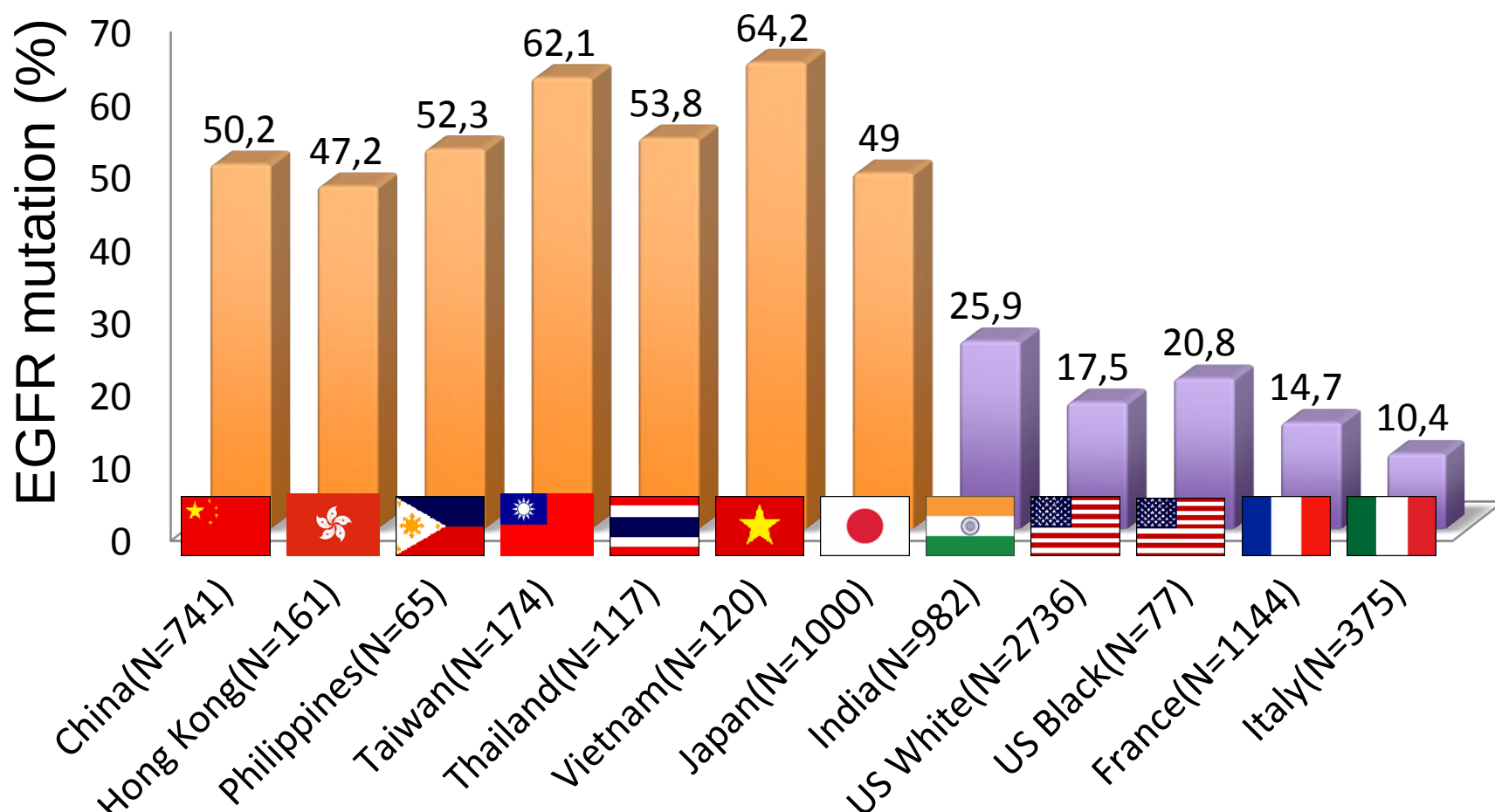
*Age-standardized to the 2000 IARC World population, ages 40-79, only presented if 10 or more events occurred within specified age-range

Incidences of EGFR mutations according to patient backgrounds

(compiled from the literature N=2880, Mitsudomi et al., Cancer science 2007)



EGFR Mutation frequency according to ethnic/geographic backgrounds

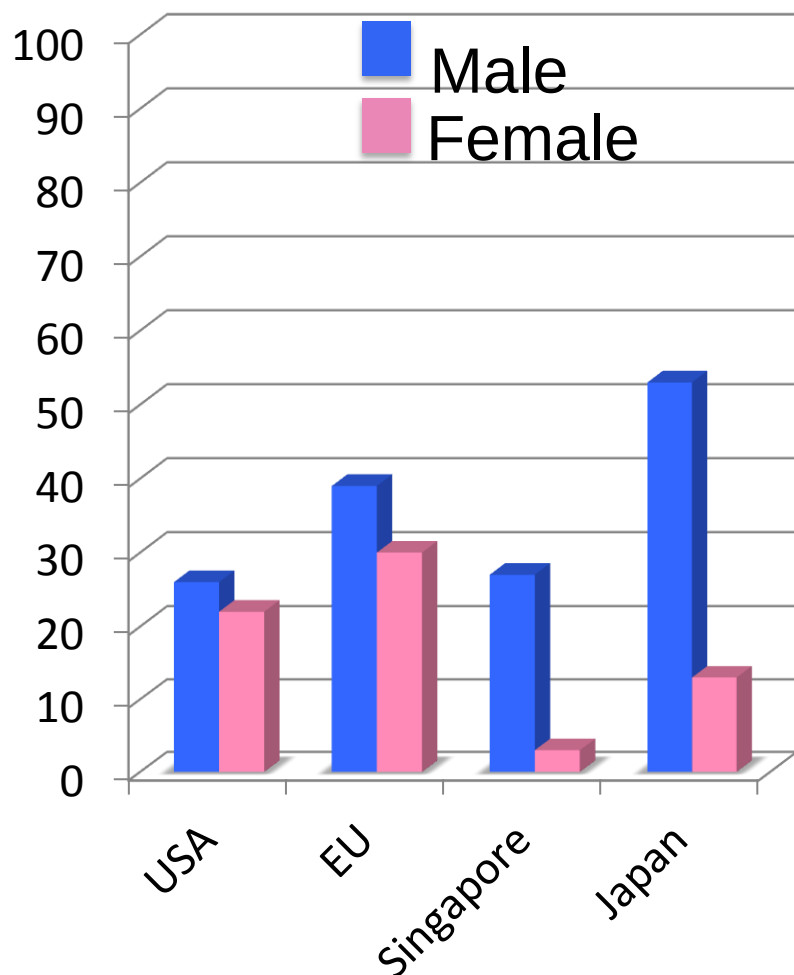


Shi et al., JTO, 2014; Chougule et al., PLoS One, 2013; Yatabe personal comm, Dogan et al., Clin Cancer Res, 2012, Valey et al., Int J Oncol, 2013, Marchetti et al., JCO, 2005

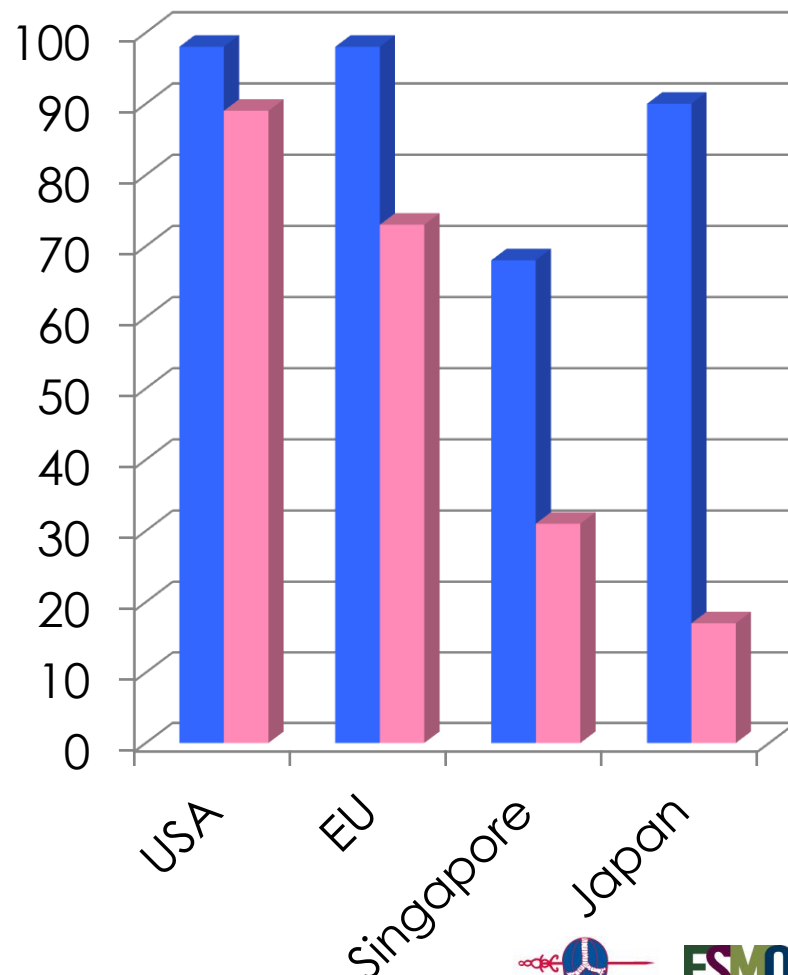
Smoking rate by sex and countries in lung cancer cases and controls

Low smoking rate in Asian female lung cancer patients

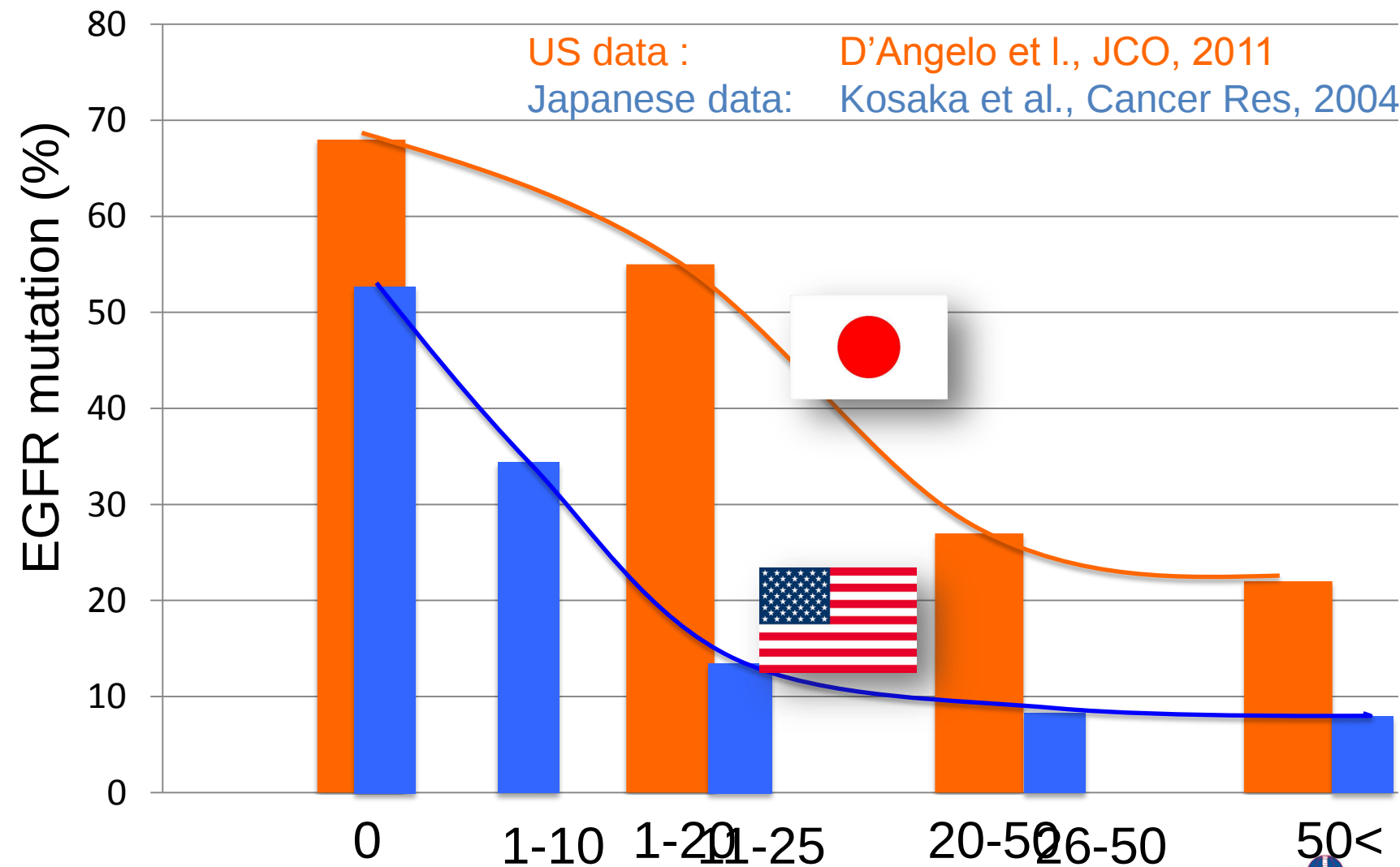
Control



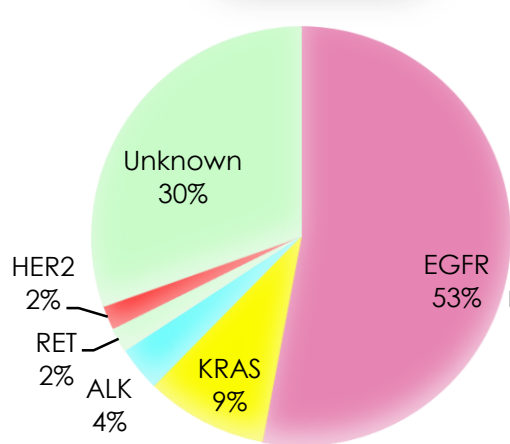
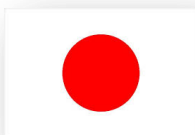
Lung cancer



Incidence of EGFR mutations in Pack-year: Comparison of US data with Japanese data

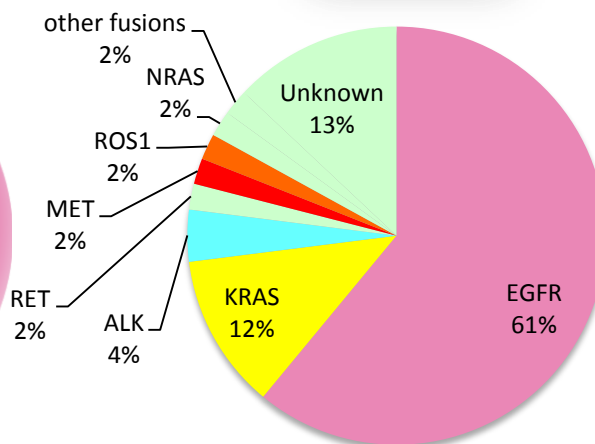


Incidence of driver gene mutations in adenocarcinoma of the lung



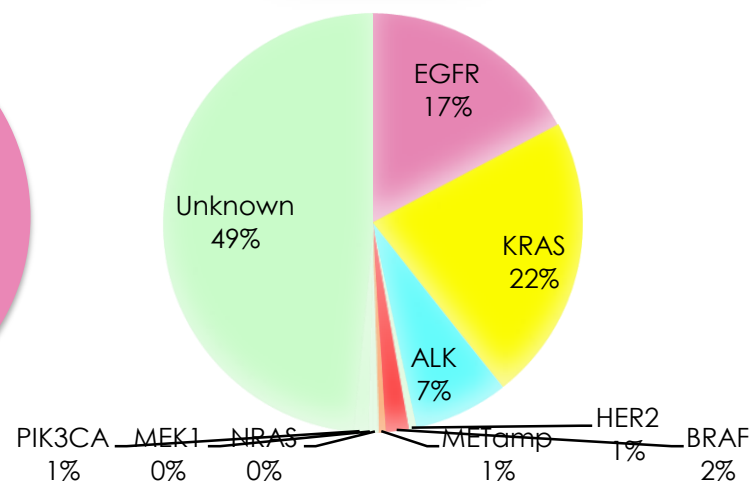
Kohno et al, Nat Med, 2012

N=300



Seo et al., genome Res, 2012

N=200



Kris et al., ASCO 2012

N=516

Does smoking prevent EGFR mutations?

Case control study at ACC to assess effect of smoking on EGFR mutations

- Lung cancer cases 435

152 EGFR mutation, 283 wt

- Non-cancer controls 2175

1:5 age-sex matched

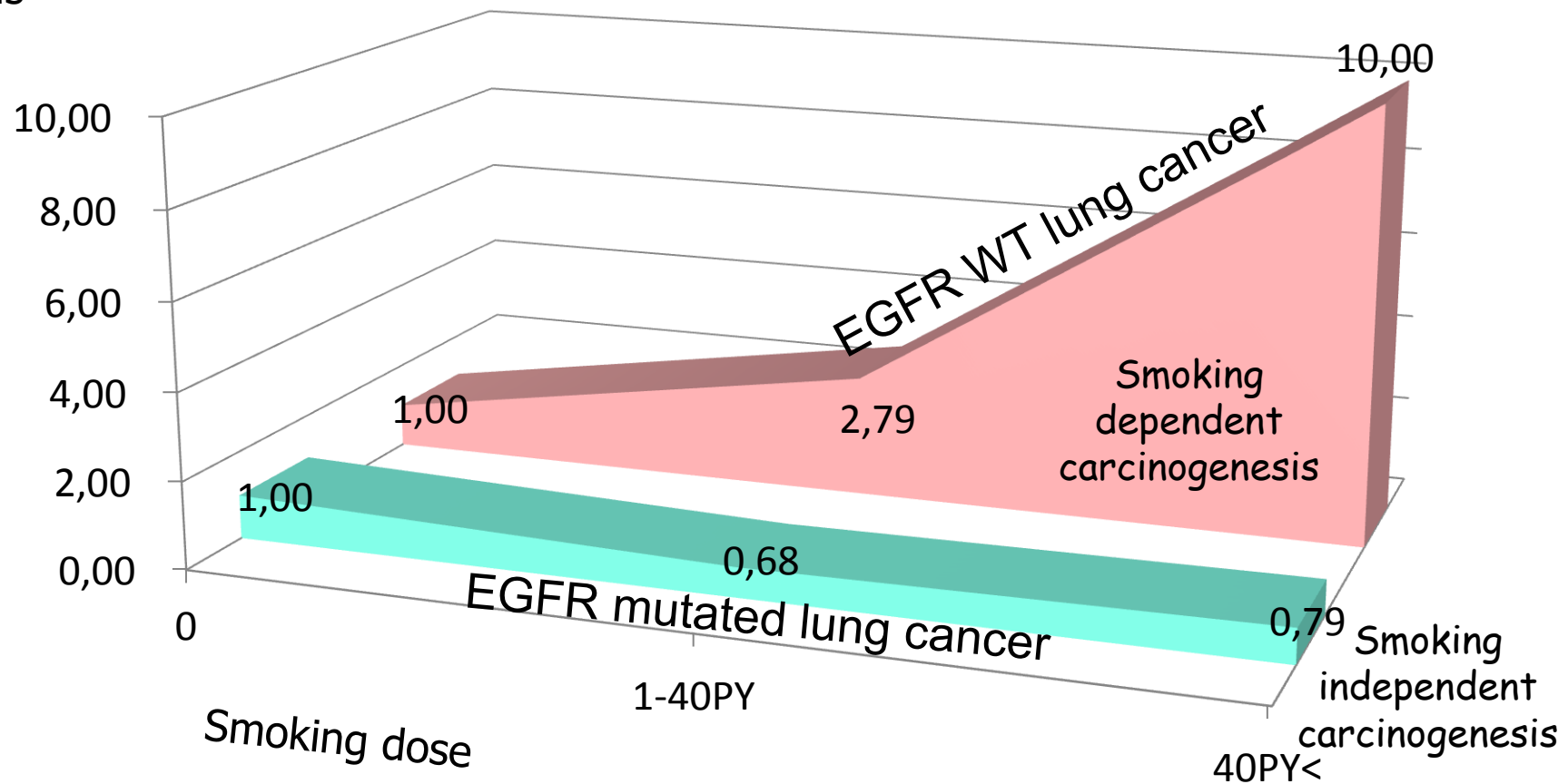
Comprehensive life-style data by The Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC)

Matsuo et al., Cancer Science 98:96-101,2007

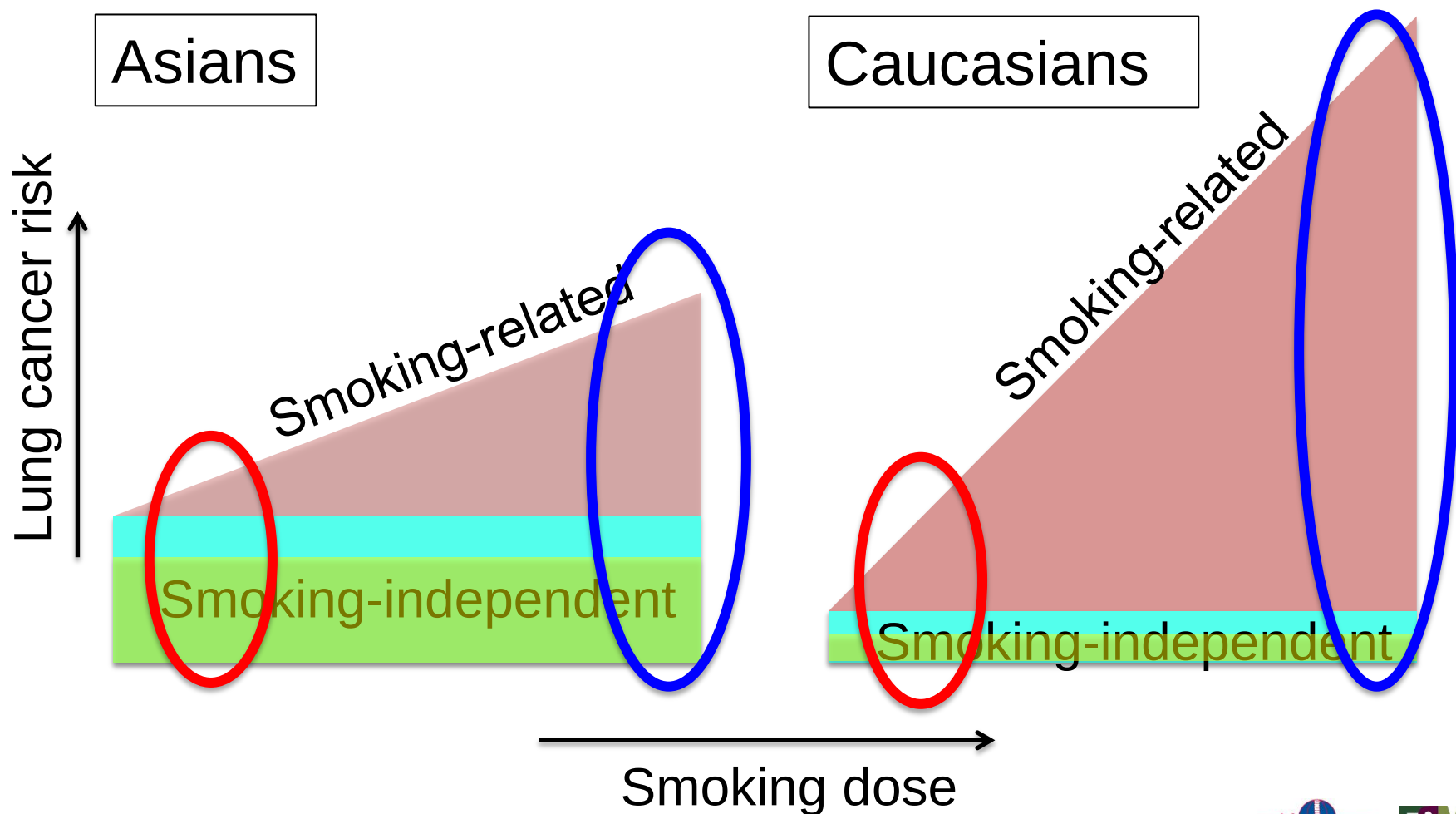
EGFR mutation occurs independent of smoking! Smoking dilutes EGFR mutated lung cancer by EGFR WT lung cancer

Matsuo et al., Cancer Science 98:96-101,2007

odds



EGFR mutation, smoking and ethnicity



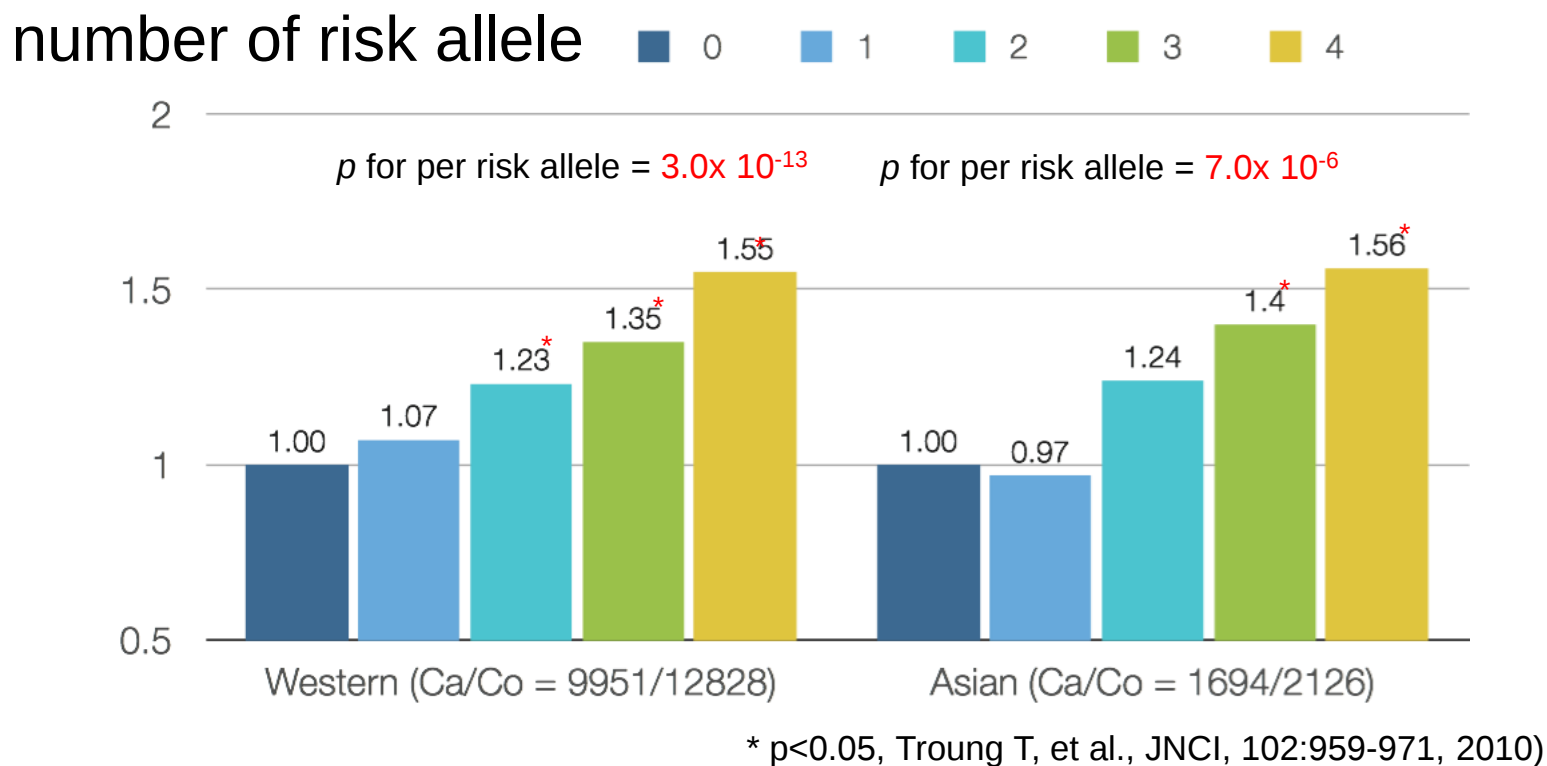
GWAS of lung cancer vs controls

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
Thorgeirsson 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)	2,331 controls (smokers and never smokers)	Affymetrix [906,703]	3q28 TP63, 5p15 TERT-CLPTMIL, 13q12 MIPEP-TNFRSF19, 22q12 MTMR3-HORMAD2-LIF
Ahn 2012 (38)	444 cases (smokers and never smokers)	444 controls (smokers and never smokers)	Affymetrix [906,703]	18p11 FAM38B
Dong 2012 (39)	653 cases (smokers and never smokers)	653 controls (smokers and never smokers)	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

5p15 TERT-CLPTMIL

15q25 CHRNA3

5p15: Consistent results between Western and Asians

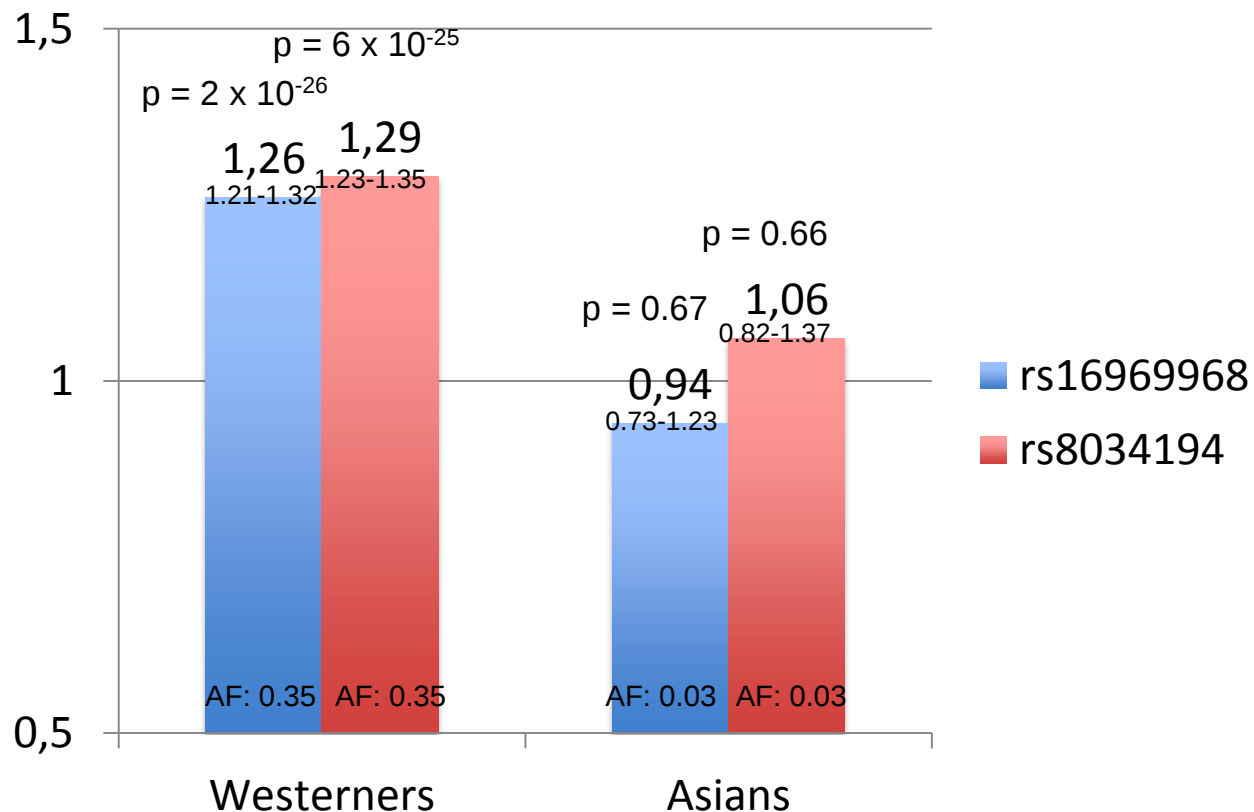


rs2736100: telomerase reverse transcriptase gene (*TERT*)

rs407210: cleft lip and plate transmembrane1-like gene (*CLPTM1L*)

15q25: inconsistent results between Western and Asians

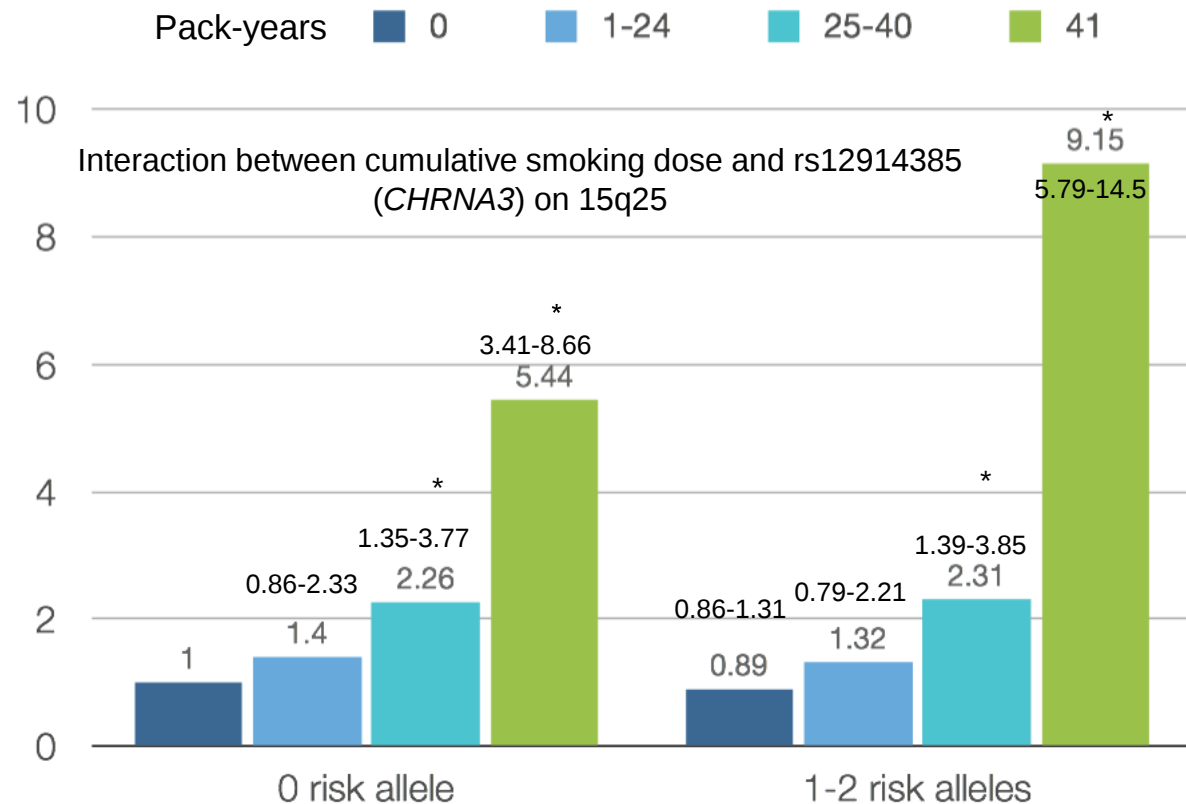
Per-allele models of the logistic regression



Troung T, et al., JNCI, 102:959-971, 2010)
 rs16969968: nicotine acetylcholine receptor $\alpha 5$ gene (*CHRNA3*) rs8034194: (*LOC123688*)

Effect of smoking on the risk of lung cancer was stronger in with 15q25 risk allele carriers in Japanese population.

P for interaction : 0.021



* p<0.05

Association between a GWAS identified locus and the risk of lung cancer in Japanese Population

Ito H, et al. JTO 7:790-797, 2012

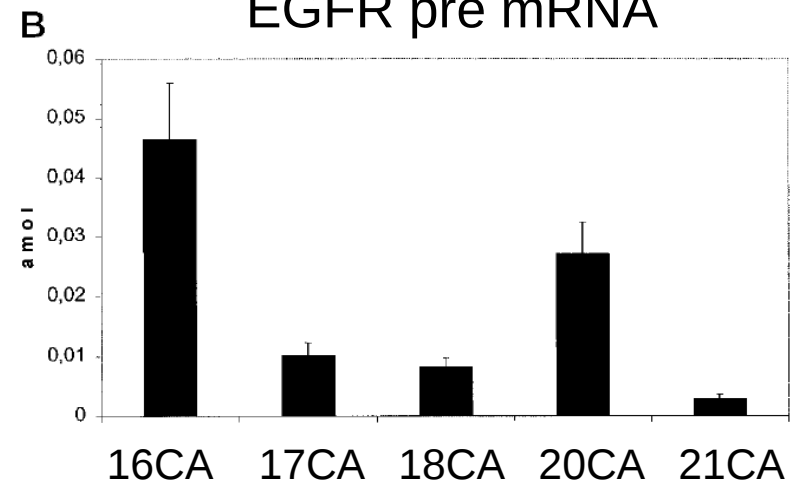
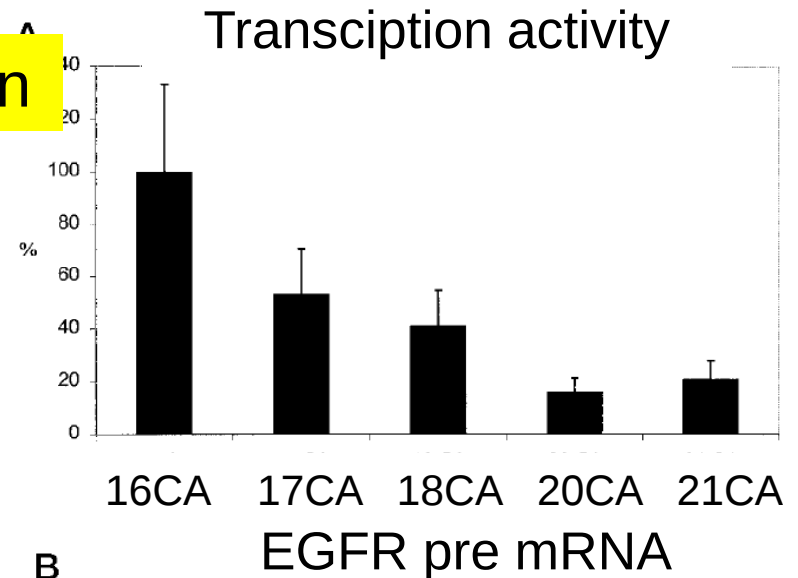
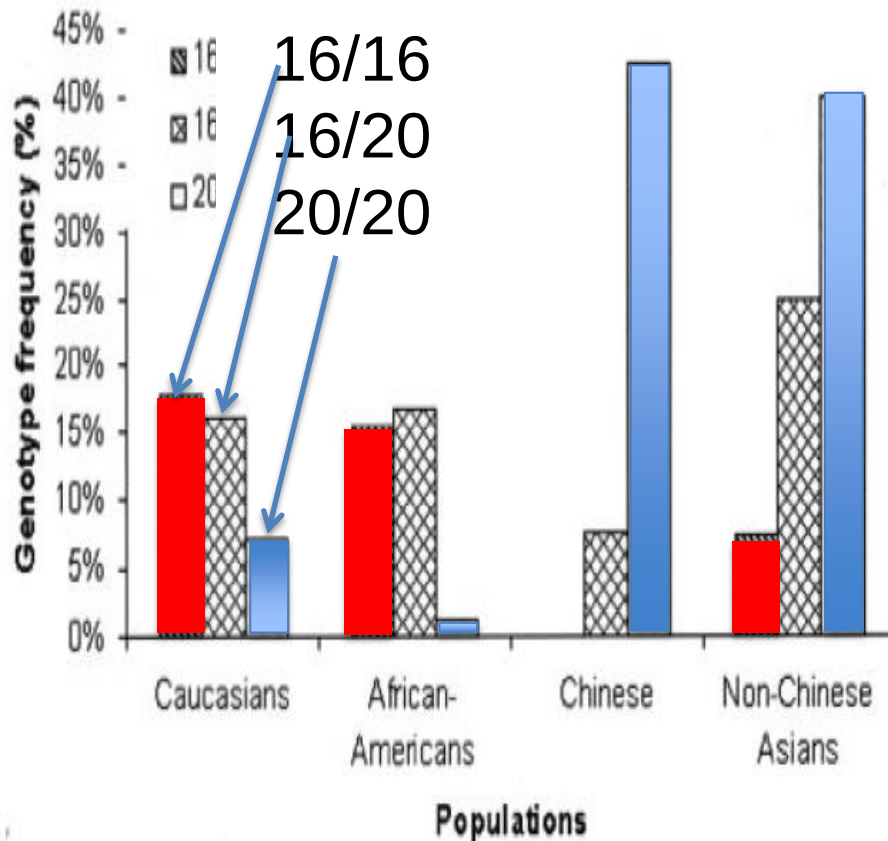
What makes Asian people susceptible for EGFR mutation?

Polymorphisms within the EGFR gene

- **CA-SSR1(CA simple sequence repeat 1) in intron 1**
 - East-Asians have longer repeats (Liu et al, CCR 2003)
 - Shorter repeats are associated with increased transcription and protein expression (Gebhardt, JBC 1999, gebhardt Hitol Hist Pathol 2000)
- **2 SNPs in the promoter lesion with increased transcription and expression of EGFR mRNA**
 - -216(G/G to G/T or T/T)
 - SP1 binding site
 - Less common in Asians (Liu, Cancer Res 2005)
 - -191(C/C to C/A or A/A)
 - Promoter regions
 - Less common in Asians(Liu, Cancer Res 2005)

CA-SSR1: ethnic difference, transcription and expression

Asians → shorter repeat → low protein



Liu et al, Clin Cancer Res,9:1009-1012, 2003

Gebhardt et al, JBC,9:13176-13180, 1999

Polymorphisms, Mutations, and Amplification of the *EGFR* Gene in Non-Small Cell Lung Cancers

Masaharu Nomura¹, Hisayuki Shigematsu¹, Lin Li², Makoto Suzuki¹, Takao Takahashi¹, Pila Estess³, Mark Siegelman³, Ziding Feng², Harubumi Kato⁴, Antonio Marchetti⁵, Jerry W. Shay⁶, Margaret R. Spitz⁷, Ignacio I. Wistuba⁸, John D. Minna^{1,9,10}, Adi F. Gazdar^{1,3*}

PLoS Med 4(4): e125, 2007

- Variant forms of SNP216 (G/T or T/T) and SNP191 (C/A or A/A) (associated with higher protein production in experimental systems) were less frequent in East Asians than in individuals of other ethnicities ($p < 0.001$).
Asians → SNP infrequent → low protein
- Both alleles of CA-SSR1 were significantly longer in East Asians than in individuals of other ethnicities ($p < 0.001$).
Asians → longer repeat → low protein

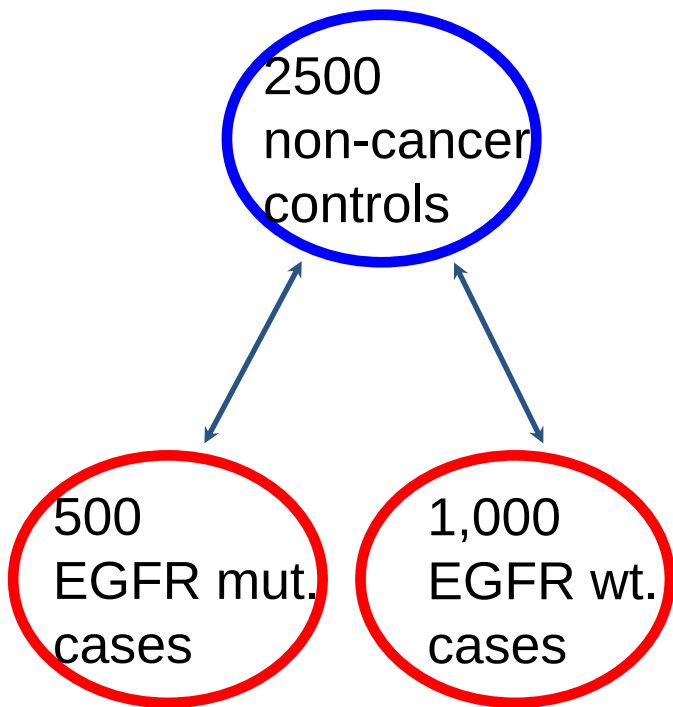
These findings suggest that the cells of most East Asians make less EGFR protein than do the cells of individuals of other ethnicities. If a certain critical level of EGFR is required to drive the cell toward a malignant phenotype, mutations of the TK domain and autonomous activation of downstream signaling may target East Asians, the subgroup with possibly lower intrinsic protein production.

Preliminary results of GWAS for EGFR mutated lung cancer

- Purpose
 - To explore genomic locus that explains difference in incidence of EGFR mutations between Japanese and Caucasians
- Materials and Methods
 - Collaborative GWAS study for NSCLC with confirmed EGFR mutational status
 - GWAS using whole genome scan data obtained by Illumina 610Quad
 - Five Centers
 - Aichi Cancer Center
 - Kyoto University
 - Okayama University
 - Hyogo University
 - Kagawa University
 - Multi-phase study
 - Phase I: Screening phase
 - Phase II: Validation phase

Courtesy of Prof. K. Matsuo@Kyushu University/Aichi Cancer Center

Phase I analysis



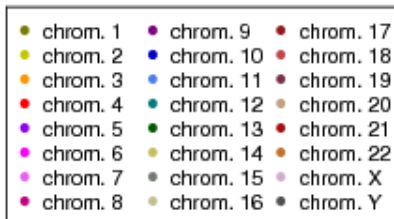
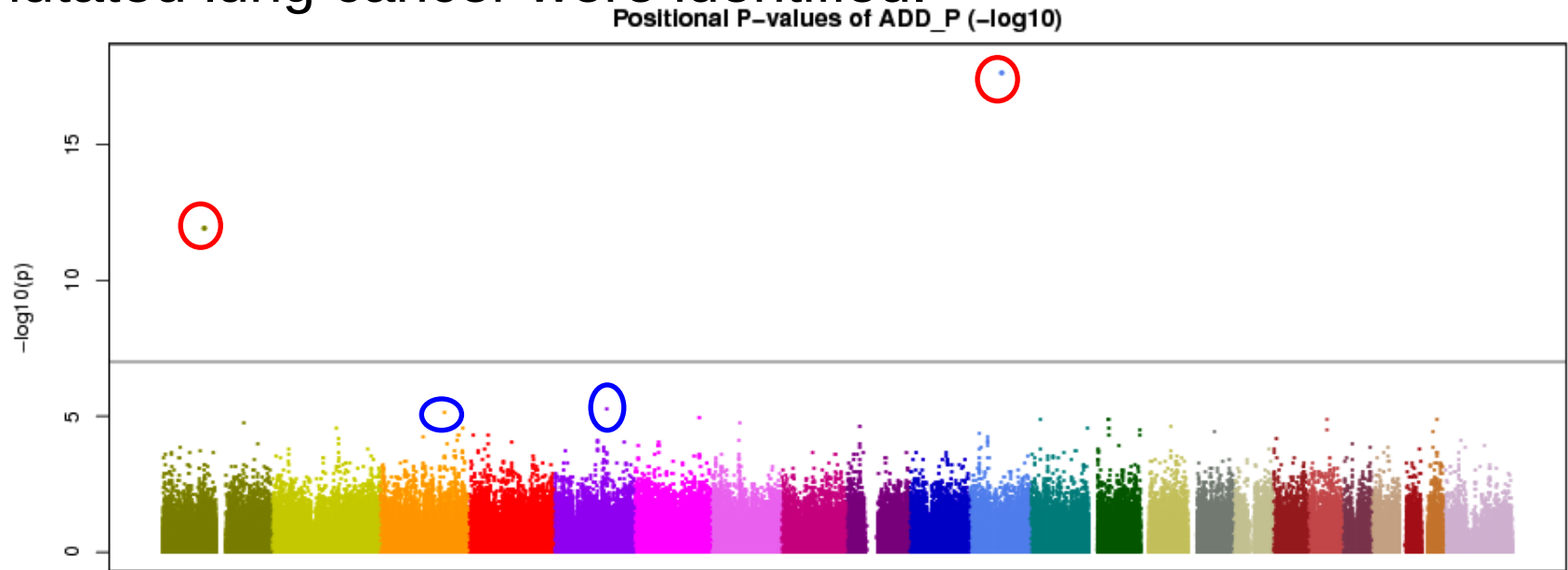
- Illumina platform.
 - 6 millions of SNPs
 - Screen for loci for
 - EGFR mut. LC
 - EGFR wt. LC
- Genotyping is on-going.
- In this presentation, preliminary results in ACC data are shown
 - Case-Control study
 - Cases: 118 EGFR mutated lung adenocarcinoma treated at ACC
 - Controls: Non-cancer control 1194

Courtesy of Prof. K. Matsuo@Kyushu University/Aichi Cancer Center

Manhattan plot (ACC cohort)

EGFR mutated LC vs. control

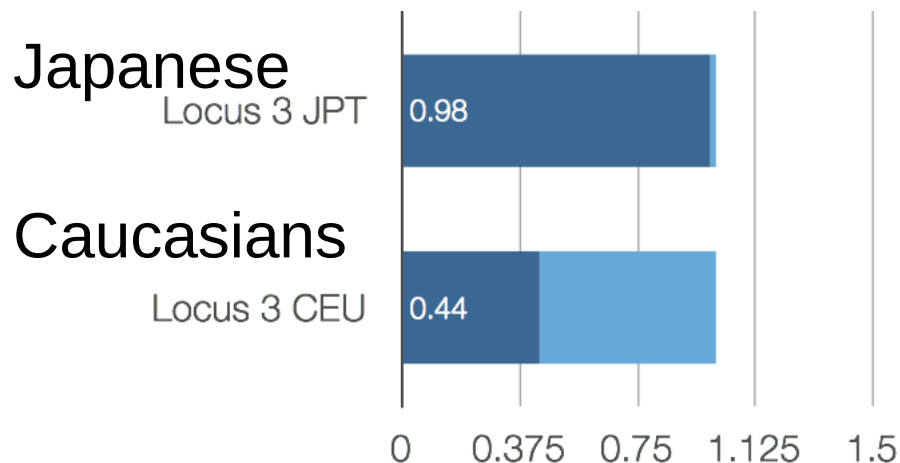
2 significant and 2 marginally significant loci for EGFR mutated lung cancer were identified.



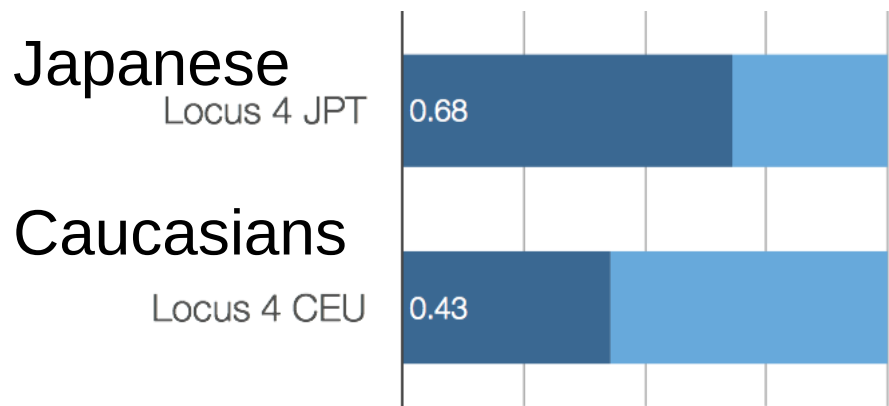
After QC and GC.

Courtesy of Prof. K. Matsuo@Kyushu University/Aichi Cancer Center

Comparison of allele freq. of risk allele in HapMap database.



- ORs for having risk allele for two of four loci were around 3.
- Allele freq. of risk allele for these loci is common in Japanese than in Caucasian.
- This is accordant with the fact EGFR mut lung cancer is more prevalent in Japanese than in Caucasian.



Courtesy of Prof. K. Matsuo@Kyushu University/Aichi Cancer Center

Conclusions

- In smokers, the risk for lung cancer is higher in Caucasians, while in never smokers, the risk is higher in Asians
- EGFR mutations are dependent on smoking status, ethnicities, sex and histologic types
- Risk for EGFR mutated lung cancer is independent of smoking dose.
- Three polymorphisms within the EGFR gene is responsible for lower EGFR protein level in Asians that may be relevant to high EGFR mutation rate
- GWAS reproducibly identified risk alleles at 5p15 and 15q25 in Caucasians.
- While 5p15 allele has similar effect on both Caucasians and Asians, effect of 15q25 appears different
- Preliminary GWAS efforts revealed several loci that is significantly associated with EGFR mutated lung cancer which is more frequent in Asians