Stephen J. Chanock, M.D.
Director, Division of Cancer Epidemiology and Genetics

The Complexity of Cancer Susceptibility & Cancer Genomics

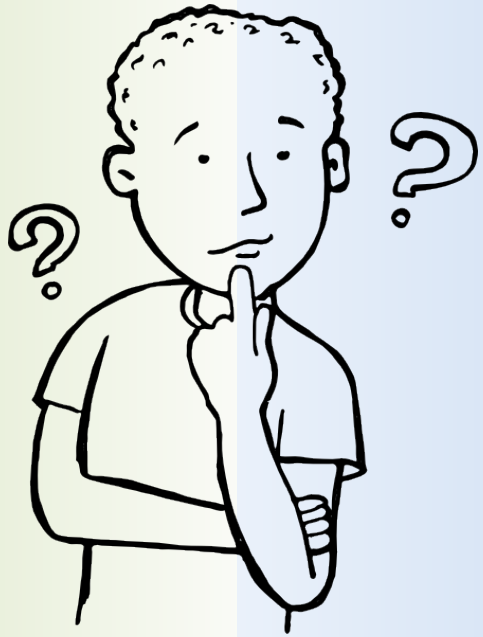
Radiation Epidemiology & Dosimetry Course

National Cancer Institute www.dceg.cancer.gov/RadEpiCourse

Etiology of Cancer

Environment

Lifestyle



Genetic Susceptibility

Mutations

SNPs

STR's

Chromosomes

(Epigenetics)

100%

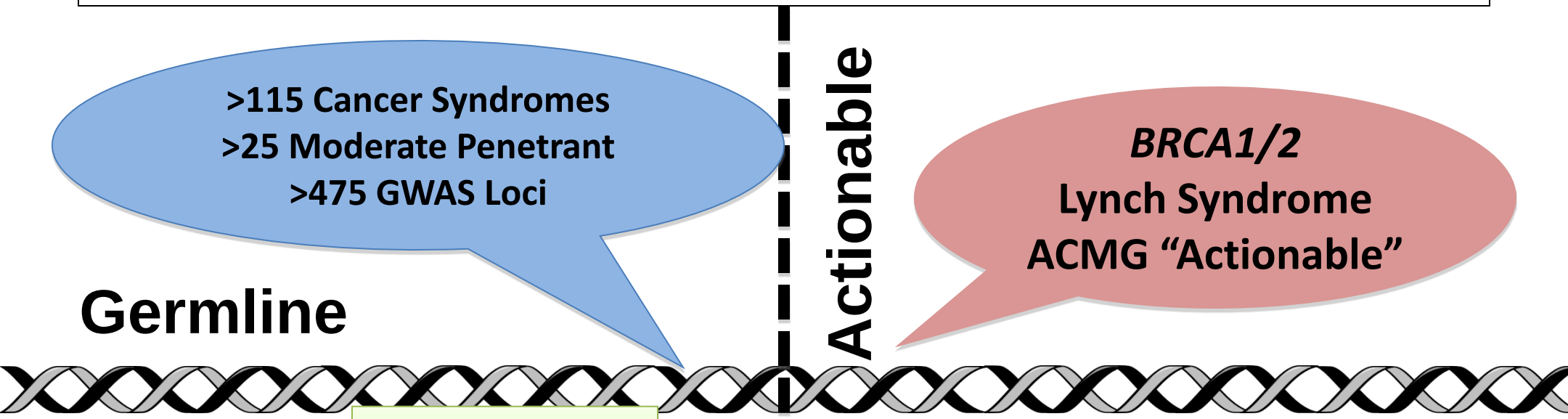
"Triggers"

"Chance"

100%

"Set Point"

Cancer Genomics: 4 Spaces



>115 Cancer Syndromes
>25 Moderate Penetrant
>475 GWAS Loci

BRCA1/2
Lynch Syndrome
ACMG "Actionable"

Germline

Somatic

Heterogeneity
Metastases

'Drivers'
'Passengers'

TCGA/ICGC-
Cosmic Data

Targeted Therapy
HER2
EGFR
BRAF600

Discovery

Clinically Actionable

Why Study Germline Susceptibility?

Explain heritability of cancers

- Clustering - families and distinct populations
- Sporadic cancer

Risk assessment

- Individual
- Population-based

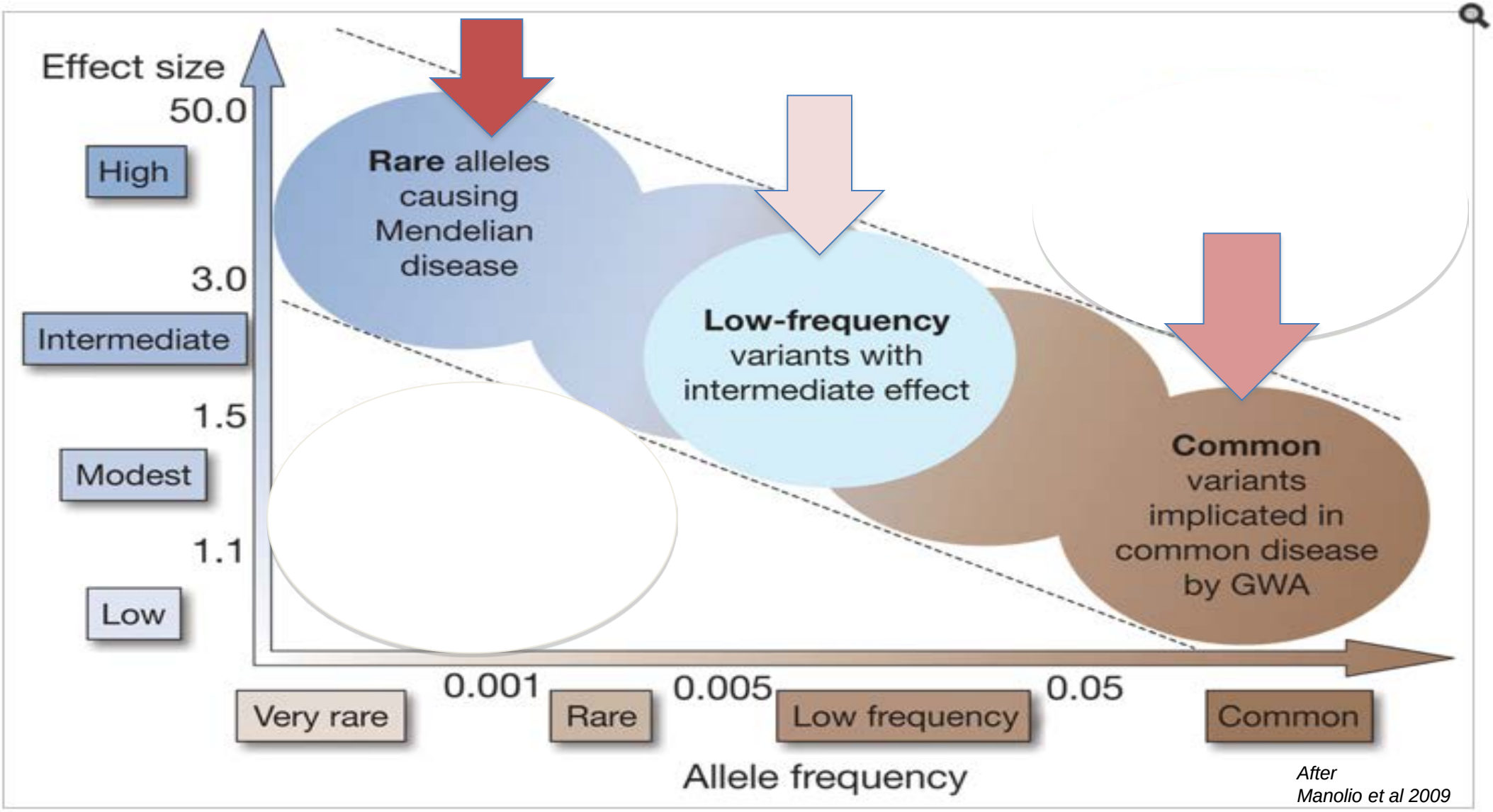
Insights into the etiology of cancer

- Gene-environment interactions
- How the germline informs somatic alterations

Pharmacogenomics

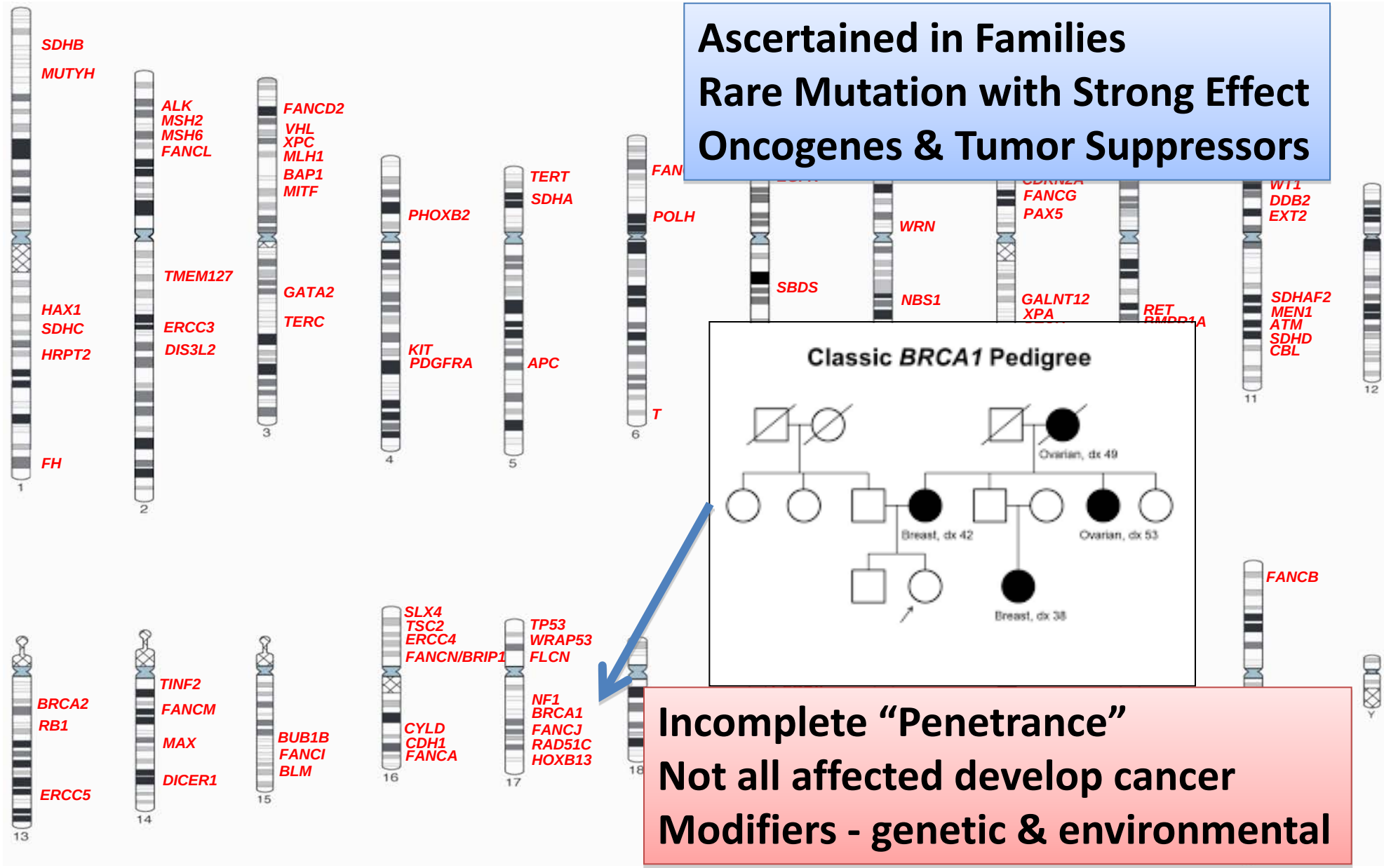
- Response
- Toxicity profiles

Architecture of Genetic Susceptibility of Cancer Defining 'Distinct' Spaces



After Manolio et al 2009

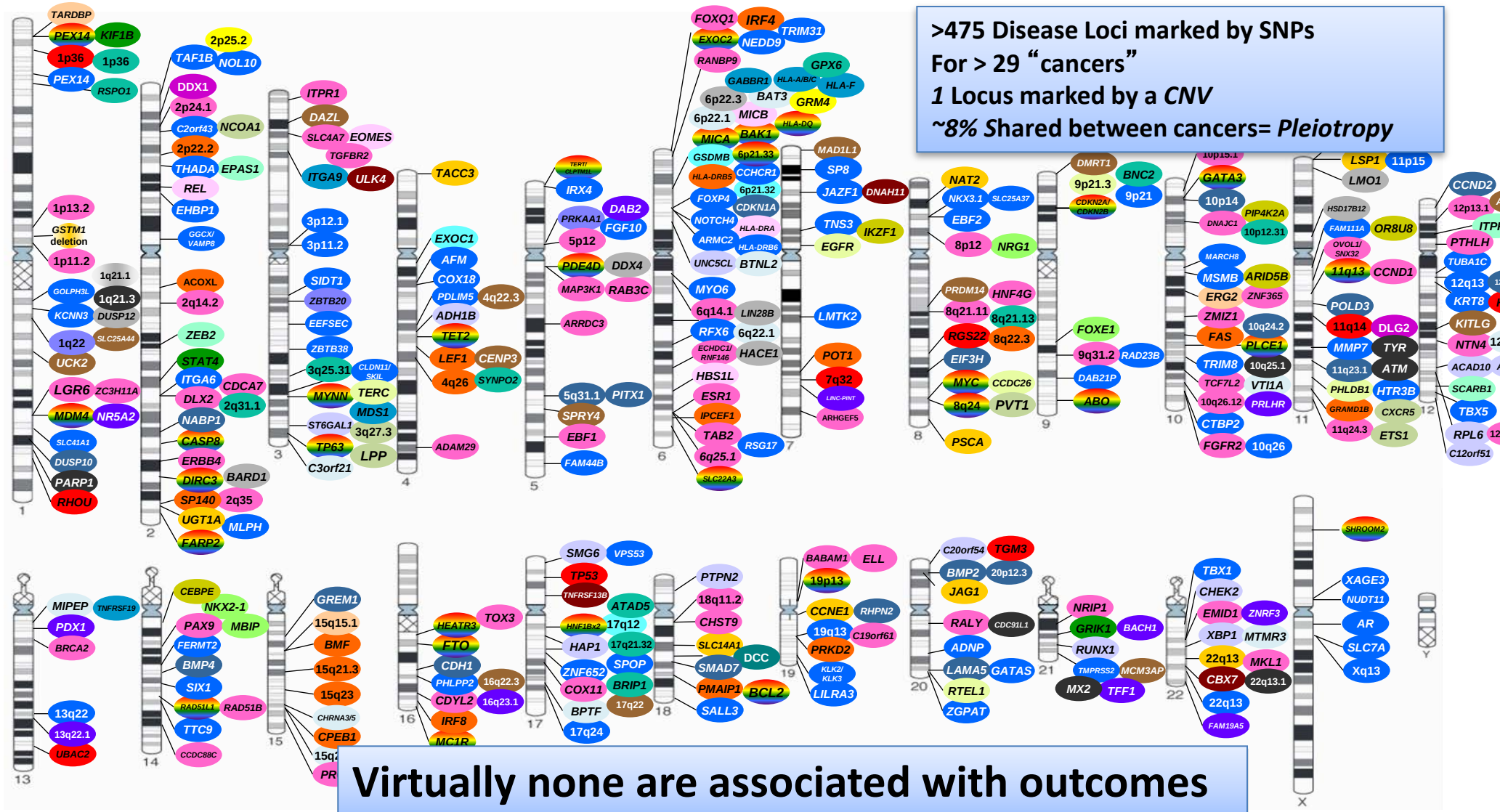
>115 Genes Mutated in Cancer Susceptibility Syndromes



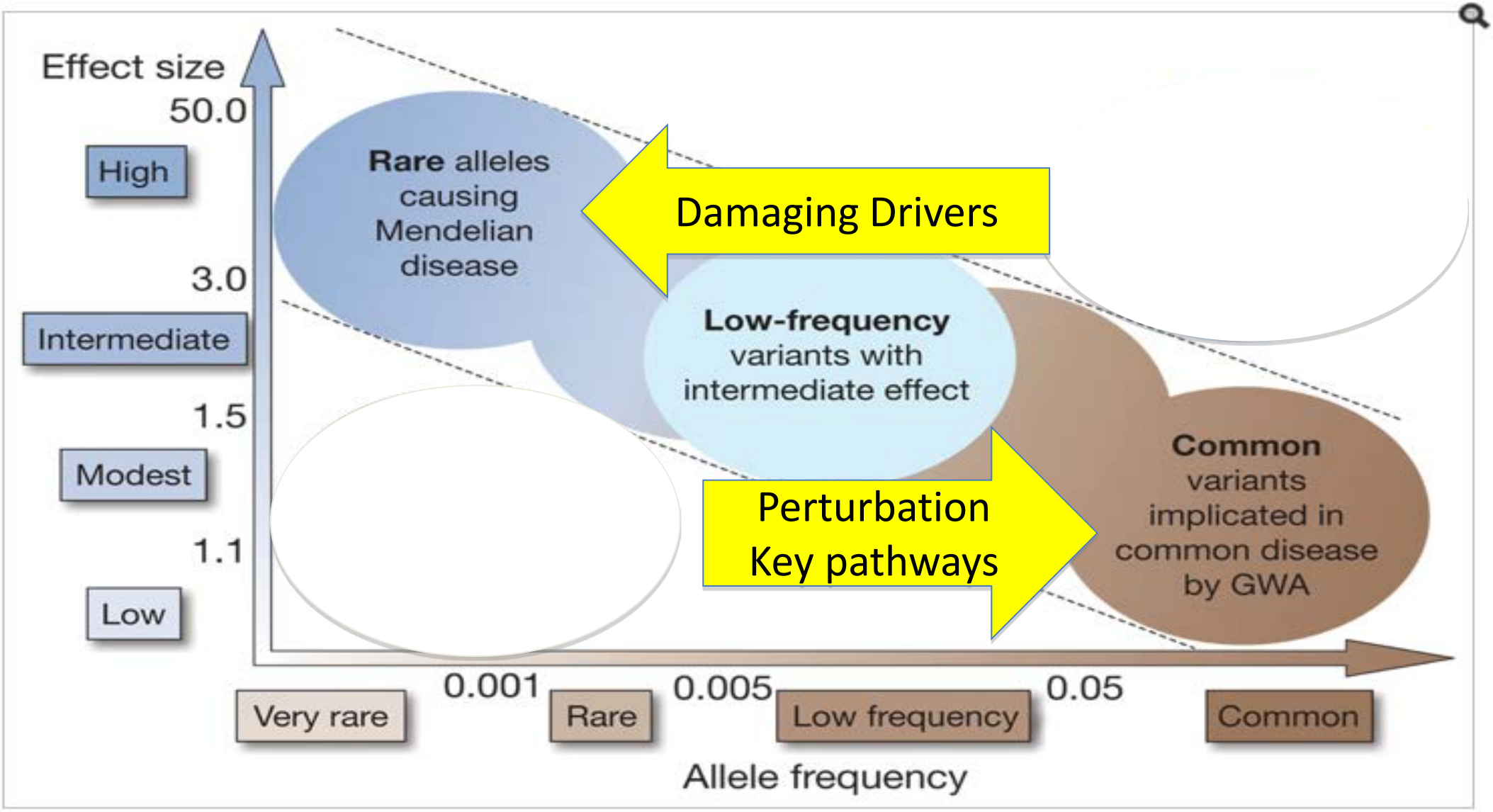
Of the 115

- Roughly 1/3 are recognized by ACMG and trigger recommendations for counseling
- Types of Mutations
  - Indels/Stop Codons, NS & Structural
- Many fit the model of ‘Autosomal Dominant’
- Ascertainment Biased by Family Studies
  - Linkage followed by targeted Sequencing
- >50% are COSMIC ‘drivers’ in somatic databases

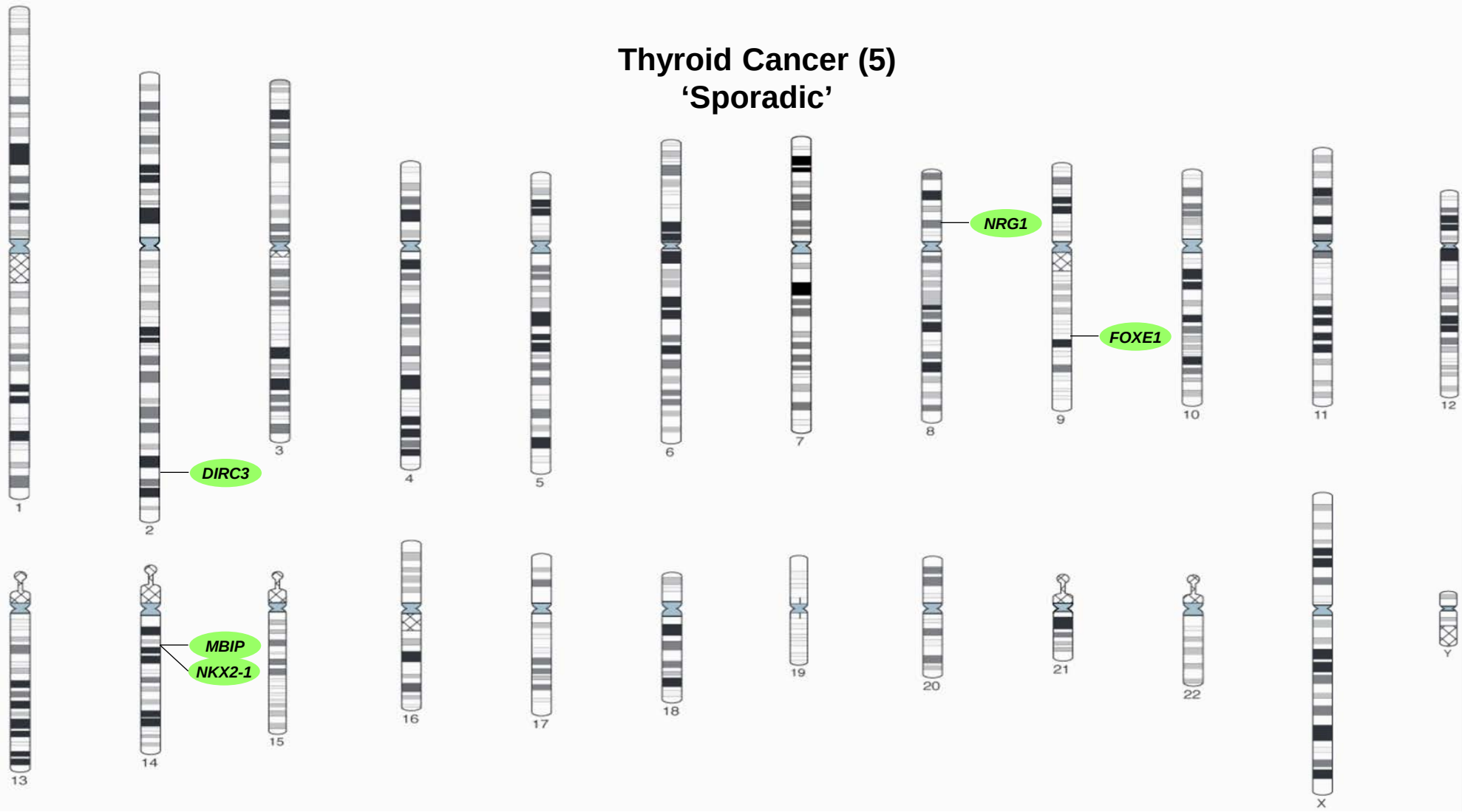
Published Cancer GWAS Etiology Hits: March 2015



Architecture of Genetic Susceptibility of Cancer Defining Distinct Spaces

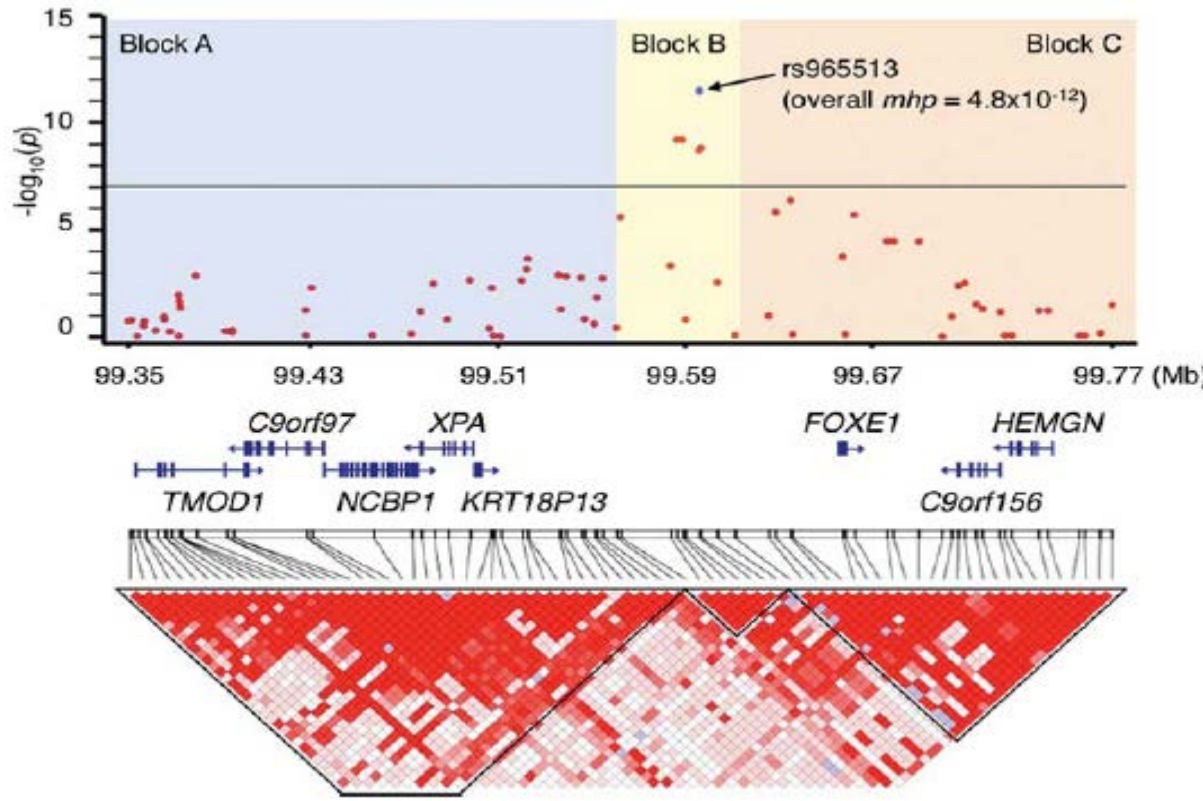


Published Cancer GWAS Etiology Hits: May 2015



9q22.33 (FOXE1)

- FOXE1- thyroid-specific transcription factor with pivotal roles in thyroid morphogenesis
- increased risk of papillary and follicular thyroid cancer
- associated in radiation exposed (Chernobyl) and unexposed thyroid cancer cases



14q13.3 (NKX2-1)

- NKX2-1- also thyroid-specific transcription factor with pivotal roles in thyroid morphogenesis
- altered NKX2-1 expression levels in thyroid tumors
- signal in both papillary and follicular, but not replicated in radiation exposed thyroid cases

9q22.33 and 14q13.3 Risk Alleles Result in Reduced TSH Levels

Type of measurement	Individuals (*n*)	Effect per risk allele (95% CI)	*P* value
Results for rs965513[A] on 9q22.33			
Thyroid stimulating hormone (TSH)	12,035	–5.9% (–7.4%, –4.4%)	2.9×10^{-14}
Free thyroxine (T_4)	7,108	–1.2% (–1.8%, –0.6%)	6.1×10^{-5}
Free triiodothyronine (T_3)	3,593	+1.2% (+0.4%, +2.0%)	3.0×10^{-3}
Results for rs944289[T] on 14q13.3			
Thyroid stimulating hormone (TSH)	11,925	–1.7% (–3.2%, –0.2%)	0.030
Free thyroxine (T_4)	6,931	+0.5% (–0.1%, +1.0%)	0.098
Free triiodothyronine (T_3)	3,564	–0.3% (–1.1%, +0.5%)	0.44

Common SNP Variants Influence Risk For

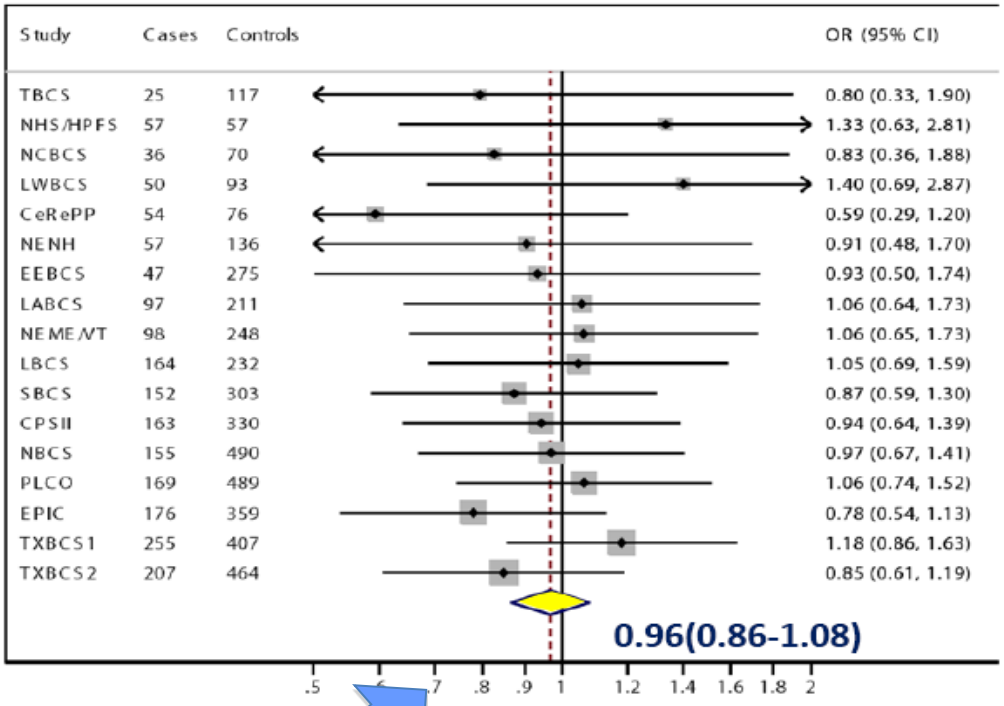
Interactions with Known Carcinogens

Radiation-induced Injury

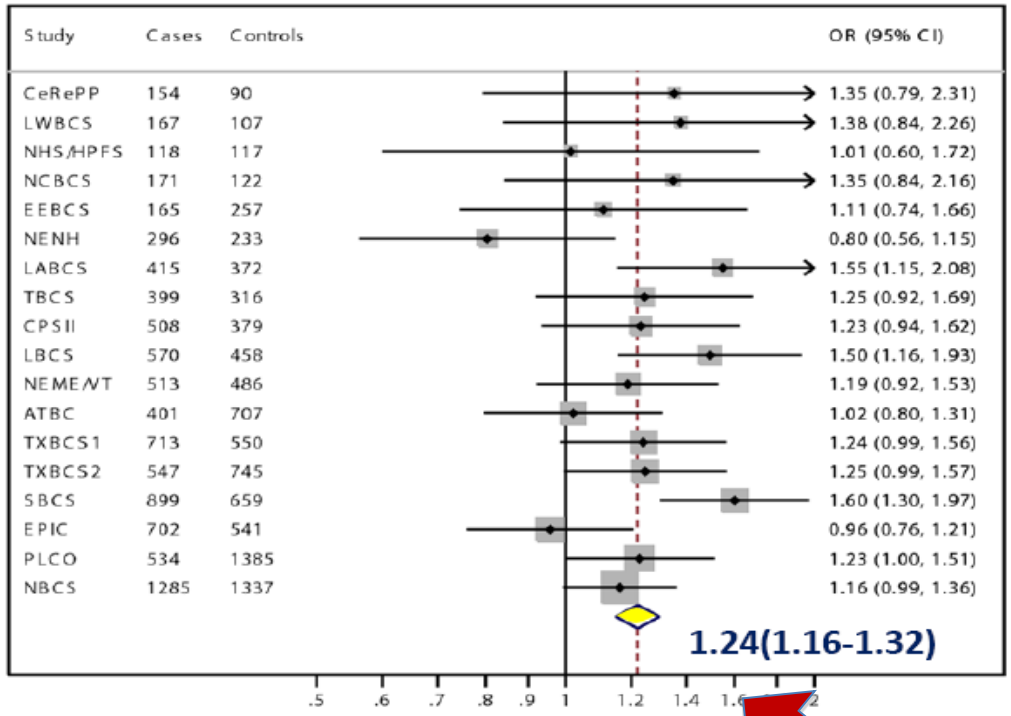
Therapeutic Effects

Gene-Environment Interaction for Bladder Cancer Risk: NAT2 Slow Acetylation Increases Risk only for Smokers

Never Smokers



Ever Smokers

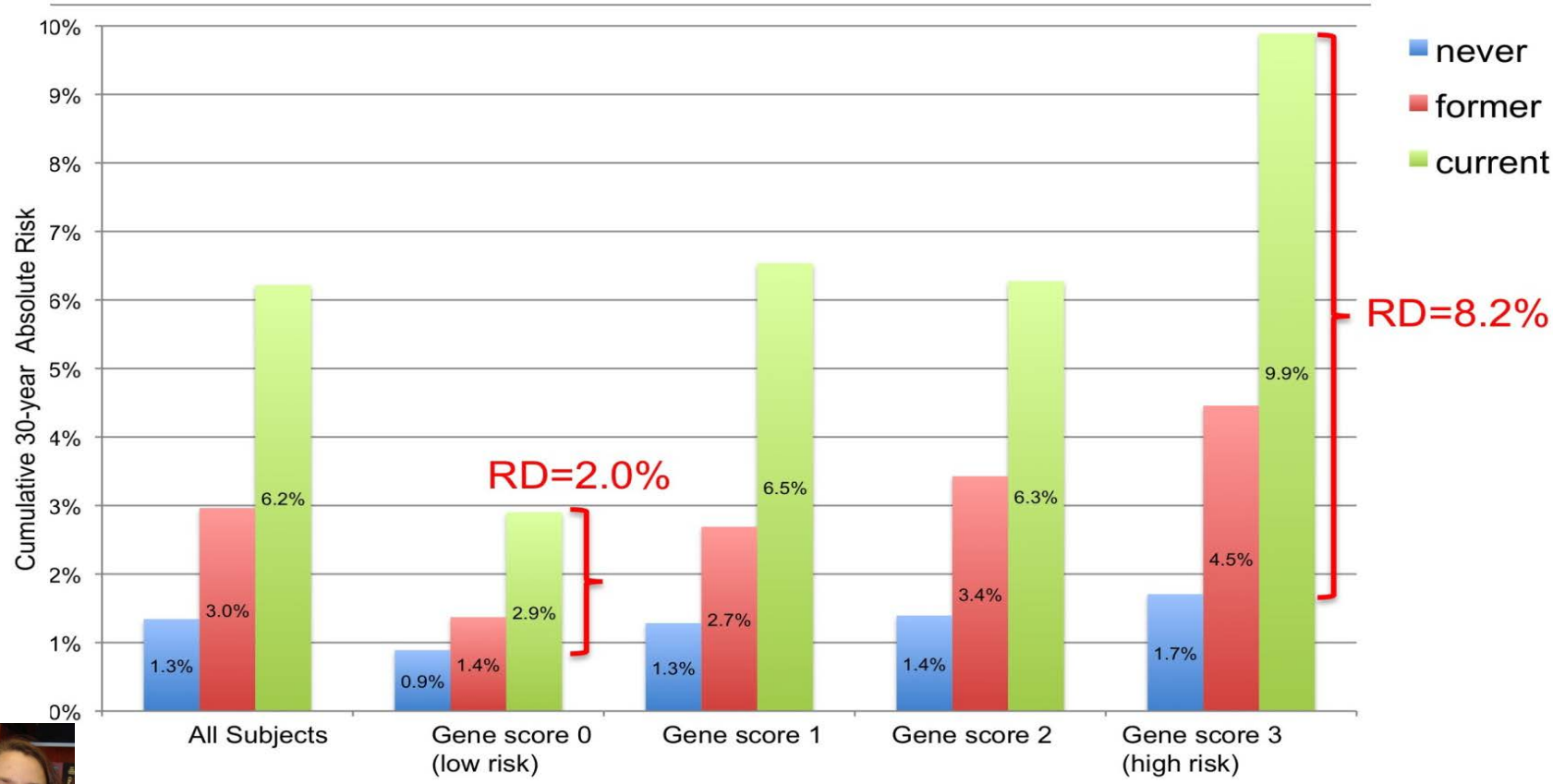


P-interaction = 2.8×10^{-4}

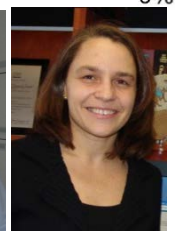
Rothman et al., *Nat Genet* 2010

Cumulative 30-year Absolute Risk for Bladder Cancer in a 50 Year Old Male in the U.S., Overall and by Quartiles(based on smoking + 12 SNPs)

RD = risk differences for current vs. never smokers



Nat Rothman



M Garcia-Closas

Garcia-Closas et al, *Cancer Research* 2013

Furburg and Bochner, *Nature Review Urology* 2013

Thought Experiment:

If 100,000 smokers with high genetic risk stopped smoking

Eliminate 5,400 cases of bladder cancer

If 100,000 smokers with low genetic risk stopped smoking

Eliminate 1,500 cases of bladder cancer

Possible example of how genetic & environmental risk stratification might translate into targeted prevention

"Precision Prevention"

Variants at 6q21 implicate *PRDM1* in the etiology of therapy-induced second malignancies after Hodgkin's lymphoma

Timothy Best[1], Dalin Li[2], Andrew D Skol[3], Tomas Kirchhoff[4], Sarah A Jackson[3], Yutaka Yasui[5], Smita Bhatia[6], Louise C Strong[7], Susan M Domchek[8], Katherine L Nathanson[8], Olufunmilayo I Olopade[3], R Stephanie Huang[3], Thomas M Mack[2,9], David V Conti[2], Kenneth Offit[4], Wendy Cozen[2,9], Leslie L Robison[10] & Kenan Onel[1,11]

Survivors of pediatric Hodgkin's lymphoma are at risk for radiation therapy–induced second malignant neoplasms (SMNs). We identified two variants at chromosome 6q21 associated with SMNs in survivors of Hodgkin's lymphoma treated with radiation therapy as children but not as adults. The variants comprise a risk locus associated with decreased basal expression of *PRDM1* (encoding PR domain containing 1, with ZNF domain) and impaired induction of the PRDM1 protein after radiation exposure. These data suggest a new gene-exposure interaction that may implicate PRDM1 in the etiology of radiation therapy-induced SMNs.

Patients treated successfully for Hodgkin's lymphoma in childhood develop SMNs, with a cumulative incidence of 18.4% by 30 years after treatment and an absolute excess risk of 6.9 per 1,000 person-years of follow-up[1]. This high prevalence makes SMNs the second leading cause of mortality in Hodgkin's lymphoma survivors. SMNs primarily affect organs in the involved mediastinal radiation therapy field, including the thyroid, skin, gastrointestinal tract and female breast[2,3]. Risk is positively associated with cumulative radiation dose and inversely correlated with age at treatment[4,5].

Despite the clinical importance of this devastating late consequence of radiation therapy exposure, little is known about predisposing risk factors. We performed a genome-wide association study (GWAS) to identify variants associated with radiation therapy–induced SMNs in Hodgkin's lymphoma survivors. In studies of sporadic cancers, nongenetic heterogeneity can obscure genetic associations[6], but here radiation therapy exposure is common to patients with Hodgkin's lymphoma who did and did not develop SMNs. Thus, we hypothesized that limiting our study to radiation therapy–treated survivors would improve our power to detect the genetic contribution to SMN risk.

The discovery set consisted of 100 SMN cases and 89 SMN-free controls (**Supplementary Table 1a** and **Supplementary Table 2**). All cases and controls were diagnosed with Hodgkin's lymphoma as children (median age: 15.6, range: 8–20) and treated with 25–44 Gy radiation therapy with or without alkylating chemotherapy[7]. Cases developed SMNs with a mean latency of 20.0 years (s.d. = 5.8 years, range: 6–34). Controls were followed for at least 27 years (median: 32 years, range: 27–38) to ensure that the maximal contamination of controls by future cases was <2%. The **Supplementary Methods** contain a detailed description of the study populations and experimental protocols.

After genotype quality control, we successfully genotyped 665,313 single nucleotide polymorphisms (SNPs) in 96 cases and 82 controls. We compared allele frequencies between cases and controls using a Chi-square test of homogeneity. A quantile-quantile plot of the expected and observed distribution of *P* values revealed no evidence for systematic genotype calling error or hidden population substructure (genomic control $\lambda = 1.007$) (**Supplementary Fig. 1**)[8]. Principal component analysis using Eigenstrat indicated cases and controls were of European descent (**Supplementary Fig. 2**)[9].

We empirically determined the threshold for a genome-wide false positive rate of 0.05 by permutation ($P < 1.0 \times 10^{-7}$). At this threshold, our study had 80% power to detect a SNP with a frequency of 35% and an odds ratio (OR) of 3.5 (**Supplementary Fig. 3**). Three SNPs (rs4946728, rs1040411 and rs8083533) achieved genome-wide significance (**Supplementary Fig. 4** and **Table 1**). rs4946728 and rs1040411 mapped to chromosome 6q21, intergenic between *ATG5* (encoding autophagy protein 5) and *PRDM1*. The strongest evidence for association in this region was for rs4946728 ($P = 1.09 \times 10^{-8}$, $OR_{allelic} = 4.22$ (95% confidence interval (CI) = 2.53–7.05)). rs8083533 mapped to 18q11.2, intronic to *TAF4B* (encoding transcription initiation factor TFIID subunit 4B) ($P = 4.98 \times 10^{-8}$, $OR_{allelic} = 3.78$ (95% CI = 2.31–6.18)). Logistic regression, adjusting for gender, age at diagnosis, year of Hodgkin's lymphoma diagnosis, gonadal radiation (in females) and alkylating chemotherapy exposure, indicated that these risk variables had no effect on the observed associations (**Supplementary Table 3**).

We sought to replicate these findings in an independent set of 62 cases with SMNs and 71 SMN-free controls, all treated for Hodgkin's lymphoma in childhood with 25–44 Gy mediastinal radiation therapy (**Supplementary Table 1b**). We observed significant associations with SMNs for both SNPs on chromosome 6q21, rs4946728 ($P = 0.002$) and rs1040411 ($P = 0.03$), but not for rs8083533 ($P = 0.82$) (**Table 1**). In the

[1]Committee on Cancer Biology, University of Chicago, Chicago, Illinois, USA. [2]Department of Preventive Medicine, Keck School of Medicine and Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, California, USA. [3]Department of Medicine, University of Chicago, Chicago, Illinois, USA. [4]Department of Medicine, Clinical Genetics Service, Memorial Sloan-Kettering Cancer Center, New York, New York, USA. [5]Department of Public Health Sciences, University of Alberta, Edmonton, Alberta, Canada. [6]Department of Population Sciences, City of Hope, Duarte, California, USA. [7]Department of Genetics, The University of Texas MD Anderson Cancer Center, Houston, Texas, USA. [8]Abramson Cancer Center, University of Pennsylvania, Philadelphia, Pennsylvania, USA. [9]Department of Pathology, Keck School of Medicine and Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, California, USA. [10]Department of Epidemiology and Cancer Control, St. Jude Children's Research Hospital, Memphis, Tennessee, USA. [11]Department of Pediatrics, University of Chicago, Chicago, Illinois, USA. Correspondence should be addressed to K. Onel (konel@uchicago.edu).

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Table 1 Association results for the three stages and the meta-analysis at the *TANC1* locus

			RADIOGEN				RAPPER				Gene-PARE				RADIOGEN + RAPPER + Gene-PARE			
					$STAT_{late}$				$STAT_{late}$				$STAT_{late}$		Meta-analysis $STAT_{late}$			
SNP	Position[a]	Allele[b]	MAF	R^2[c]	β (SE)[d]	P	MAF	R^2	β (SE)[d]	P	MAF	R^2	β (SE)[d]	P	Fixed-effects β[e]	Fixed-effects P	Q	I^2
rs10497203	159,851,423	C	0.03	0.92	0.40 (0.08)	1.78×10^{-6}	0.02	0.90	0.42 (0.11)	2.08×10^{-4}	0.04	1.00	0.31 (0.14)	2.60×10^{-2}	0.39	8.84×10^{-11}	0.82	0
rs7582141	159,899,489	T	0.04	0.99	0.37 (0.07)	7.55×10^{-7}	0.02	0.90	0.42 (0.11)	2.08×10^{-4}	0.04	G	0.27 (0.13)	3.10×10^{-2}	0.37	4.64×10^{-11}	0.76	0
rs6432512	159,899,913	T	0.04	G	0.34 (0.07)	2.83×10^{-6}	0.02	0.90	0.42 (0.11)	2.08×10^{-4}	0.04	1.00	0.27 (0.13)	3.21×10^{-2}	0.35	1.97×10^{-10}	0.73	0
rs264663[f,g]	159,910,206	T	0.02	G	0.61 (0.10)	6.85×10^{-9}							–					
rs264651[h]	159,929,431	G	0.03	G	0.40 (0.08)	4.36×10^{-7}	0.02	0.66	0.36 (0.10)	5.33×10^{-4}			–					
rs264588	159,936,391	A	0.03	0.92	0.38 (0.08)	1.53×10^{-6}	0.02	0.90	0.40 (0.12)	7.06×10^{-4}	0.04	1.00	0.27 (0.13)	3.21×10^{-2}	0.37	3.08×10^{-10}	0.81	0
rs264631	159,950,865	G	0.03	0.88	0.36 (0.08)	3.17×10^{-6}	0.02	0.90	0.40 (0.12)	7.06×10^{-4}	0.04	0.97	0.27 (0.13)	3.21×10^{-2}	0.36	6.40×10^{-10}	0.83	0

R^2, imputation accuracy info metric; β, linear regression coefficient; SE, standard error; Q, Cochrane's Q statistic P value; I^2, heterogeneity index.
[a]According to GRCh37/hg19. [b]Minor and risk allele. [c]G, genotyped. [d]Linear regression coefficient or effect size from unadjusted analysis (for each SNP_j, β_j is calculated from $STAT_{late} = \beta_0 + \beta_j \times SNP_j + E$). [e]Effect size resulting from the meta-analysis of unadjusted effect sizes for each cohort. [f]rs264663 was not genotyped in the RAPPER cohort and could not be imputed because this SNP was monomorphic in the reference panel. [g]rs264663 did not fulfill quality control criteria (genotyping call rate < 0.95%) in the Gene-PARE cohort. [h]rs264651 did not fulfill quality control criteria (Hardy-Weinberg equilibrium P value < 1×10^{-6}) in the Gene-PARE cohort.

Health Science Centre, Christie Hospital, Manchester, UK. [8]Institute of Cancer Research and Royal Marsden National Health Service (NHS) Foundation Trust, Sutton, UK. [9]Cancer and Other Non-Infectious Diseases, Medical Research Council (MRC) Clinical Trials Unit, London, UK. [10]Clinical Trials and Statistics Unit, Institute of Cancer Research, London, UK. [11]Department of Oncology, University of Cambridge, Oncology Centre, Cambridge University Hospitals NHS Foundation Trust, Cambridge, UK. [12]Center of Excellence in Genomic Medicine Research (CEGMR), King Abdulaziz University, Jeddah, Saudi Arabia. Correspondence should be addressed to A.V. (ana.vega@usc.es).

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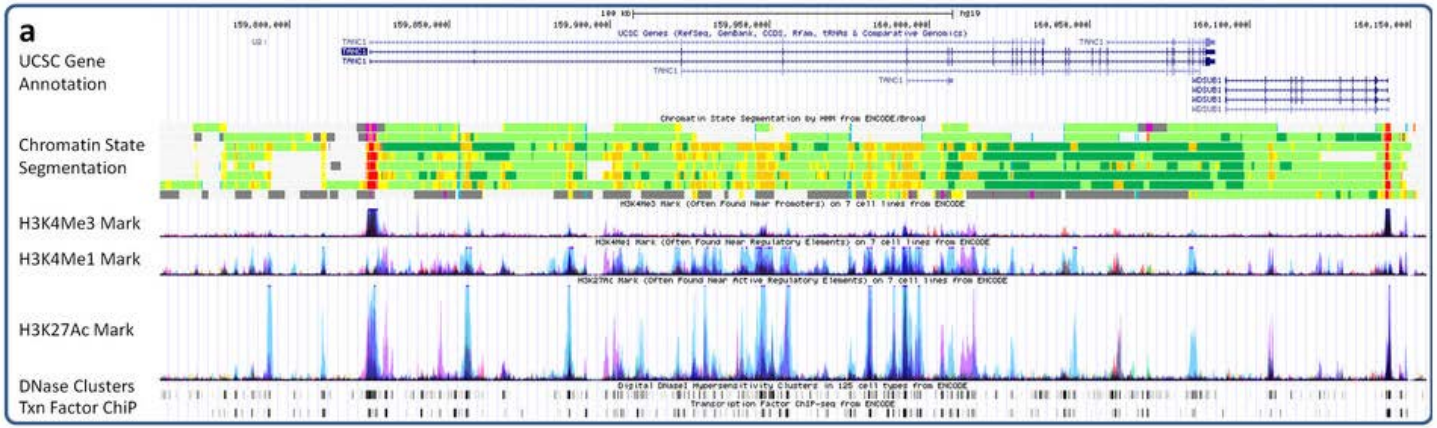
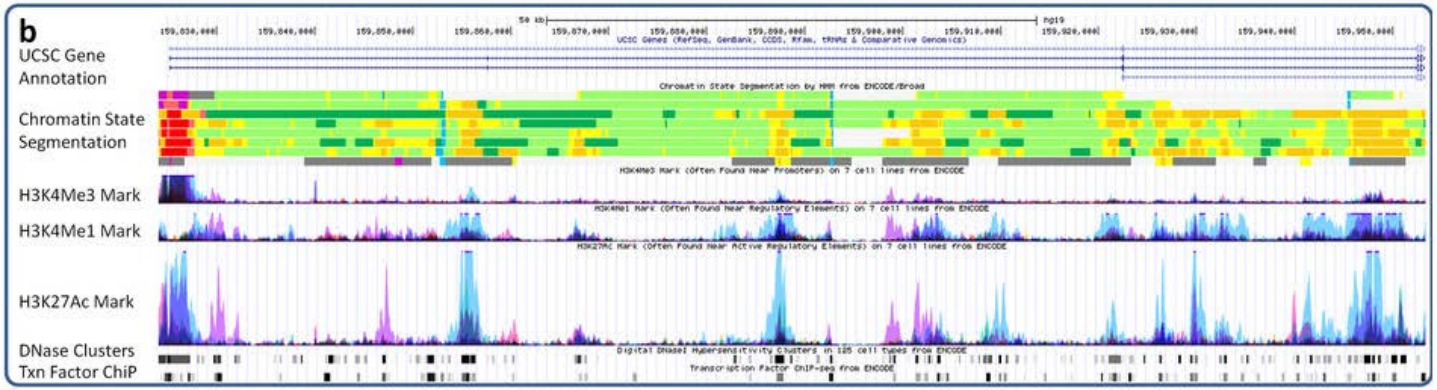
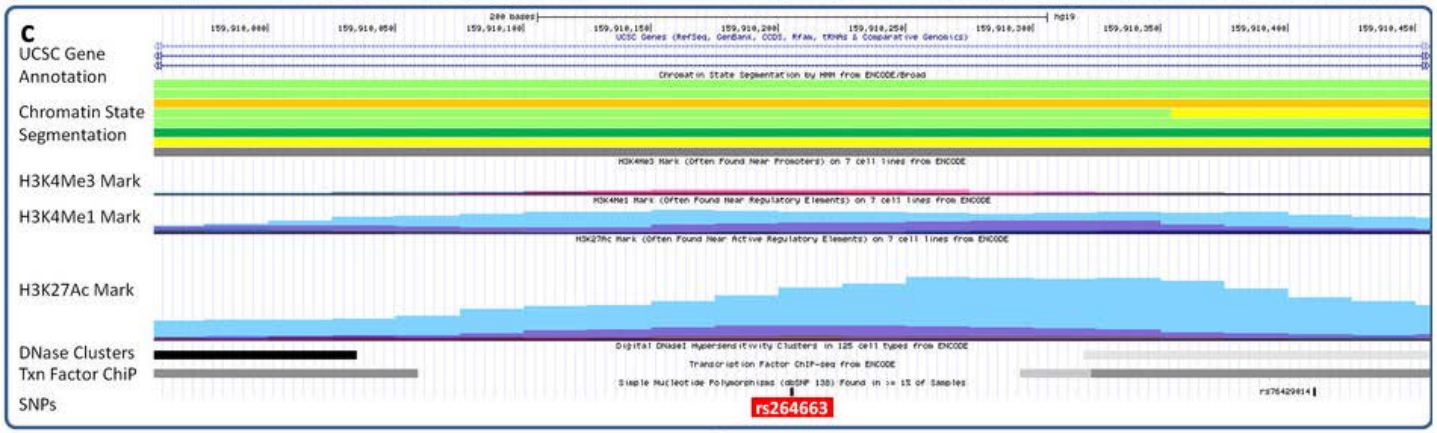
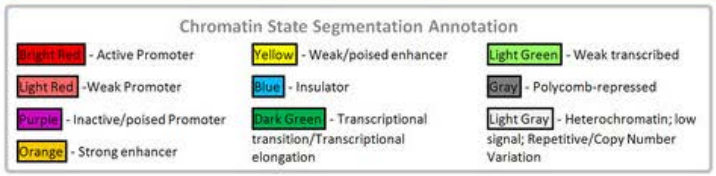
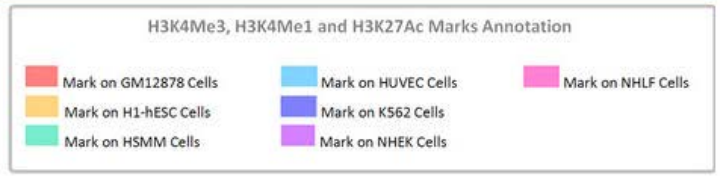
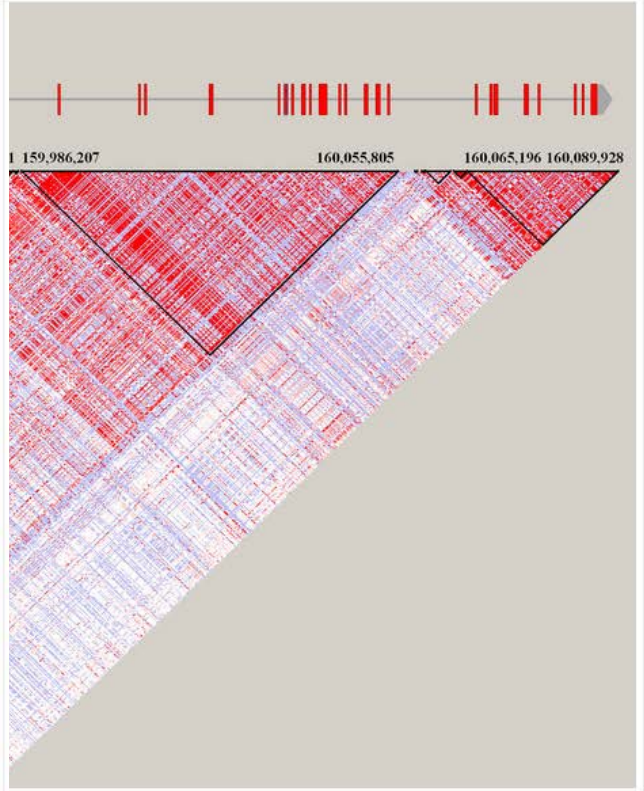


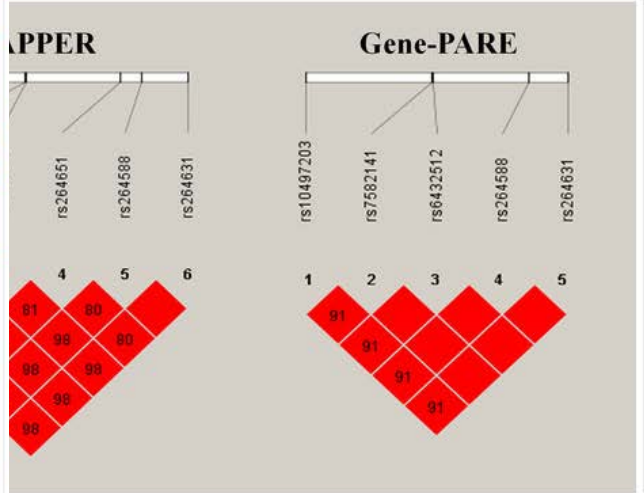






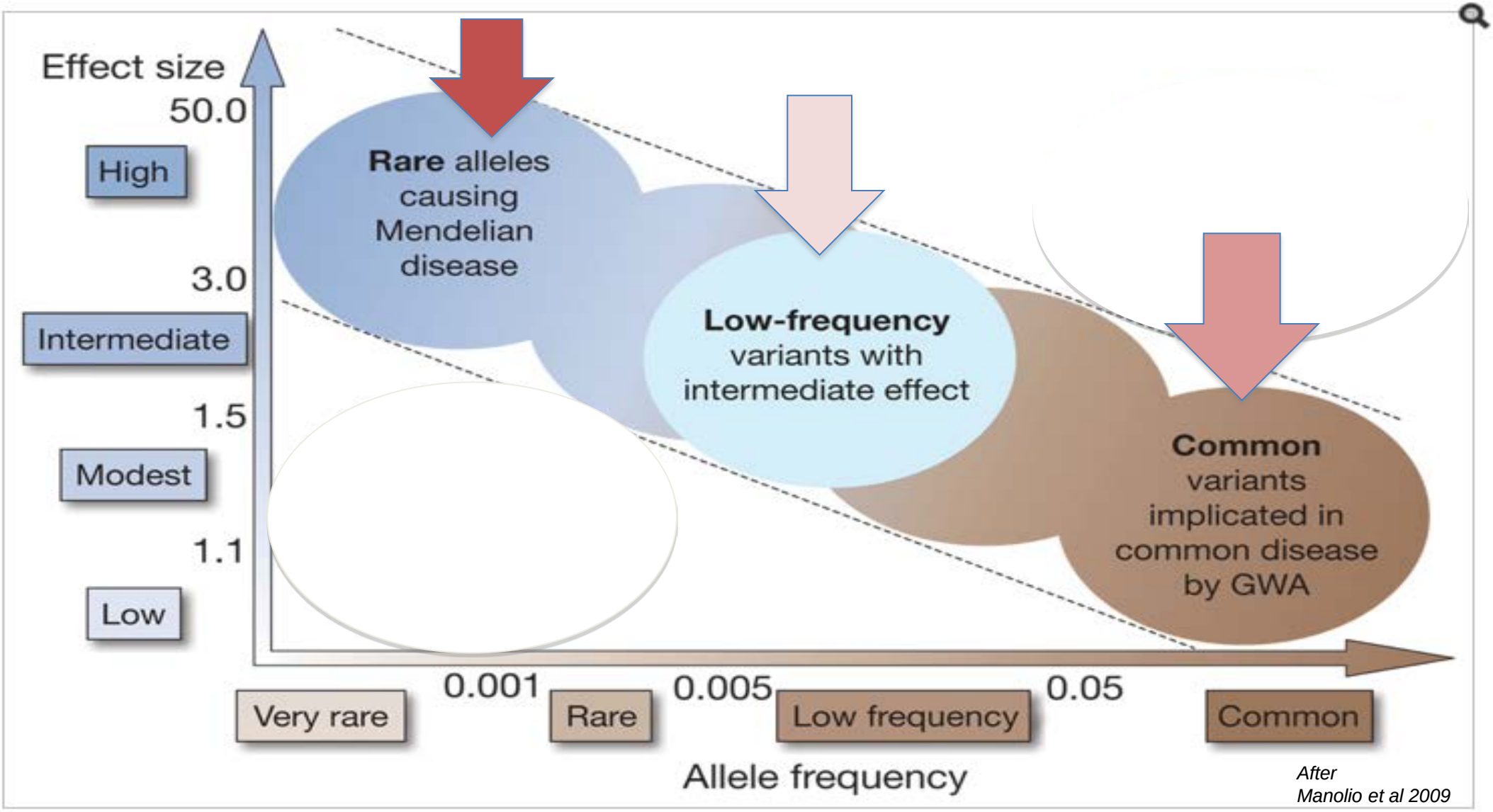






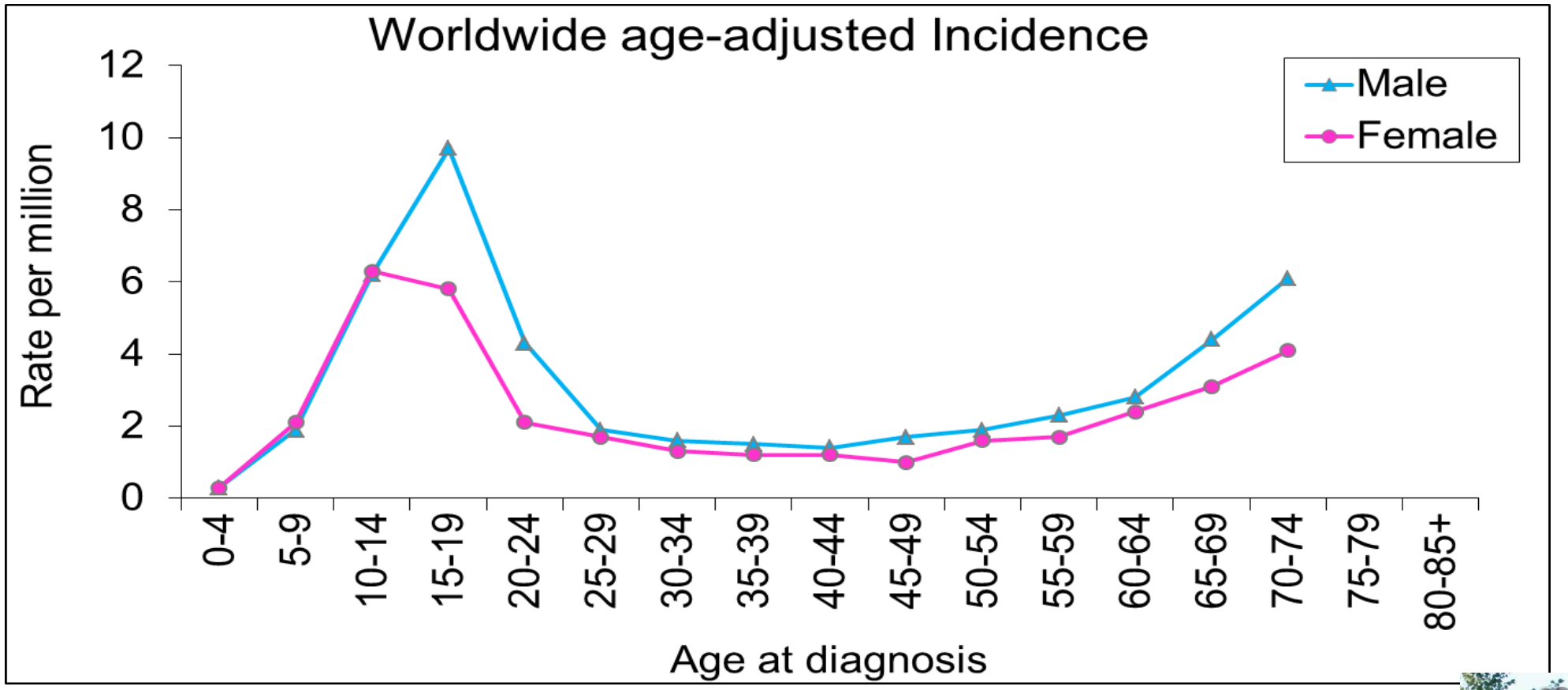
nkage disequilibrium values correspond to the r^2 parameter. **(a)** Haploview
significant SNPs are placed. The left bottom diagram shows the linkage
loview analysis of the replicated SNPs in the three cohorts (RADIOGEN,

Architecture of Genetic Susceptibility of Cancer Defining 'Distinct' Spaces



After Manolio et al 2009

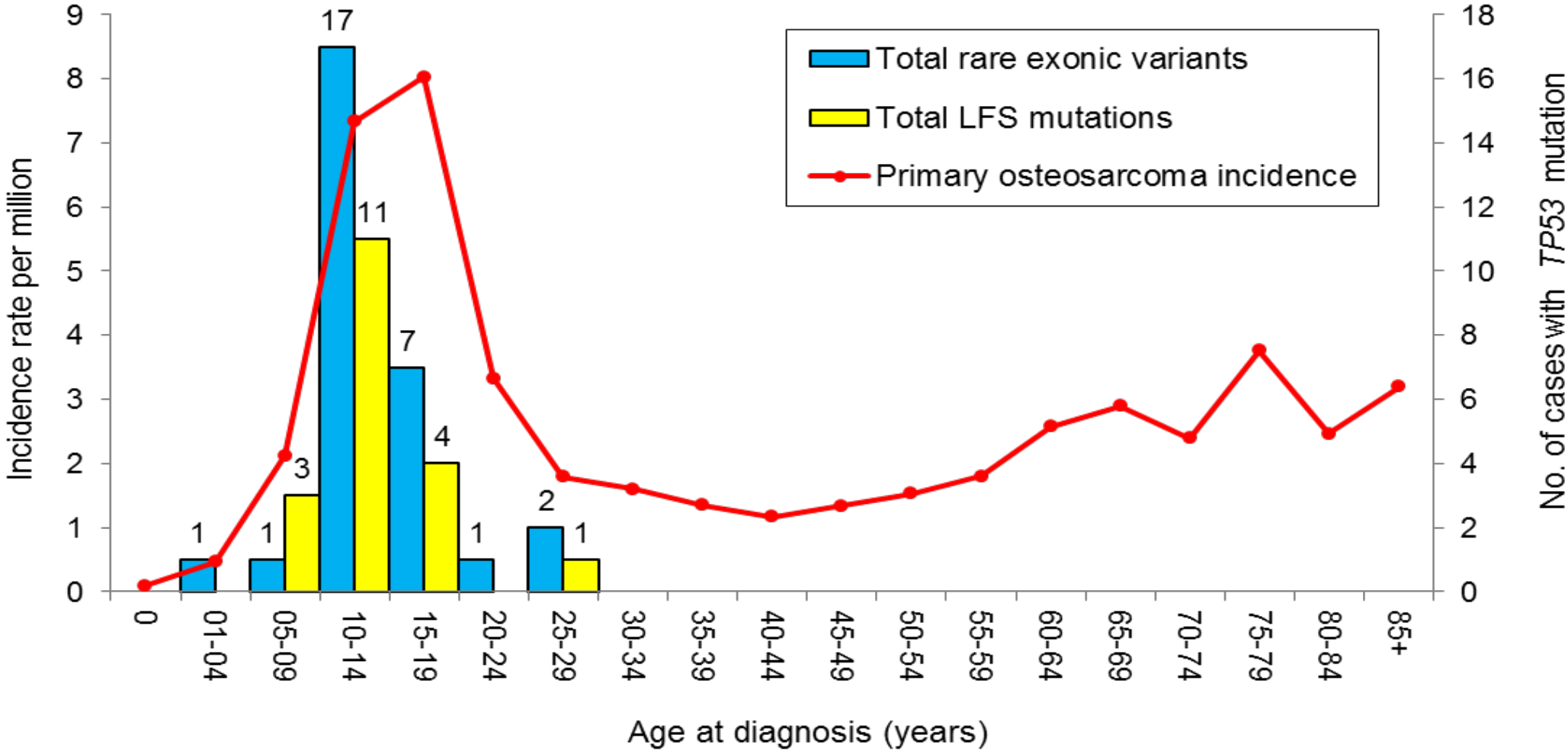
Germline Susceptibility to Osteosarcoma: Most common primary bone tumors



Li-Fraumeni (*TP53*)
Rothmund-Thomson Syndrome *(RECQL4*)
Hereditary Retinoblastoma (*RB*) - as 2nd cancer

Lisa Mirabello

Number of Patients with Germline *TP53* Variants in 765 Unselected Osteosarcoma Cases



Mirabello et al JNCI 2015

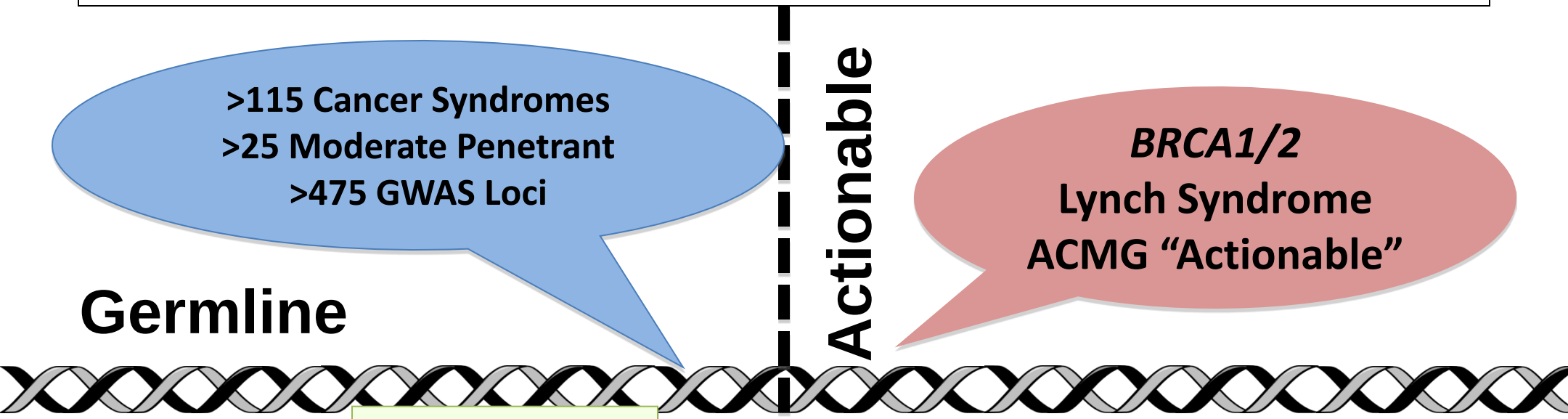
Frequency of Germline *TP53* Variants in 765 Unselected Osteosarcoma Cases

Consider genetic counseling & *TP53*-mutation testing in *young* patients with osteosarcoma,
Especially if there is history of cancer in close relatives

LFS-associated	3.13%	4.76%	3.84%	1.96%	0
Rare exonic	4.97%	3.18%	6.14%	5.88%	0
Total	8.10%	7.94%	9.98%	7.84%	0

***P*< 0.001**

Cancer Genomics: 4 Spaces



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BRCA1/2
Lynch Syndrome
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Germline

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Heterogeneity
Metastases

'Drivers'
'Passengers'

TCGA/ICGC-
Cosmic Data

Targeted Therapy
HER2
EGFR
BRAF600

Discovery

Clinically Actionable

Pipeline for Comprehensive Characterization: The Cancer Genome Atlas (TCGA) >20 Cancers

Tissue Sample

Pathology QC

DNA & RNA Isolation, QC

Sequencing

Expression, CNA & LOH, Epigenetics

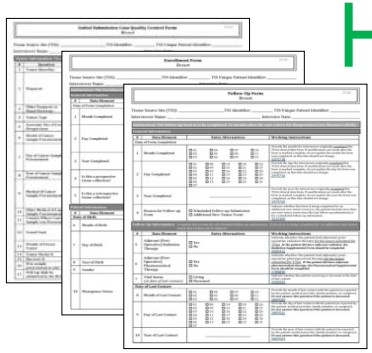
Data Storage at DCC & CGHub

GDAC

Integrative Analysis

Comprehensive Characterization of a Cancer Genome

3 months – 2 years



~90d

SNP 6.0 ~45d

Methylation ~60d

miRNAseq ~105d

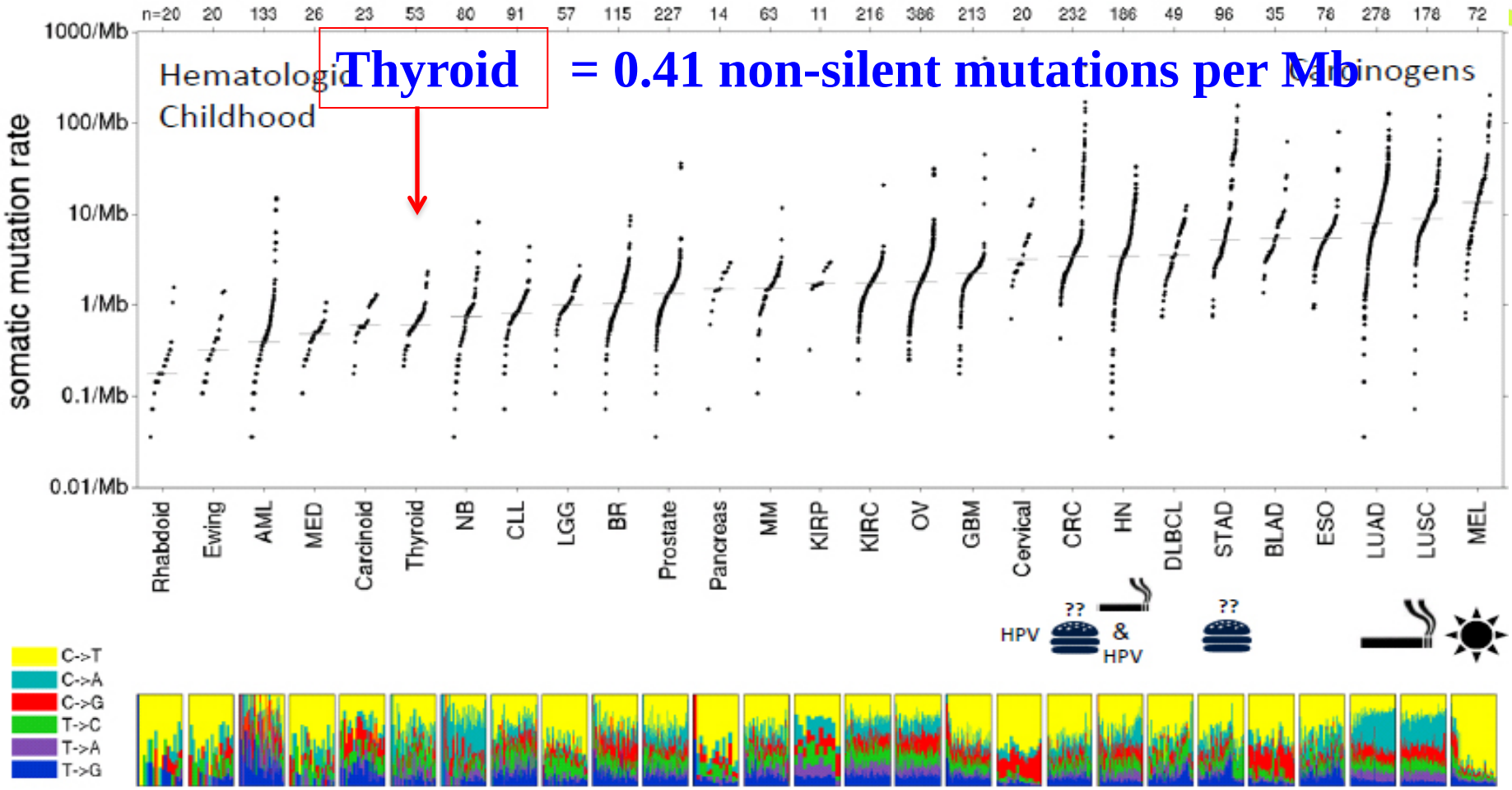
mRNAseq ~120d

DNAseq Exome ~180d

~12-24 months

Target: 500 cases for Major Cancers and 50-100 for Rarer Cancers

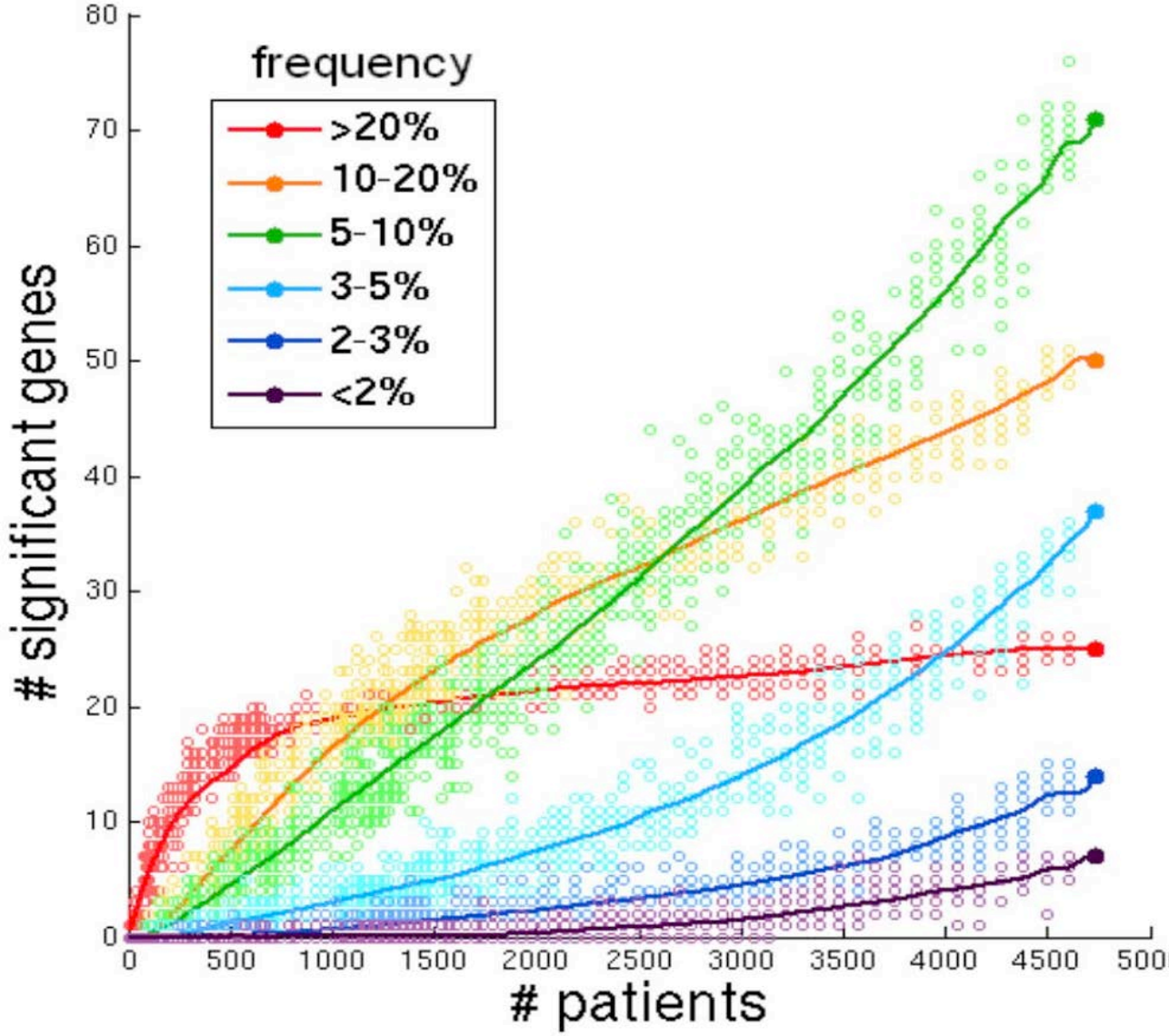
Lessons Learned from the Data
The Cancer Genome Atlas (TCGA)



M. Lawrence & G. Getz/Broad Institute

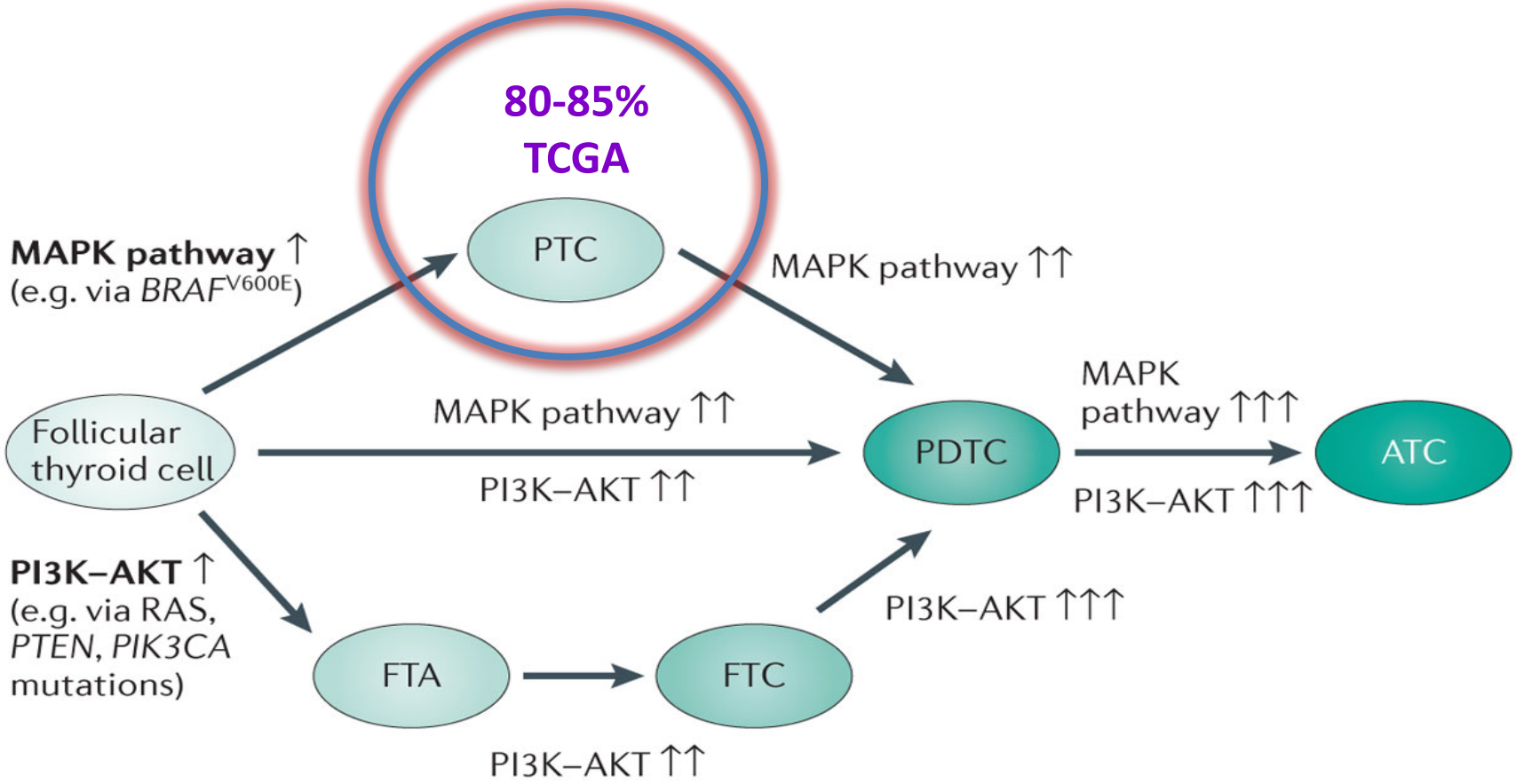
The Cancer Genome Atlas

Many Cancer Drivers With <20% Prevalence Remain Undiscovered



Lawrence et al, Nature 2014

Simple model of thyroid cancer progression



Loss of differentiation

Mingzhao Xing, JHU

Driver Mutations in Thyroid -2013

BRAF

RAS

PTEN

PICK3A

CTNNB1

TP53

ALK

Table 2 | **Gene mutations in thyroid tumours**

Mutations	Types of thyroid tumours	Approximate prevalence (%)*	Primary signalling pathways affected	Functional impact on the protein and tumour	Refs
BRAF^{V600E}	CPTC	45	MAPK	Activating; promoting tumorigenesis, invasion, metastasis, recurrence and mortality	6–12, 16–18
	FVPTC	15			
	TCPTC	80–100			
	ATC	25			
BRAF^{K601E}	FVPTC	5	MAPK	Activating; probably similar to *BRAF*^{V600E}	14
HRAS, *KRAS*, *NRAS*	FTA	20–25	MAPK and PI3K–AKT	Activating; promoting tumorigenesis, invasion and metastasis of PDTC and FTC	26,31,33, 35,148, 184–187, 206–211
	FTC	30–45			
	FVPTC	30–45			
	PDTC	20–40			
	ATC	20–30			
PTEN (mutation)	FTA	0	PI3K–AKT	Inactivating the gene but activating the PI3K pathway; promoting tumorigenesis and invasiveness	26,31–33, 212
	FTC	10–15			
	ATC	10–20			
	PTC	1–2			
PTEN (deletion)	FTC	30	PI3K–AKT	Inactivating the gene but activating the PI3K pathway; promoting tumorigenesis and invasiveness	212
PIK3CA	FTA	0–5	PI3K–AKT	Activating; promoting tumorigenesis and invasiveness	25,26, 31–35
	FTC	5–15			
	ATC	15–25			
	PTC	1–2			
AKT1	Metastatic cancer	15	PI3K–AKT	Unclear; seems to favour metastasis	35
CTNNB1	PDTC	25	WNT–β-catenin	Activating; promoting tumour progression	37,38
	ATC	60–65			
TP53	PDTC	25	p53-coupled pathways	Inactivating; promoting tumour progression	39,40
	ATC	70–80			
IDH1	FTC	5–25	IDH1-associated metabolic pathways	Inactivating; impact on tumours is unclear	41,42
	FVPTC	20			
	CPTC	10			
	ATC	10–30			
ALK	ATC	10	MAPK and PI3K–AKT	Activating; probably promoting tumour progression	43
EGFR	CPTC	5	MAPK and PI3K–AKT	Activating; impact on tumours is unclear	44
NDUFA13 (also known as *GRIM19*)	HCTC	15	Component of complex I of the mitochondrial respiratory chain	Presumably inactivating; affecting mitochondrial metabolism and cell death	45

ALK, anaplastic lymphoma kinase; ATC, anaplastic thyroid cancer; CPTC, conventional PTC; *CTNNB1*, β-catenin; *EGFR*, epidermal growth factor receptor; FTA, follicular thyroid adenoma; FTC, follicular thyroid cancer; FVPTC, follicular-variant PTC; HCTC, Hürthle cell thyroid cancer; *IDH1*, isocitrate dehydrogenase 1; *NDUFA13*, NADH dehydrogenase (ubiquinone) 1α subcomplex 13; PDTC, poorly differentiated thyroid cancer; PTC, papillary thyroid cancer; TCPTC, tall-cell PTC.
*The values represent estimated overall prevalence of the indicated mutations.

Molecular pathogenesis and mechanisms of thyroid cancer

Mingzhao Xing

Resource

496 primary Papillary Thyroid Cancers
391 on all major platforms
All 'Sporadic'

Cell

Integrated Genomic Characterization of Papillary Thyroid Carcinoma

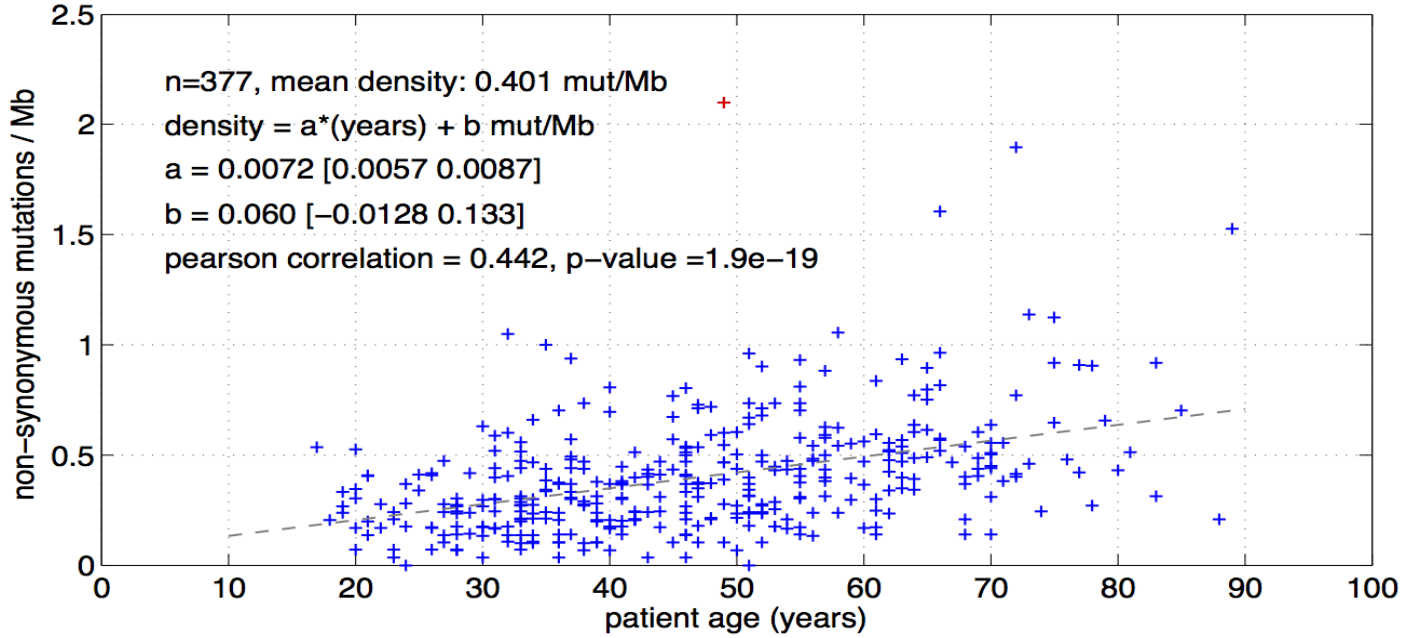
The Cancer Genome Atlas Research Network[1,*]
[1]The Cancer Genome Atlas Program Office, National Cancer Institute at NIH, 31 Center Drive, Bldg. 31, Suite 3A20, Bethesda, MD 20892, USA
*Correspondence: giordano@umich.edu, gadgetz@broadinstitute.org
http://dx.doi.org/10.1016/j.cell.2014.09.050

SUMMARY

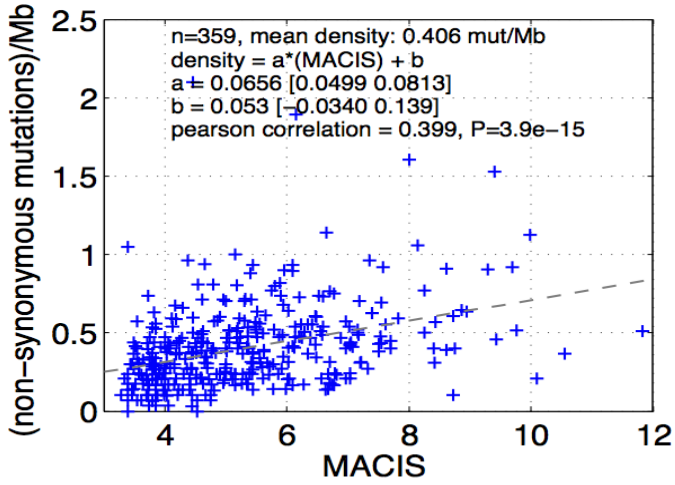
Papillary thyroid carcinoma (PTC) is the most common type of thyroid cancer. Here, we describe the genomic landscape of 496 PTCs. We observed a low frequency of somatic alterations (relative to other carcinomas) and extended the set of known PTC driver alterations to include *EIF1AX*, *PPM1D*, and *CHEK2* and diverse gene fusions. These discoveries reduced the fraction of PTC cases with unknown oncogenic driver from 25% to 3.5%. Combined

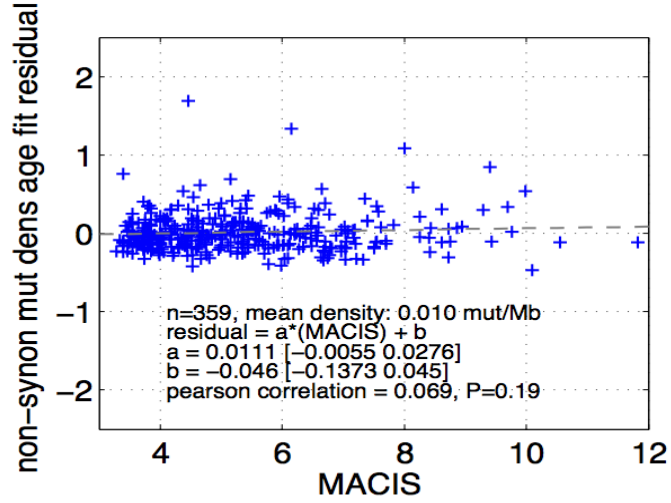
Previous genetic studies report a high frequency (70%) of activating somatic alterations of genes encoding effectors in the mitogen-activated protein kinase (MAPK) signaling pathway, including point mutations of *BRAF* and the *RAS* genes (Cohen et al., 2003; Kimura et al., 2003; Lemoine et al., 1988; Suárez et al., 1988), as well as fusions involving the *RET* (Grieco et al., 1990) and *NTRK1* tyrosine kinases (Pierotti et al., 1995). These mutations are almost always mutually exclusive (Soares et al., 2003), suggesting similar or redundant downstream effects. The various MAPK pathway alterations are strongly associated with distinct clinicopathological characteristics (Adeniran et al., 2006), and gene expression (Giordano et al., 2005) and DNA

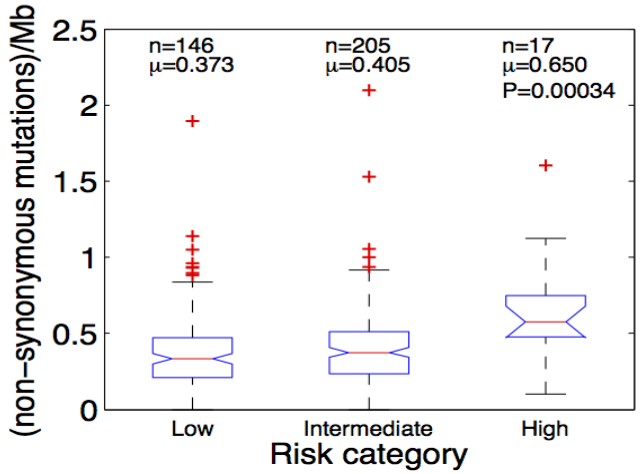
TCGA Thyroid- DNA Sequencing
Mutation density increases with age

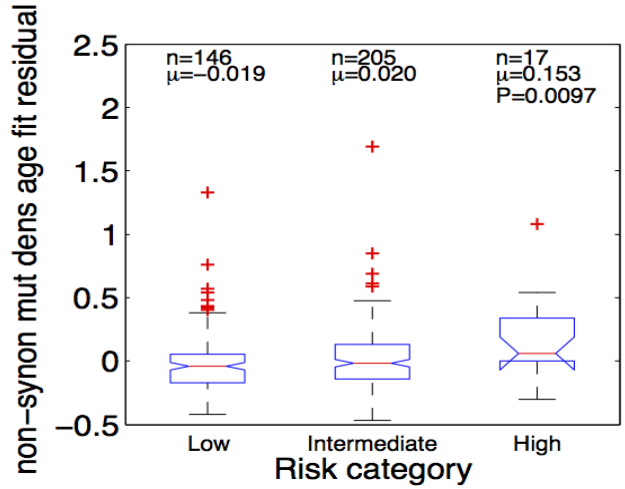


Mutation density associated with high risk of recurrence (after correcting for age)









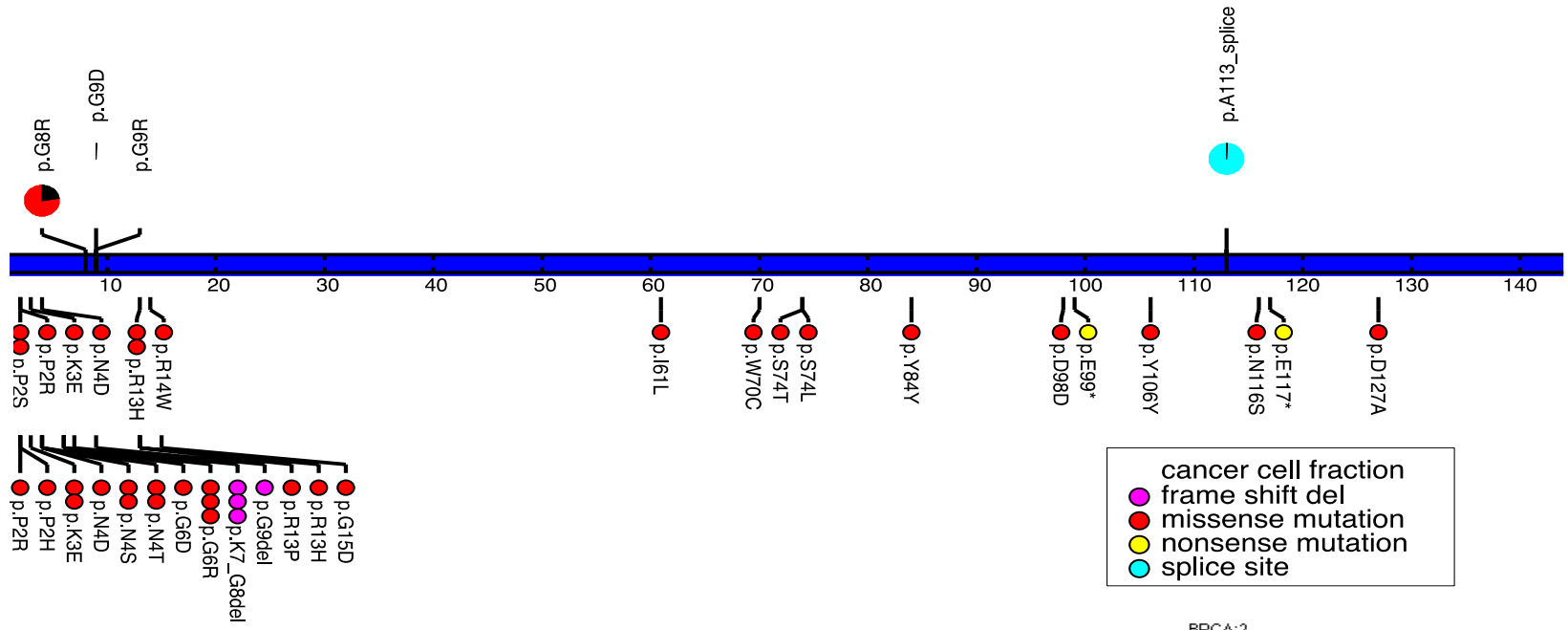
New Finding: EIF1AX

Translation initiation factor 1A, X-linked

THCA:
6 mutations

COSMIC:
19 mutations
Endometrium, breast, colon, lung, esophagus, ovary and prostate

Uveal melanoma
20 mutations

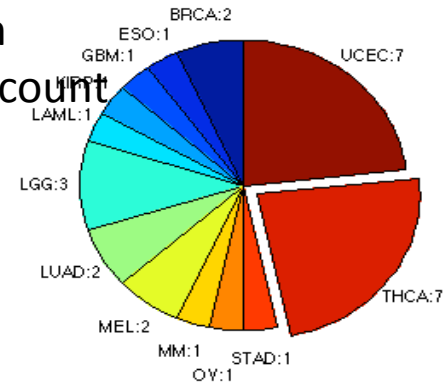


Exome sequencing identifies recurrent somatic mutations in *EIF1AX* and *SF3B1* in uveal melanoma with disomy 3

Marcel Martin[1,2], Lars Maßhöfer[3], Petra Temming[4], Sven Rahmann[1], Claudia Metz[5], Norbert Bornfeld[5], Johannes van de Nes[6], Ludger Klein-Hitpass[7], Alan G Hinnebusch[8], Bernhard Horsthemke[3], Dietmar R Lohmann[3,9] & Michael Zeschnigk[3,9]

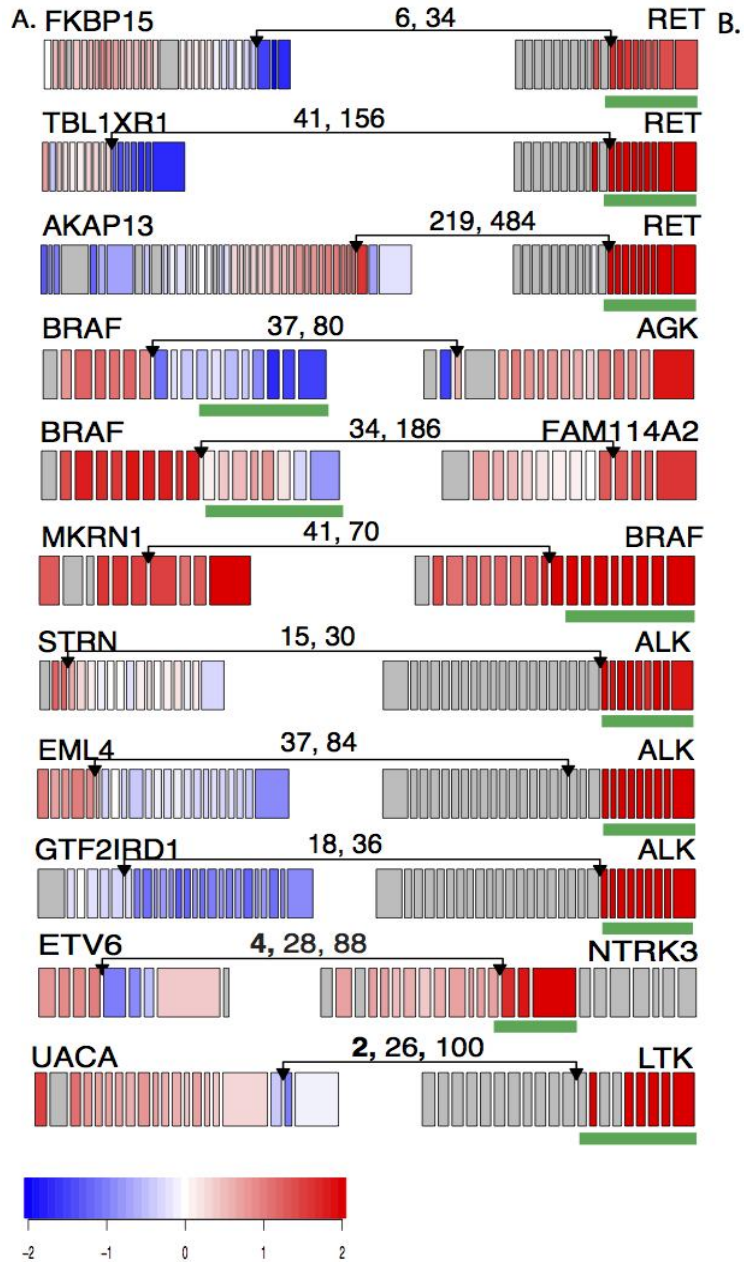
NATURE GENETICS VOLUME 45 | NUMBER 8 | AUGUST 2013

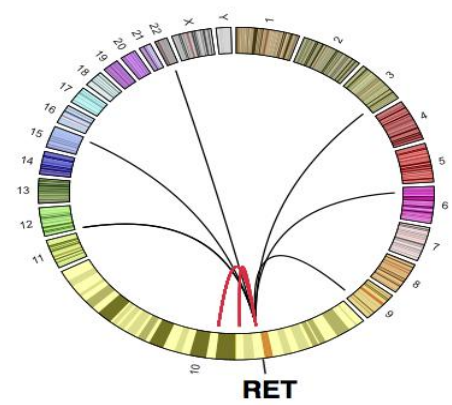
Pan-can
EIF1AX count

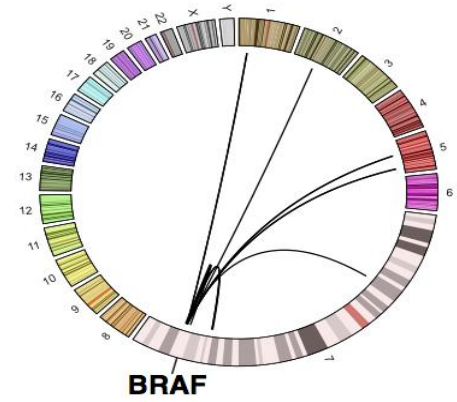


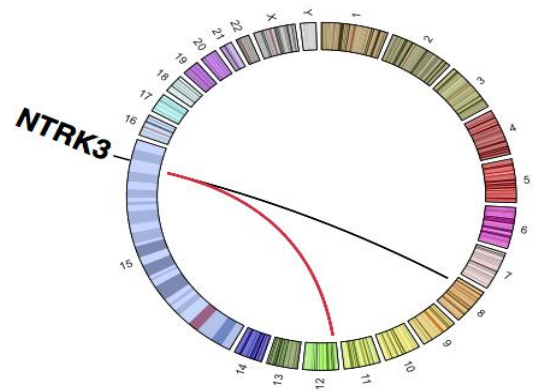
Large Structural Events: Gene Fusions

- New *RET* partners
- Diverse *BRAF* fusions
- ALK fusions, diverse
  - (*EML4-ALK*)
- *ETV6-NTRK3*



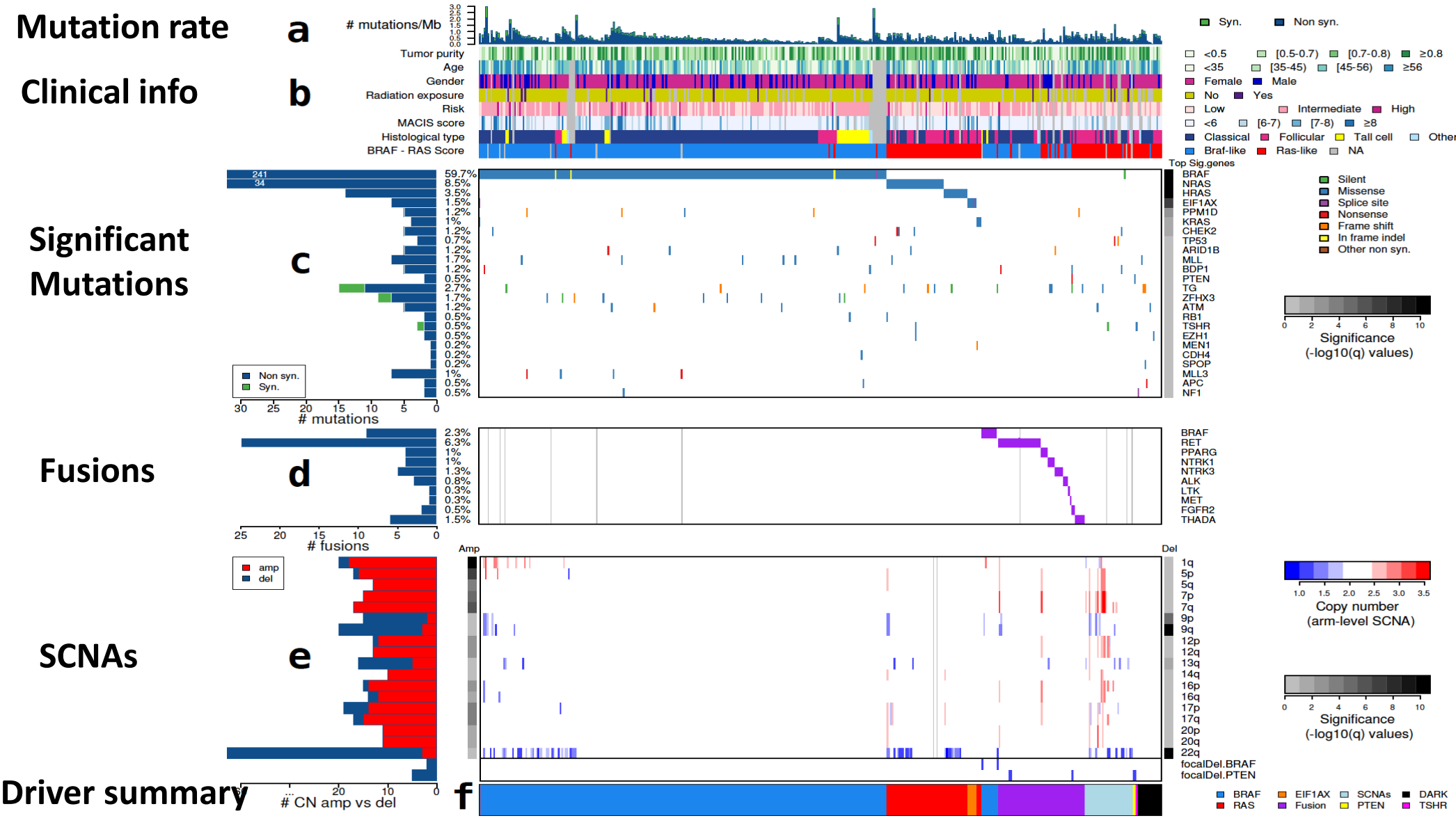






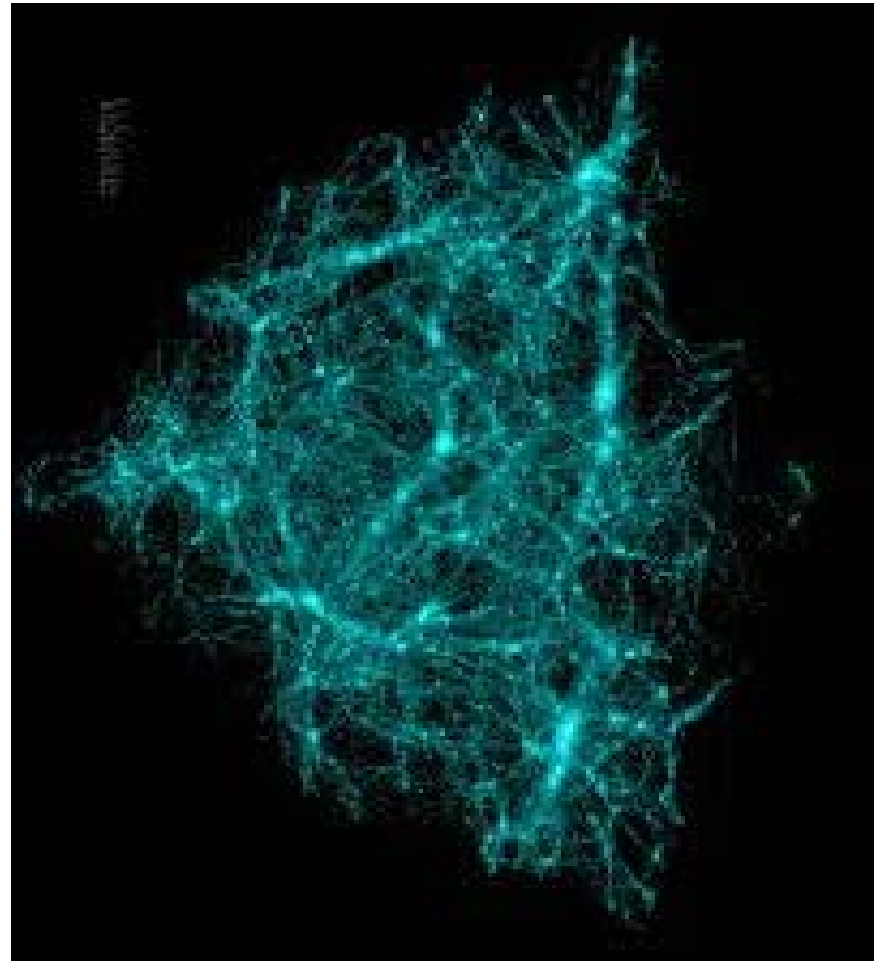
Angela Hadjipanayis, Harvard
Katie Hoadley, UNC
Chip Stewart, Broad Institute

Overview of Somatic Alterations



The “Dark Matter” of the Cancer Genome

- Regions of the genome that we cannot easily interpret
- Examples:
  - regulatory regions
  - intergenic regions
  - repeat-rich DNA
  - “non-focal” copy number alterations



TERT Promoter Mutations in Thyroid Cancer

Highly prevalent *TERT* promoter mutations in aggressive thyroid cancers

Xiaoli Liu, Justin Bishop[1], Yuan Shan[2], Sara Pai[3], Dingxie Liu, Avaniyapuram Kannan Murugan, Hui Sun[4], Adel K El-Naggar[5] and Mingzhao Xing

Frequent Somatic *TERT* Promoter Mutations in Thyroid Cancer: Higher Prevalence in Advanced Forms of the Disease

Iñigo Landa, Ian Ganly, Timothy A. Chan, Norisato Mitsutake, Michiko Matsuse, Tihana Ibrahimpasic, Ronald A. Ghossein, and James A. Fagin

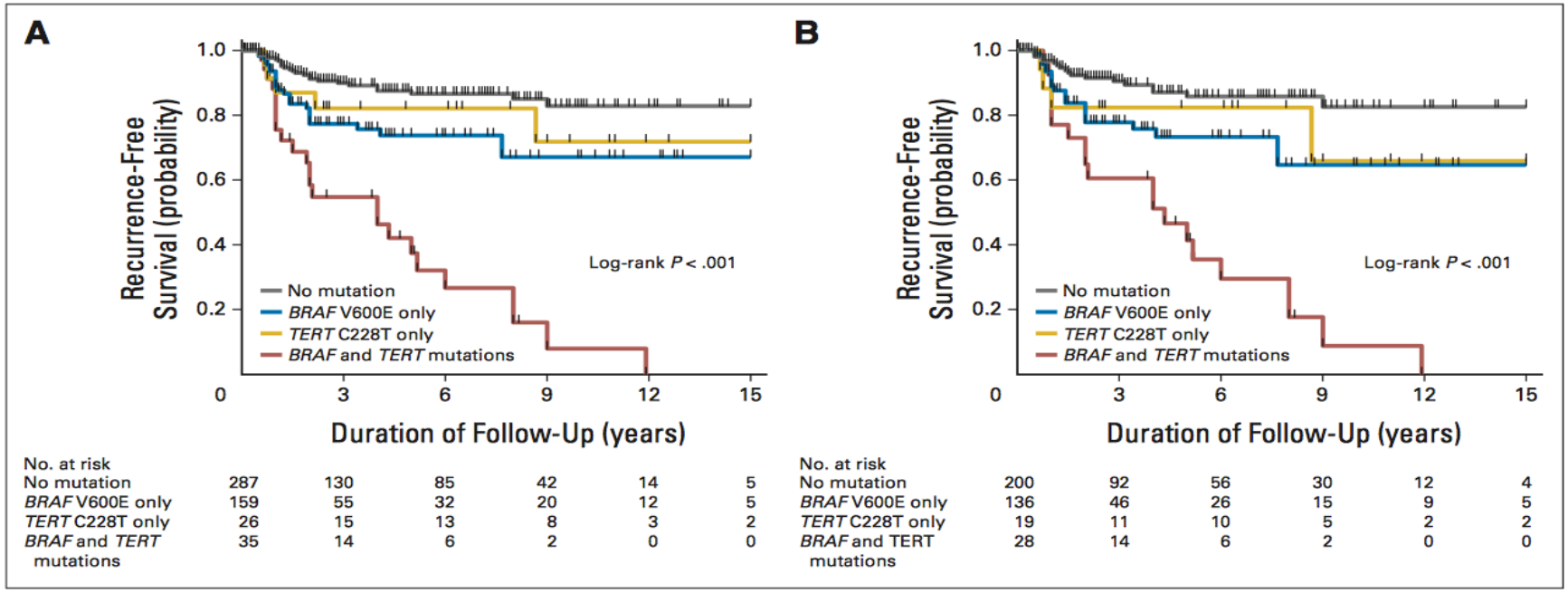


Fig 2. Kaplan-Meier analyses of the impacts of *BRAF* V600E or *TERT* C288T alone or their coexistence on disease-free survival of patients with papillary thyroid cancer (PTC). (A) Results of the analyses of patients with PTC of all types. (B) Results of the analyses of conventional variant PTC only. Four groups of patients are indicated in A and B, including patients with neither mutation (gray lines), *TERT* C228T mutation only (gold lines), *BRAF* V600E mutation only (blue lines), and coexistence of the two mutations (red lines).

BRAF V600E and *TERT* Promoter Mutations Cooperatively Identify the Most Aggressive Papillary Thyroid Cancer With Highest Recurrence

Mingzhao Xing, Rengyun Liu, Xiaoli Liu, Avaniyapuram Kannan Murugan, Guangwu Zhu, Martha A. Zeiger, Sara Pai, and Justin Bishop

TERT Promoter Mutations in TCGA Cohort

A

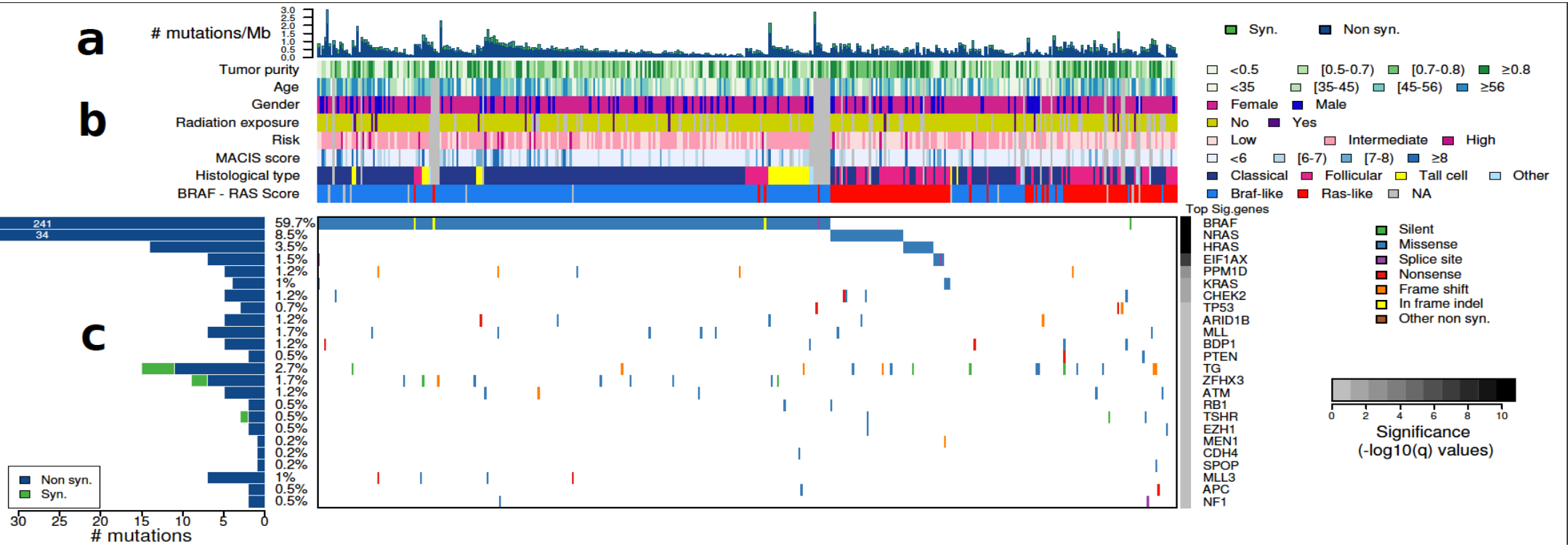
B

C

TERT promoter mutations are associated with high risk of recurrence, poor survival prediction and low thyroid differentiation

Chip Stewart and others, Broad Institute

BRAF-V600E and RAS Mutations are Mutually Exclusive

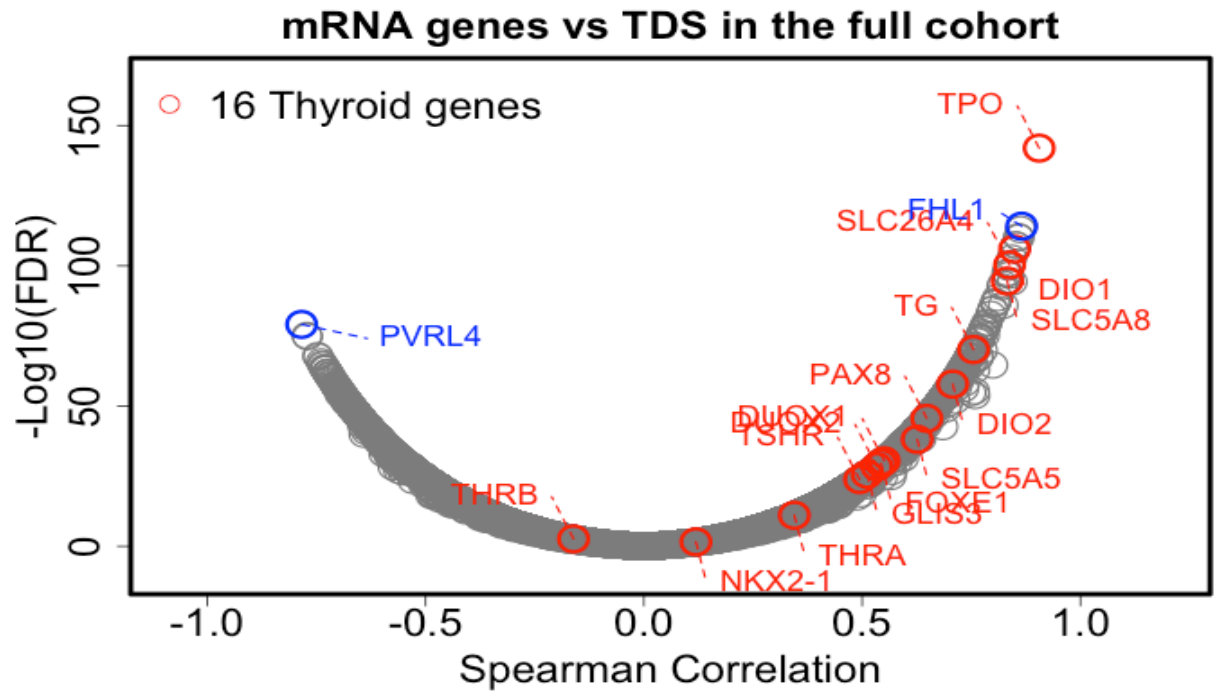


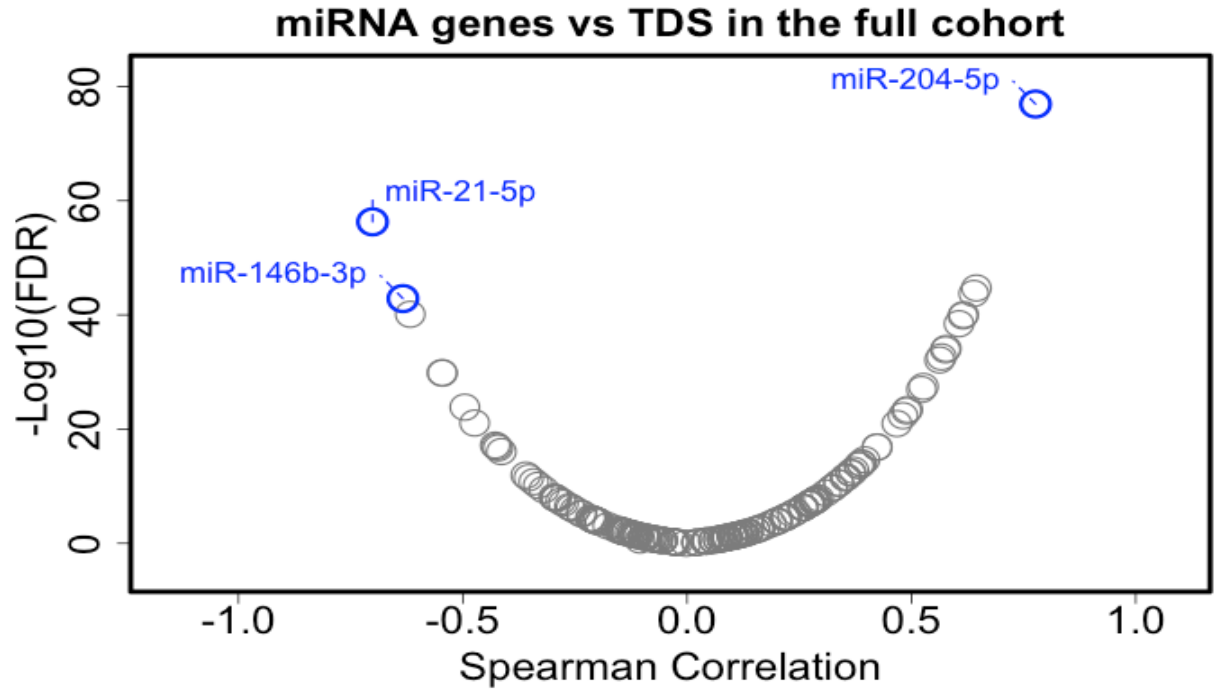
Giovanni Ciriello at MSKCC and Katie Hoadley at UNC developed a gene expression based score that measures whether a tumor has expression like a *BRAF*- or a *RAS*-mutant tumor

Thyroid Differentiation Score

Gene Expression Set

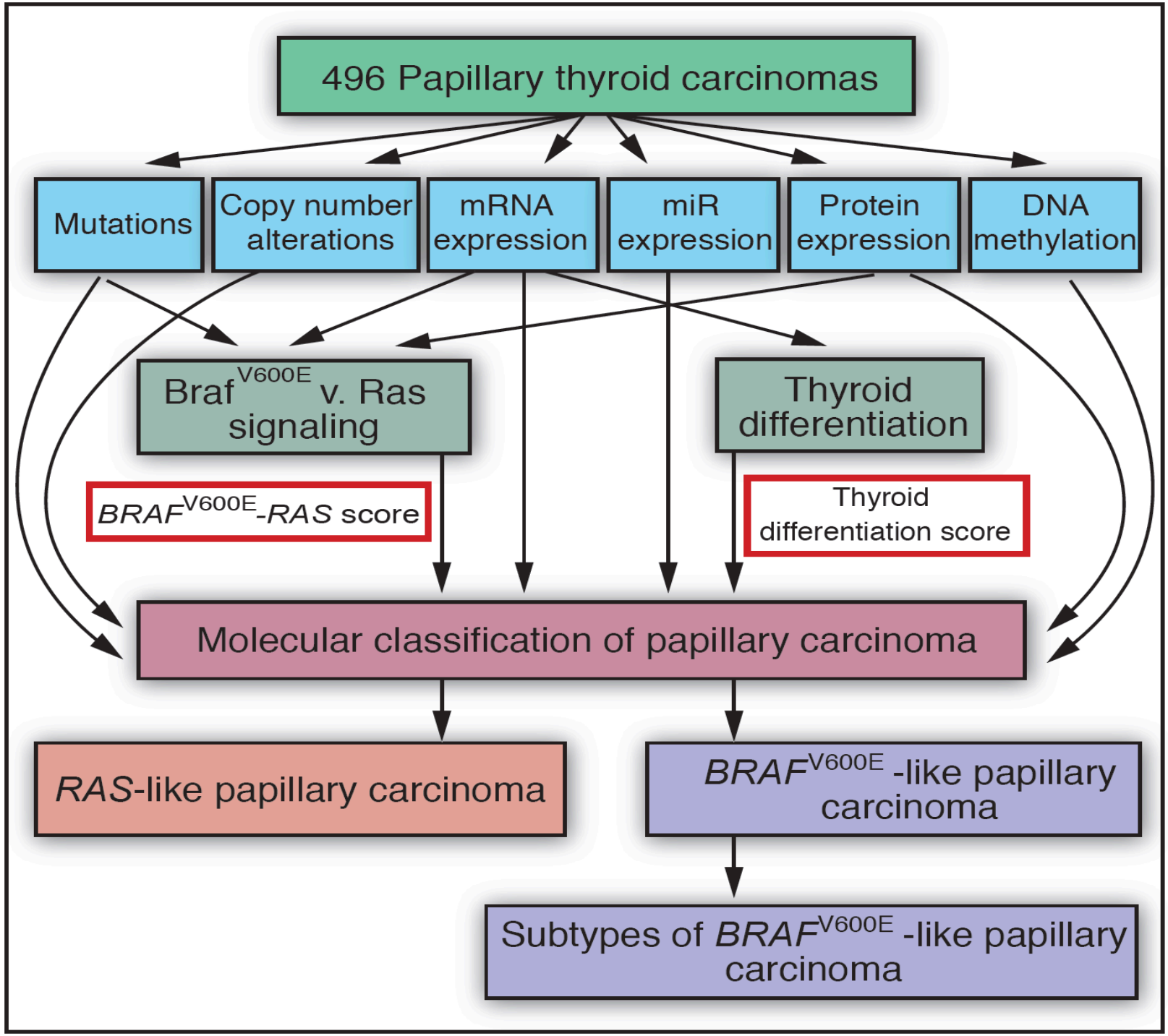
Remember miR-21 and miR-146b





Visual Summary

Thyroid TCGA



National Cancer Institute

Gene-Environment Opportunity

Full Genomic Characterization (TCGA-style) of Radiation-Related Thyroid Cancer in the Ukraine

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Focused Molecular Studies of Thyroid Cancer: UkrAm

- Somatic mutation analyses (jointly with Dr. Nikiforov)
  - Dose-related increase in *MAPK** gene rearrangements, but not in *BRAF/RAS* point mutations
  - Strong dose response for remaining 30% of tumors with no candidate-gene mutations
- Gene expression studies (jointly with Dr. Abend)
  - Differential tumor/non-tumor gene expression in relation to dose identified several candidate genes/pathways
  - Additional genes with dose-dependent gene expression either in tumor or non-tumor thyroid tissue found

*Mitogen-activated protein kinase signaling pathway

Abend et al. PlosOne, 2013 & BJC, 2013
Leeman-Neil et al. Cancer, 2013 & Cancer, 2014

Specific Aims & Analysis Plan

- **Primary –** Comprehensive characterization of the genomic alterations of radiation-related PTC
  - Compare radiation-related PTCs against sporadic PTC in TCGA to identify possible signature of radiation-related somatic alterations
  - Across levels of I-131 exposure
  - Prefer Fresh Frozen but can use FFPE

- **Secondary –** Evaluate gene-radiation interactions and contribution of genetic susceptibility to risk of PTC using germline DNA data

CTB: I-131 thyroid doses

Group	Basis for dose reconstruction	Available, N	Mean I-131 dose, Gy (range)	Mean GSD*
1	Direct thyroid measurement and personal interview	165	1.2 (0.001-13)	1.5
2	Direct thyroid measurement, but no personal interview	19	1.9 (0.008-13)	1.5
3	No direct thyroid measurement and no personal interview	1,685	0.13 (0.001-24.1)	3.3
4	Exposed *in utero*	64	0.10 (<0.001-2.1)	3.8
5	Not-exposed, born after Jan 1, 1987	310		
Total		2,243**		

*Geometric standard deviation, measure of dose uncertainty

**For 24 cases place of residence in 1986 is unknown

Likhtarov et al. Radiat Prot Dosimetry, 2013

UkrAm Pilot
Molecular-Genetic study

12 PTC cases:
***4**-low; **4**-middle; **4**-high dose groups;*
6 – from Zhitomir; **6** – from Chernigov regions;
7-Femaile; **5**-Male

Paraffin embedded blocks

Frozen tissue samples

Blood samples EDTA x2

Pathology information

Clinical information

IRB permission

Comprehensive Characterization in TCGA Pipeline

Exome Sequencing

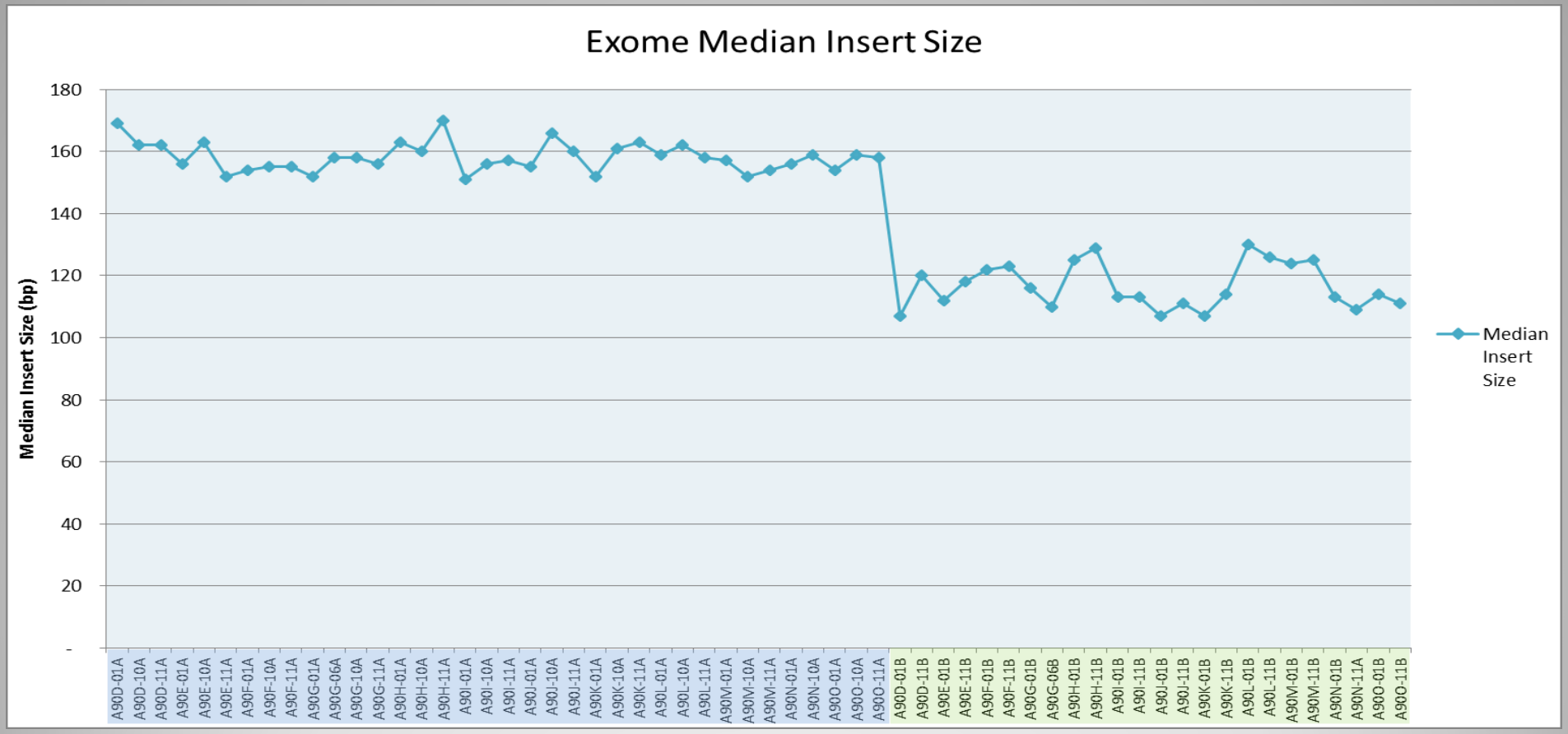
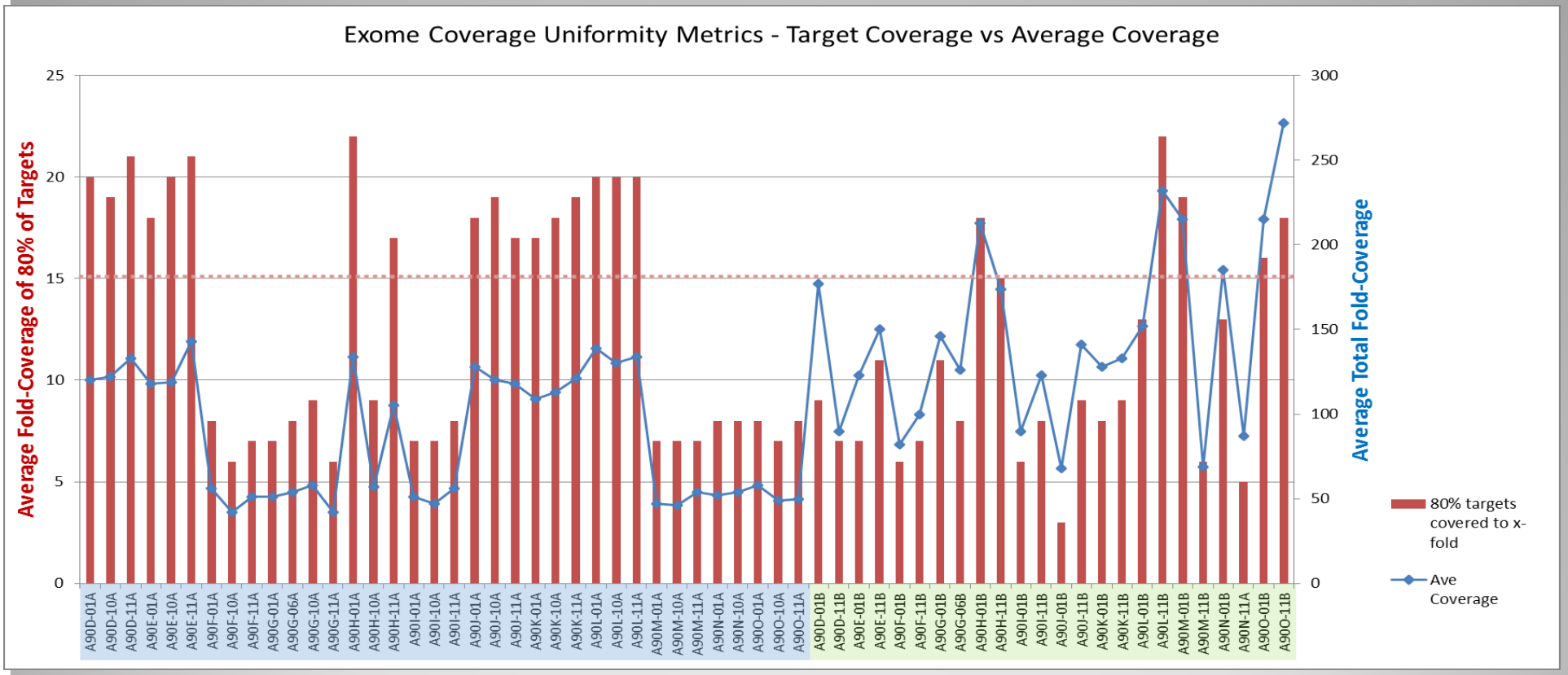


Figure 5: Median Insert Size in sequencing libraries; FF and Blood samples are shaded blue, FFPE samples are shaded green

Exome Sequencing

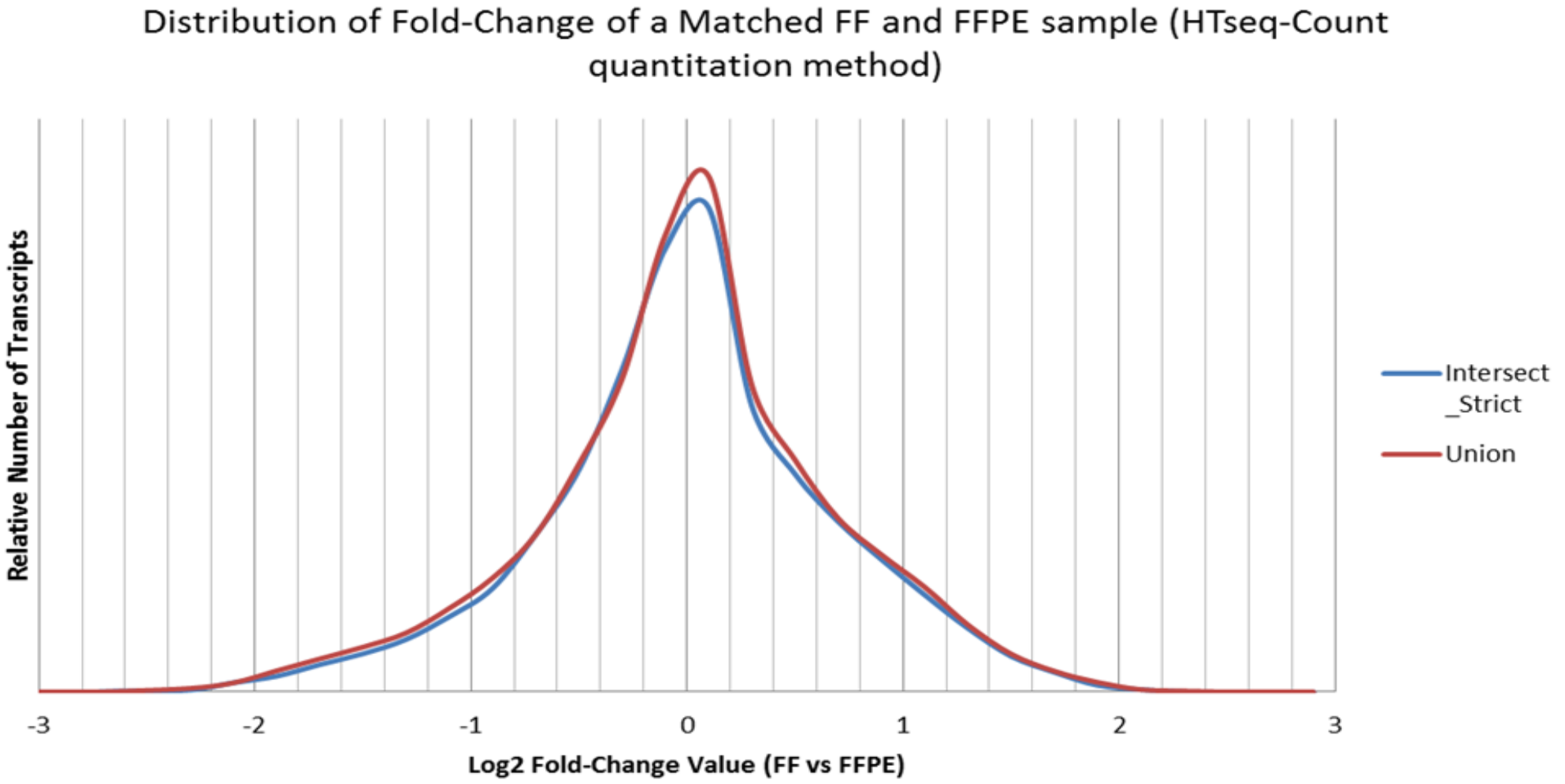


Exome Coverage Uniformity Metrics; the red dotted line represents typical target coverage for 80% of targets for germline variant exome sequencing; FF and blood sample codes are shaded blue, and FFPE sample codes are shaded green

Pilot study: Exome Analysis: Preliminary Assessment of DNA Seq

- Known Mutations
  - *BRAF (2)*
    - *V600E in RNA & Exome (1)*
  - *TP52*
  - *ALK*
  - *CHEK2*

RNA-Seq QC



Distribution of log2 fold-change between a matched FF and FFPE sample

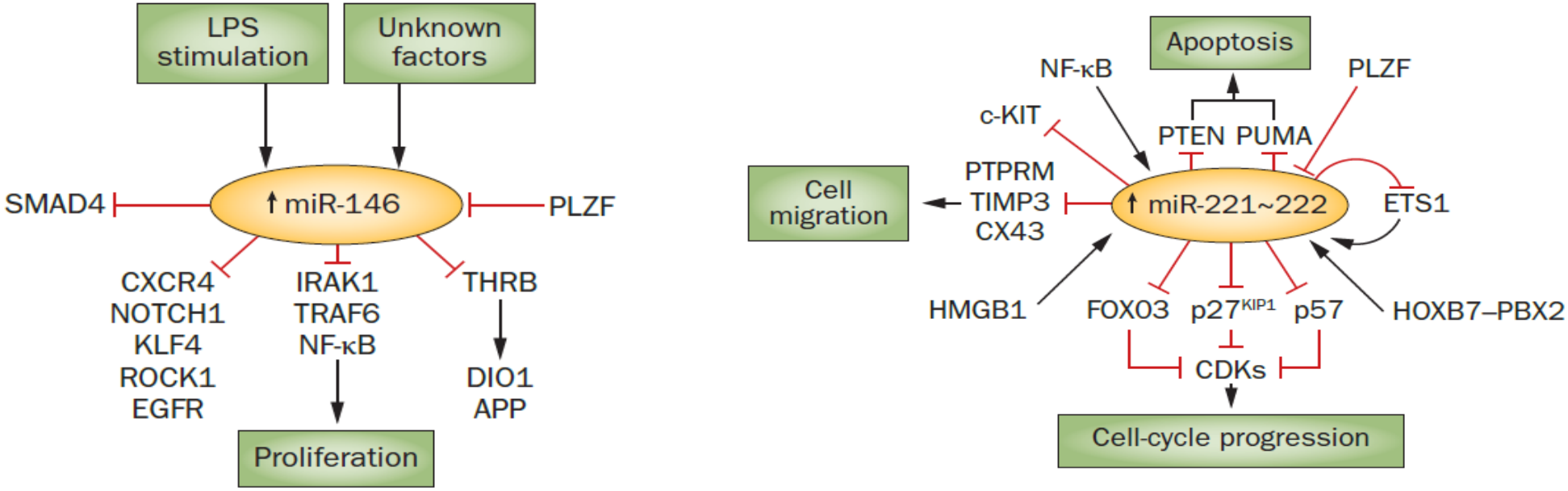
Pairwise Analysis of Matched Tumor/Normal Data - Significantly Dysregulated miRs

miRNAs Significantly Up-regulated in Tumor vs Normal *(>8-fold)*					
miR	**Follicular Variant (n=5)**	**Papillary, Other (n=4)**	**Classical subtype (n=1)**	**All Tumor Pairs (n=10)**	**Reference(s)** *(Thyroid Studies)*
hsa-mir-146b	5	3	1	**9**	*Chen, et al. 2008; He et al. 2005; Nikiforova et al. 2008*
hsa-mir-221	5	3	1	**9**	
hsa-mir-222	5	3	1	**9**	
hsa-mir-31	4	3	1	**8**	*Tetzlaff et al., 2007, Nikiforova et al. 2008*
hsa-mir-375	3	2	1	**6**	*Dettmer et al. 2013*
hsa-mir-34a	1	1	1	**3**	*Tetzlaff et al., 2007*
hsa-mir-187	1	2	0	**3**	*Nikiforova et al. 2008*
hsa-mir-891a	2	1	0	**3**	

miRNAs Significantly Down-regulated in Tumor vs Normal *(<-8-fold)*					
miR	**Follicular Variant (n=5)**	**Papillary, Other (n=4)**	**Classical subtype (n=1)**	**All Tumor Pairs (n=10)**	**Reference** *(Thyroid Studies)*
hsa-mir-486	2	4	1	**7**	*Braun et al. 2010*
hsa-mir-675	3	3	1	**7**	
hsa-mir-144	1	3	1	**5**	*Rossing et al. 2012*
hsa-mir-7-3	2	2	1	**5**	
hsa-mir-136	1	2	1	**4**	

Note: Tumors without matching normals were excluded from this analysis;
Differential expression using a pairwise approach was measured by calculating the log2 fold-change of tumor miRNA counts relative to the matched normal (DESeq/Bioconductor); miR's were ranked according to the most significant fold-change (cut-off of log2FC of <-3 and >3; equivalent to 8-fold/-8-fold change)

Significantly Up-Regulated miRs 146b, 221 & 222 – Cell Cycle Regulation

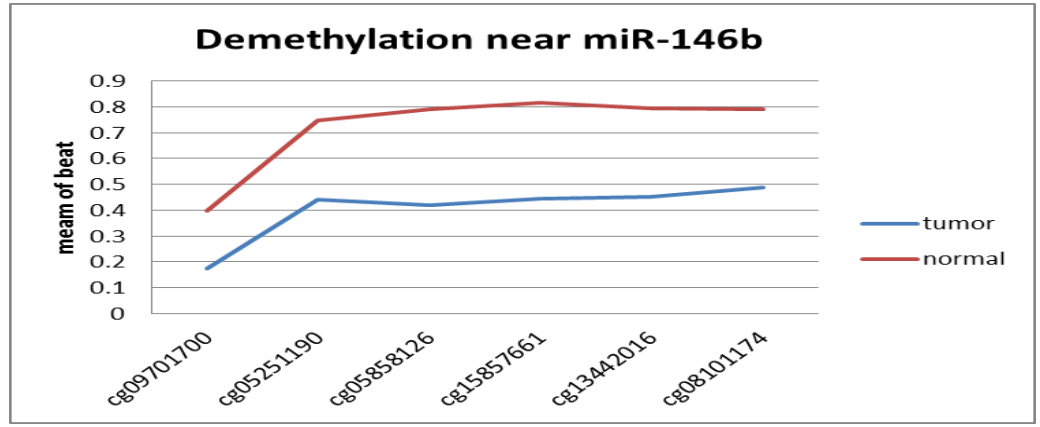


Pallante, et al. Deregulation of microRNA Expression in Thyroid Neoplasias. Nat. Rev. Endocrinol. 2013

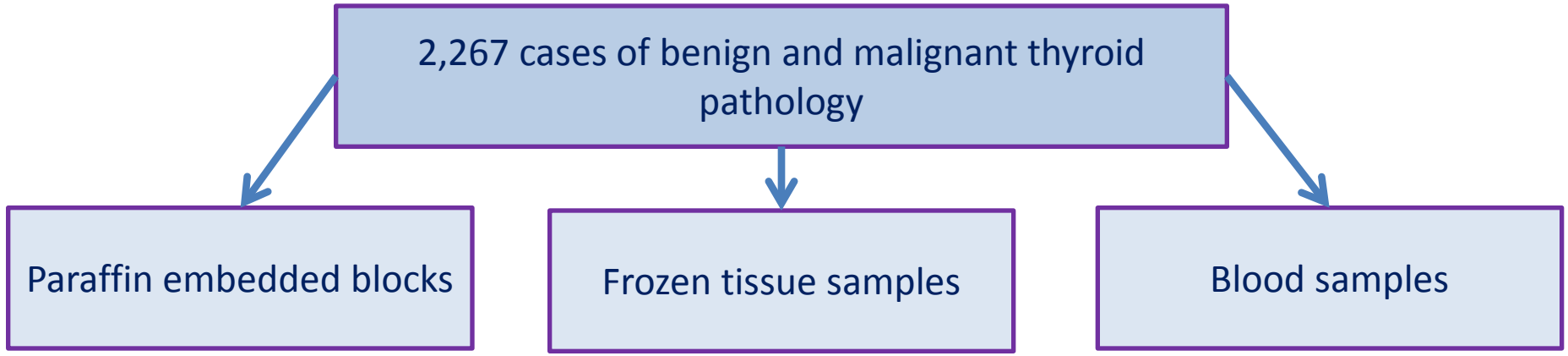
Integration of Methylation & miRNA Data – miR-146b

- *Identified reduced methylation at predicted transcription start site (TSS) of miR-146b in tumor samples*
- *Methylation was reduced by 40 - 50% on average in tumors relative to normals*
- ***Demethylation in the tumors may be contributing to the over-expression of miR-146b observed in the miRNA data***

probeID	hg19 Coordinates	arm	Gene	P.Value
cg09701700	chr10:104194843	q	MIR146B	3.46E-06
cg05251190	chr10:104196206	q	MIR146B	2.73E-04
cg05858126	chr10:104196213	q	MIR146B	1.08E-04
cg15857661	chr10:104196243	q	MIR146B	2.90E-05
cg13442016	chr10:104196339	q	MIR146B	1.88E-05
cg08101174	chr10:104196541	q	NA	4.43E-05



Ukrainian CTB Cases: 1998-2012



2,267 cases

Tumor(s) – 1-4 blocks per case
Normal – 1-3 blocks per case
Mts - 1-3 blocks per case

1,726 cases

Tumor(s) – 1-4 samples per case
Normal – 1-3 samples per case
Mts - 1-3 samples per case

919 cases

EDTA - 2 samples per case
Serum - 2 samples per case

Courtesy of Dr. Bogdanova

Target for Number of Cases

- 450 radiation-related PTC cases from CTB
  - 100 UkrAm*
  - 350 non-UkrAm*

- 550 sporadic (non-irradiated) PTC cases
  - 50 CTB* cases born after Jan 1, 1987
    - Part of this proposal
  - 500 cases from TCGA
    - Available to researchers based on *Cell* 159:676, 2014

*From Zhytomyr, Chernihiv, Kyiv region, and Kyiv city

National Cancer Institute

Parental irradiation of Ukrainian clean-up workers and evacuees and germline mutations in their offspring (TRIO Study)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
National Institutes of Health

Background

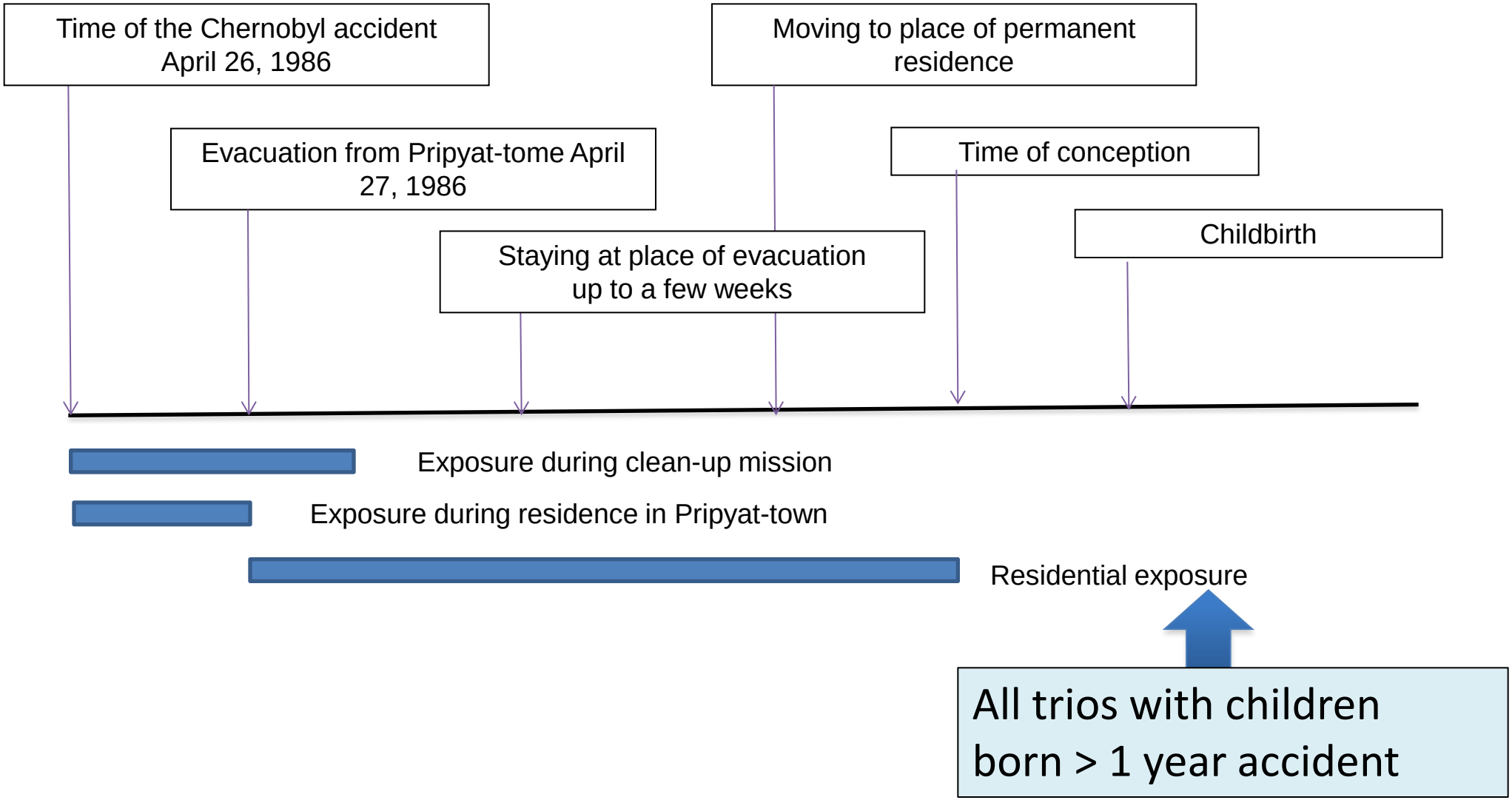
- No reliable evidence of untoward pregnancy outcomes, childhood mortality, or sex chromosome aneuploidy associated with parental radiation exposure in Japanese A-bomb F_1 or other studies
  - Statistical power in A-bomb F_1 study is low for endpoints
- Based on 7-locus mouse data (Russell et al) and non-significant indications from the A-bomb F_1 study, ICRP assumes parental radiation exposure induces a large spectrum of genetic effects in offspring
  - Doubling dose (DD) of about 1 Gy

DD = Radiation dose expected to double the spontaneous mutations rate in a generation

Objectives

- Comprehensive characterization of genomic alterations and inherited variation patterns in trios (parents and children) associated with pre-conception exposure to radiation from the Chernobyl accident:
  - ***de novo* mutations**
  - **Single nucleotide polymorphisms**
  - **Minisatellite mutations**
  - **Copy number variations**
  - **Somatic mutations**
  - **Mosaicism**
  - **Variation in telomere length**
  - **Methylation**
- Overlap of *de novo* mutations, copy number changes with genes linked to known diseases

Timeframe of Exposure



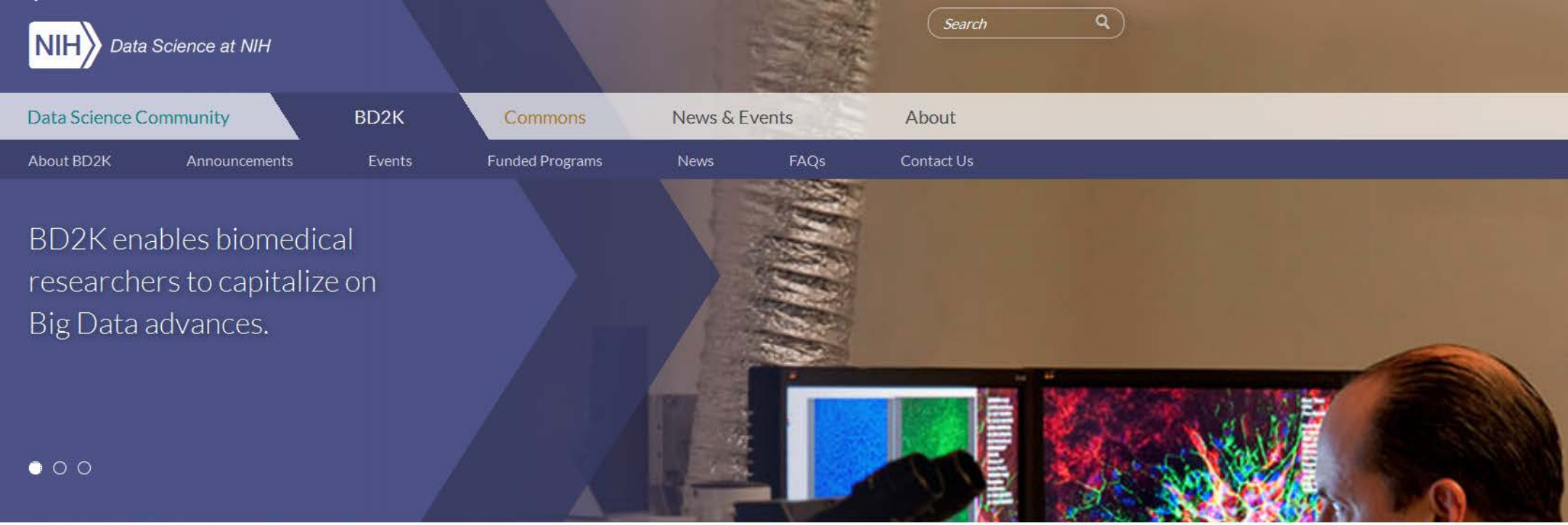
Target Trio Numbers

- Initial study: Recruit 50 trios, selected from risk categories (10 trios for each of 5 groups):
  - Exposed father, exposed mother
  - Exposed father, unexposed mother
  - Unexposed father, exposed mother
  - Unexposed father, unexposed mother
  - High dose emergency worker (fathers only, with acute radiation syndrome)
- Full study aims to recruit up to 450 trios from exposed and/or unexposed parents

Future collaborations

- Existing radiation studies
  - RERF atomic-bomb survivors
  - Mayak nuclear plant workers
  - Childhood cancer survivors

BD2K enables biomedical researchers to capitalize on Big Data advances.



Big Data to Knowledge (BD2K)

The ability to harvest the wealth of information contained in biomedical Big Data will advance our understanding of human health and disease; however, lack of appropriate tools, poor data accessibility, and insufficient training, are major impediments to rapid translational impact. To meet this challenge, the National Institutes of Health (NIH) launched the Big Data to Knowledge (BD2K) initiative in 2012.

BD2K is a trans-NIH initiative established to enable biomedical research as a digital research enterprise, to facilitate discovery and support new knowledge, and to maximize community engagement.

Read More

BD2K Recent News

- **NIH BD2K ENIGMA Center is published in Nature**

  NIH BD2K ENIGMA Center is published in Nature

  ... *read more*

- **Important Information for BD2K Centers RFA HG-13-009 Applicants**

  National Institutes of Health Director Francis S. Collins, M.D., Ph.D, announced today the... *read more*

- **NIH BD2K Seeks Input on Making Data Usable!**

  This Request for Information (RFI) solicits comments and ideas related to how community... *read more*

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Global Alliance
for Genomics & Health

Collaborate. Innovate. Accelerate.

Mission

To accelerate progress in human health by helping to establish a common framework of harmonized approaches to enable effective and responsible sharing of genomic and clinical data, and by catalyzing data sharing projects that drive and demonstrate the value of data sharing

Utility of a Cancer Knowledge System

Cancer information donor

Identify low-frequency cancer drivers

Define genomic determinants of response to therapy

Compose clinical trial cohorts sharing targeted genetic lesions

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Questions and Answers

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