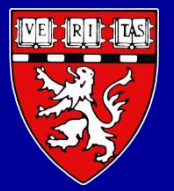
Molecular Pathological Epidemiology (MPE): Novel Integrative Pathology Science

(9/24/12. 50 min)

Shuji Ogino, MD, PhD, MS (Epidemiology)
Associate Professor of Pathology
Harvard Medical School
Dana-Farber Cancer Institute
Brigham and Women's Hospital

Associate Professor (Dept of Epidemiology)
Harvard School of Public Health

I have no conflict of interest



This lecture is co-sponsored by AMP (Association for Molecular Pathology)

Association for Molecular Pathology (AMP)

A not-for-profit international society dedicated to molecular pathology and diagnostics

AMP: Background

- Member # from 50 (in 1995) to 2200 now !!!
- Why so successful?
  - AMP encompasses ALL human disease areas, including ***genetic, infectious and neoplastic diseases.***
  - AMP includes all professionals and trainees in molecular diagnostics

Membership Benefits

- **Members around the world**
  - We have common goals
  - Online community functions every day
- **Trainees** can get networking and mentoring
- **Networking**
  - Meetings; committees; online community
- **Education**
  - Meetings; courses; webinars
- Subscription to *The Journal of Molecular Diagnostics*

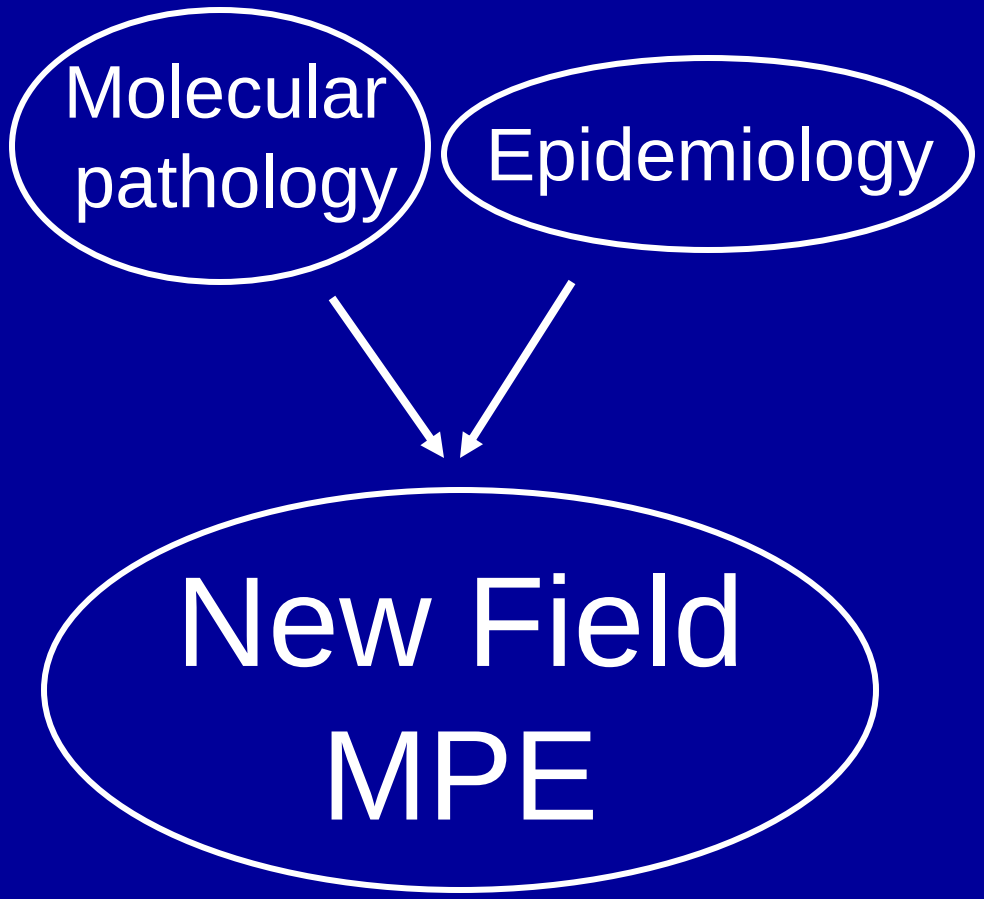
Who Should Join AMP?

- Anyone who is interested in molecular pathology and diagnostics

www.amp.org

Molecular Pathological Epidemiology (MPE)

Concepts

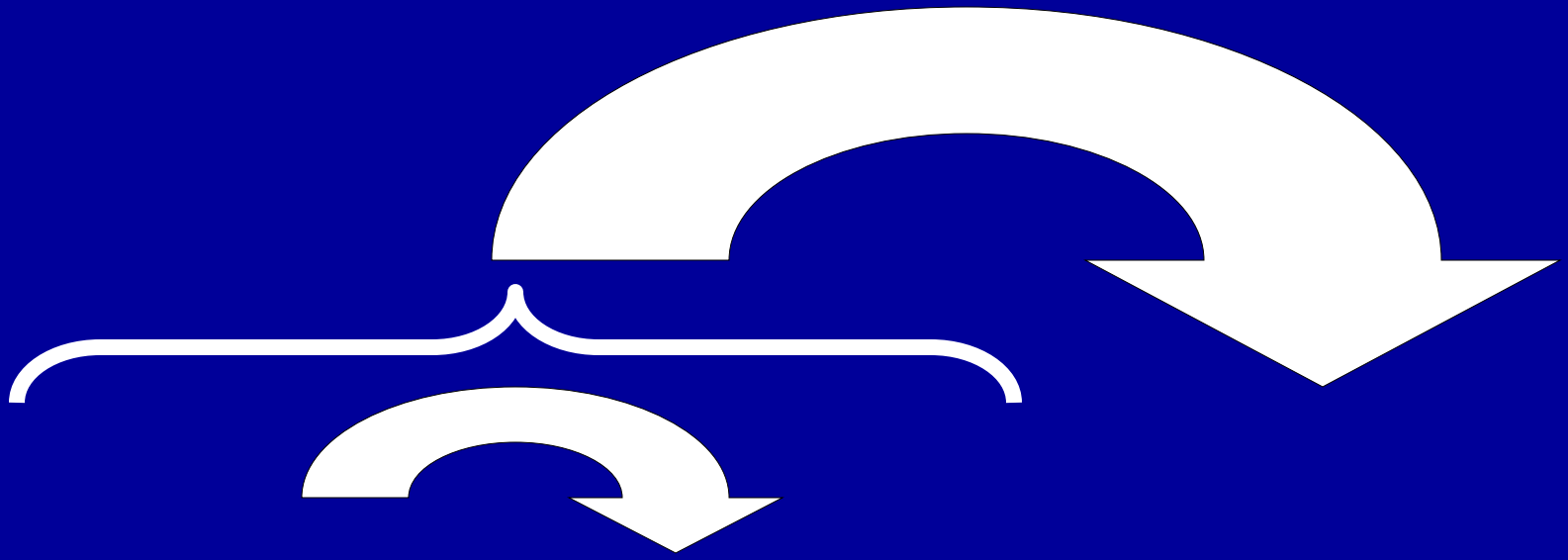


MPE = integrated (molecular pathology / epidemiology) science

Reviews on growing MPE paradigms

- Ogino et al. J Natl Cancer Inst 2010
- Ogino et al. Gut 2011
- Ogino et al. Nat Rev Clin Oncol 2011
- Ogino et al. Int J Epidemiol 2012
- Ogino et al. Expert Rev Mol Diagn. 2012
- Ogino, King, et al. Am J Epidemiol. 2012
  - Kuller. Am J Epidemiol. 2012
  - Ogino, Beck, et al. Am J Epidemiol. 2012
- *N Engl J Med 2012 in press*

Adjectival relations



Molecular Pathological Epidemiology

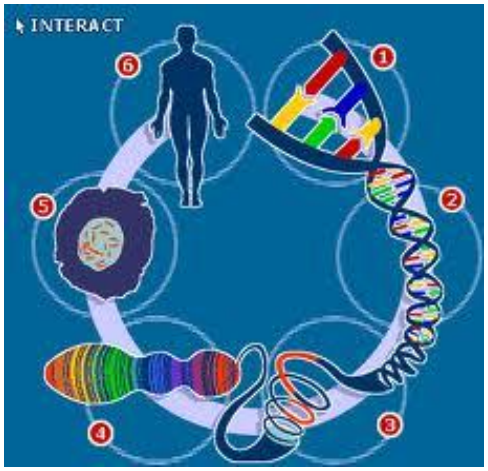
MPE (Molecular Pathological Epidemiology)

- Nice acronym is a key in science
  - DNA
  - PCR
  - SNP

Pathology and Epidemiology

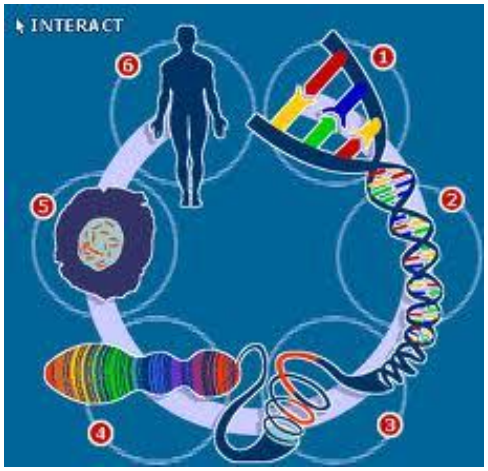
- Pathology - disease mechanisms at molecular and cellular level
- Epidemiology - disease distribution at population level
- MPE has both strengths (molecular and population-level science)

Molecular Pathological Epidemiology (MPE)



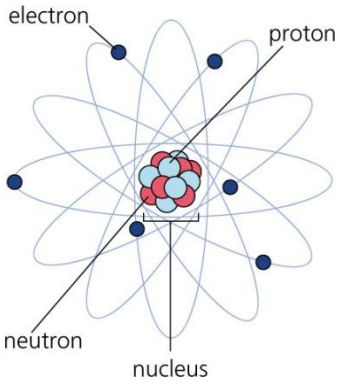
Molecular & Population-level

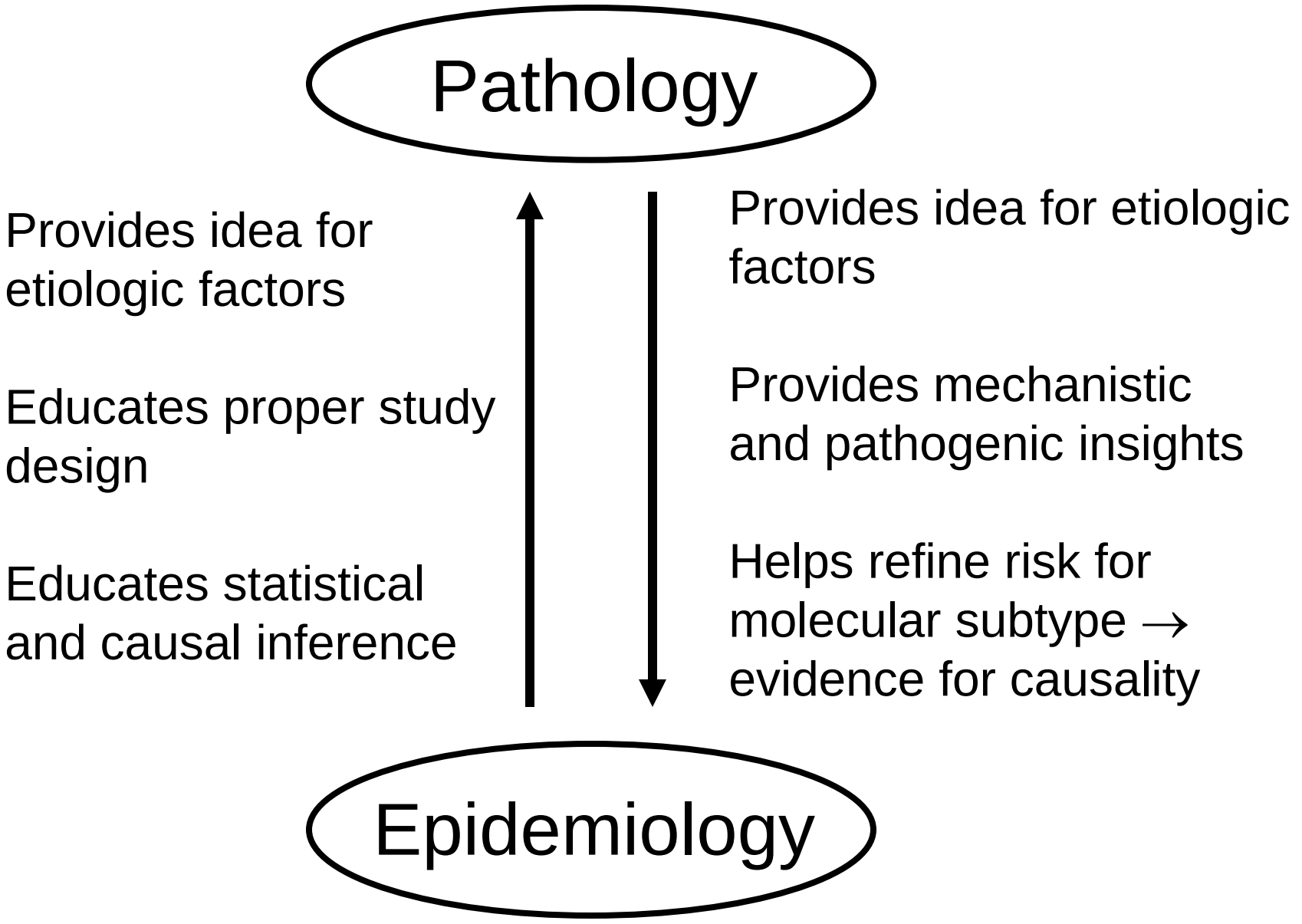
Molecular Pathological Epidemiology (MPE)



Molecular & Population-level

Physics





Ogino, et al. Am J Epidemiol. 2012

Epidemiology education is good for pathology

- Pathologists can learn
  - Proper study design
  - Sources of bias and confounding
  - Statistical analysis
  - Statistical inference

Epidemiology education is good in pathology

- You will never write “we used “UNSELECTED series of 200 breast cancers”
- All cases in your series are selected by you!
  - Selection bias

Epidemiology education is good in pathology

- What is a “P value”?

Epidemiology education is good in pathology

- Pathology will become stronger science
- Integrative Molecular Pathological Epidemiology (MPE) will develop further

MPE is based on this principle:

Each individual has
unique disease process

Each of us is unique

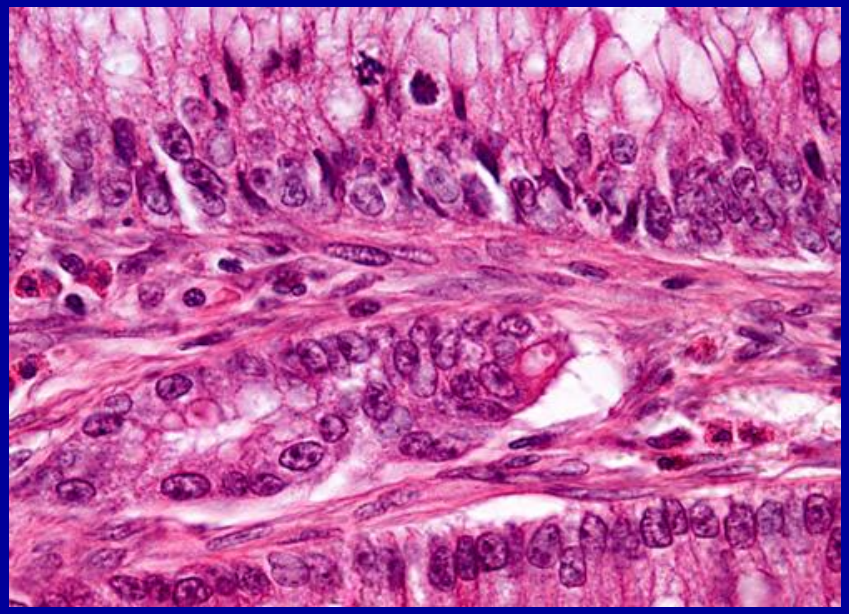
Each disease process is unique

Ogino et al. Nat Rev Clin Oncol 2011
Ogino et al. Expert Rev Mol Diagn 2012

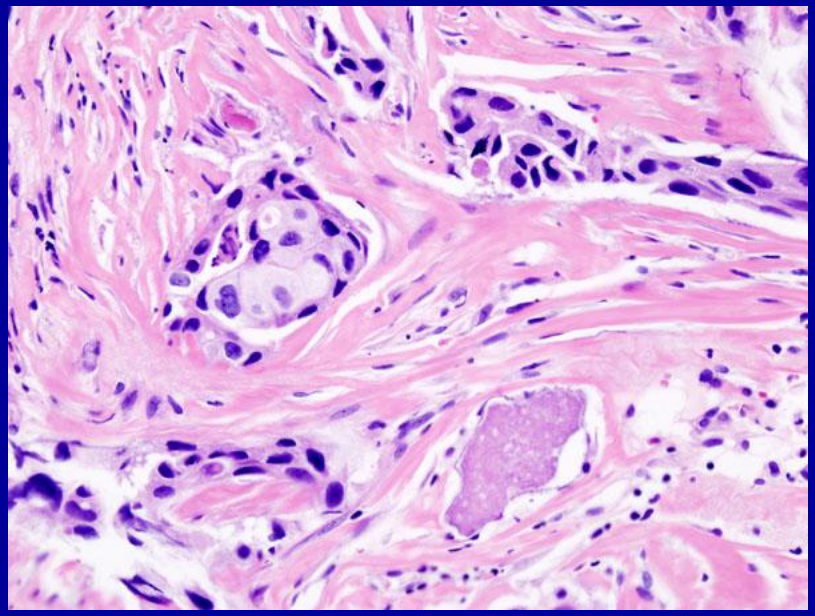
Each baby is unique

???

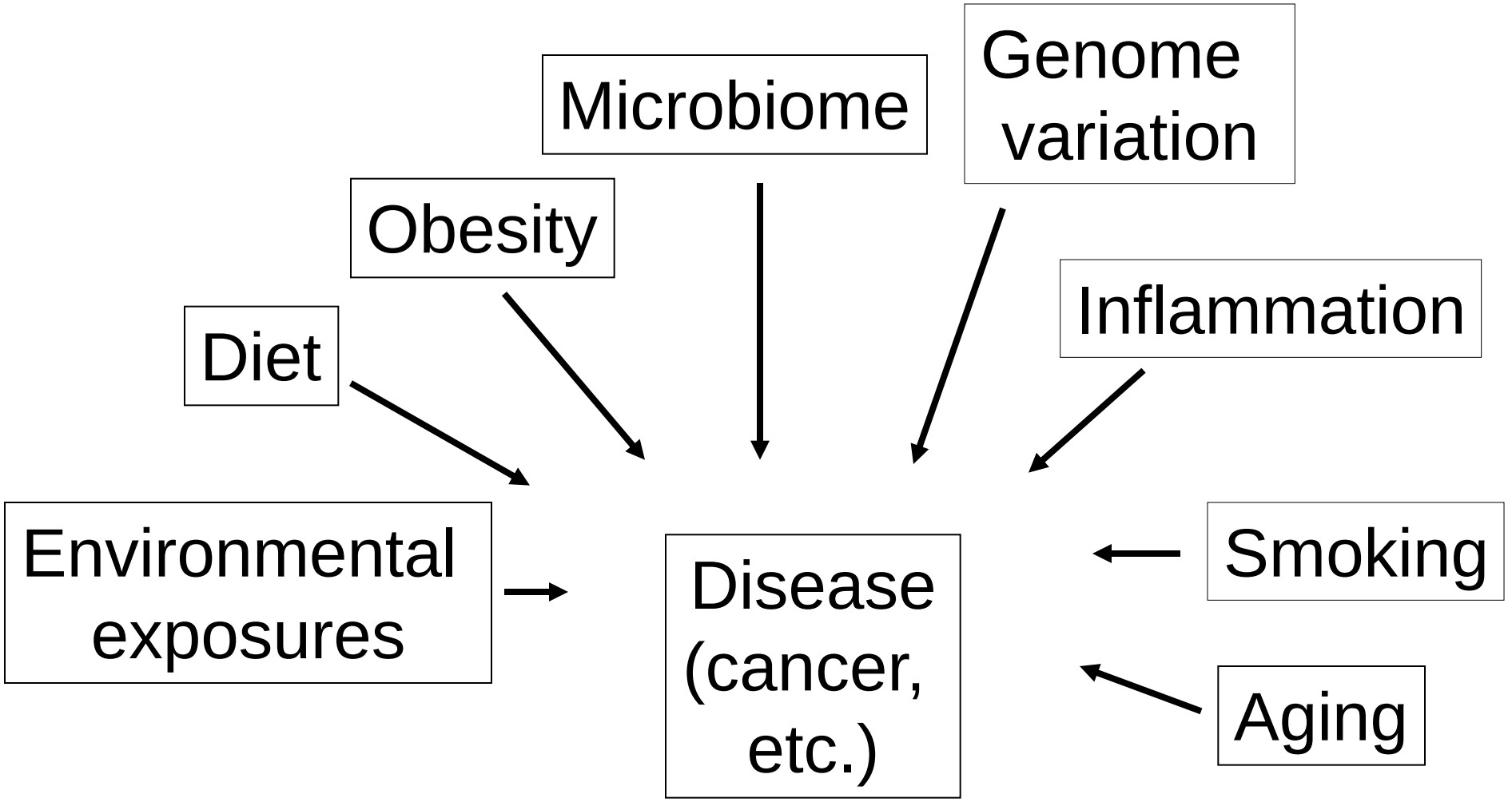
Tumor cells always interact with host cells



Colon cancer



Breast cancer


Genome variation
Microbiome
Obesity
Inflammation
Diet
Environmental exposures
Disease (cancer, etc.)
Smoking
Aging

Stroma can change tumor phenotype

- HGF from stromal cells makes melanoma resistant to RAF inhibitor
  - *Straussman et al. Nature 2012*

LETTER

doi:10.1038/nature11183

Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion

Ravid Straussman[1], Teppei Morikawa[2], Kevin Shee[1], Michal Barzily-Rokni[1], Zhi Rong Qian[2], Jinyan Du[1], Ashli Davis[1], Margaret M. Mongare[1], Joshua Gould[1], Dennie T. Frederick[3], Zachary A. Cooper[3], Paul B. Chapman[4], David B. Solit[4,5], Antoni Ribas[6,7], Roger S. Lo[7,8], Keith T. Flaherty[3], Shuji Ogino[2,9], Jennifer A. Wargo[3] & Todd R. Golub[1,10,11,12]

Stroma can change tumor phenotype

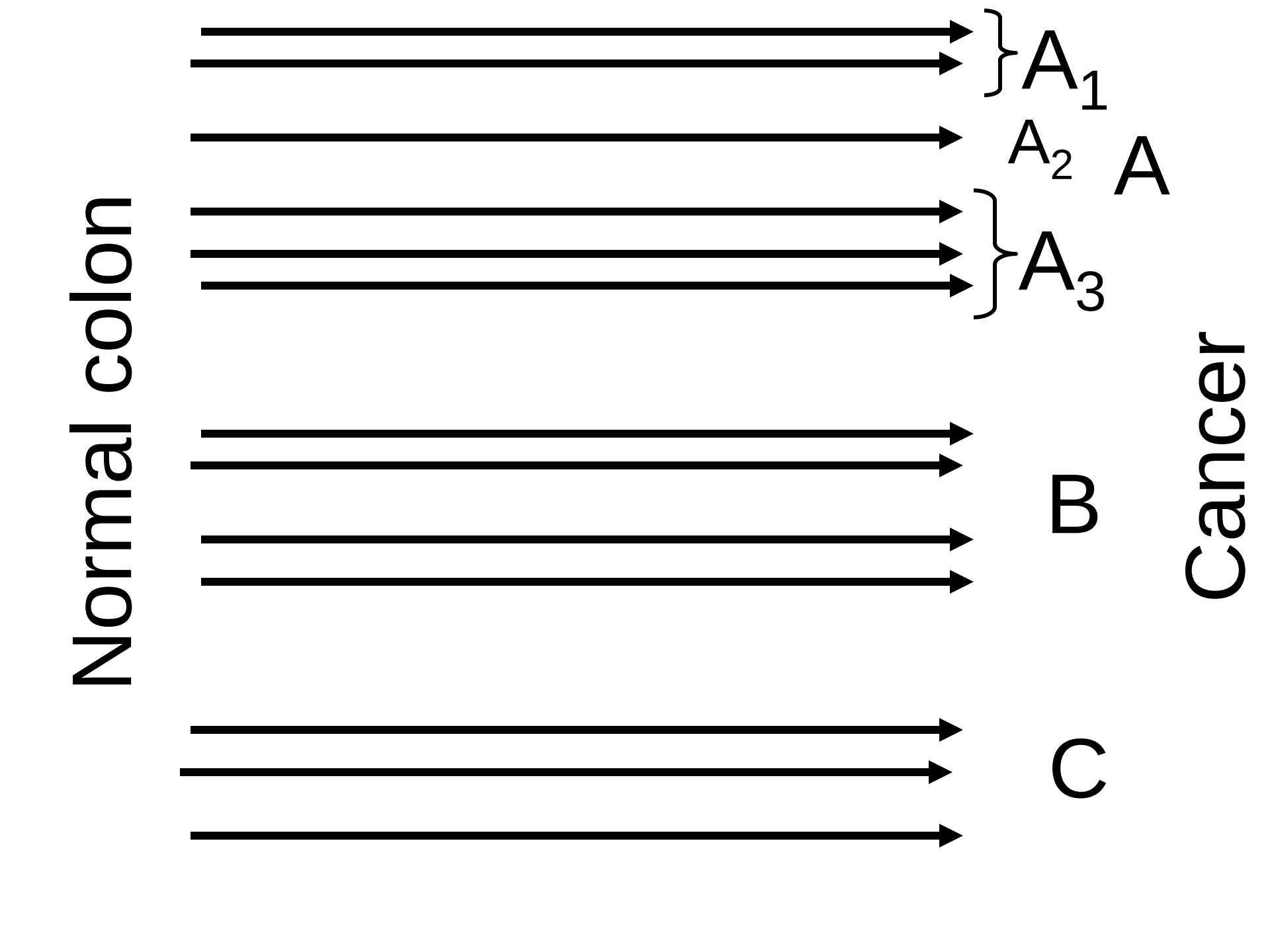
- HGF from stromal cells makes melanoma resistant to RAF inhibitor
  - *Straussman et al. Natu*

LETTER

83

Tumour micro-environme
to RAF inhibitors through

Ravid Straussman[1], Teppei Morikawa[2], Kevin Shee[1], Michal Ba
Margaret M. Mongare[1], Joshua Gould[1], Dennie T. Frederick[3], Z
Antoni Ribas[6,7], Roger S. Lo[7,8], Keith T. Flaherty[3], Shuji Ogino[2,9], Jennifer A. Wargo[3] & Todd R. Golub[1,10,11,12]


Normal colon
A1
A2
A
A3
B
Cancer
C

Molecular Pathological Epidemiology (MPE)

How to do

MPE study design

Molecular pathology

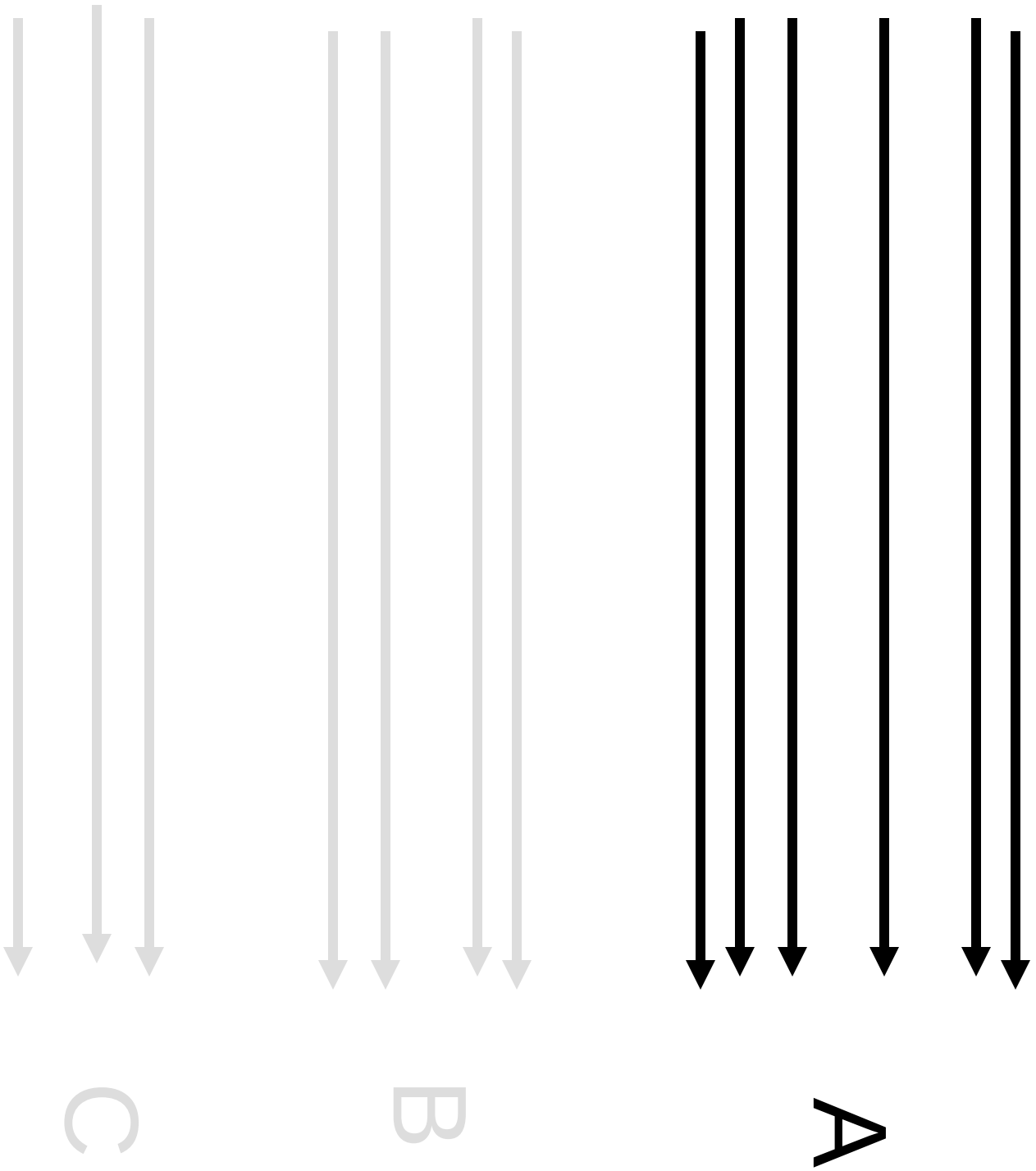
Etiologic factor

Tumor with a molecular alteration

Tumor with no alteration

Ogino et al. Gut 2011

Etiologic factor

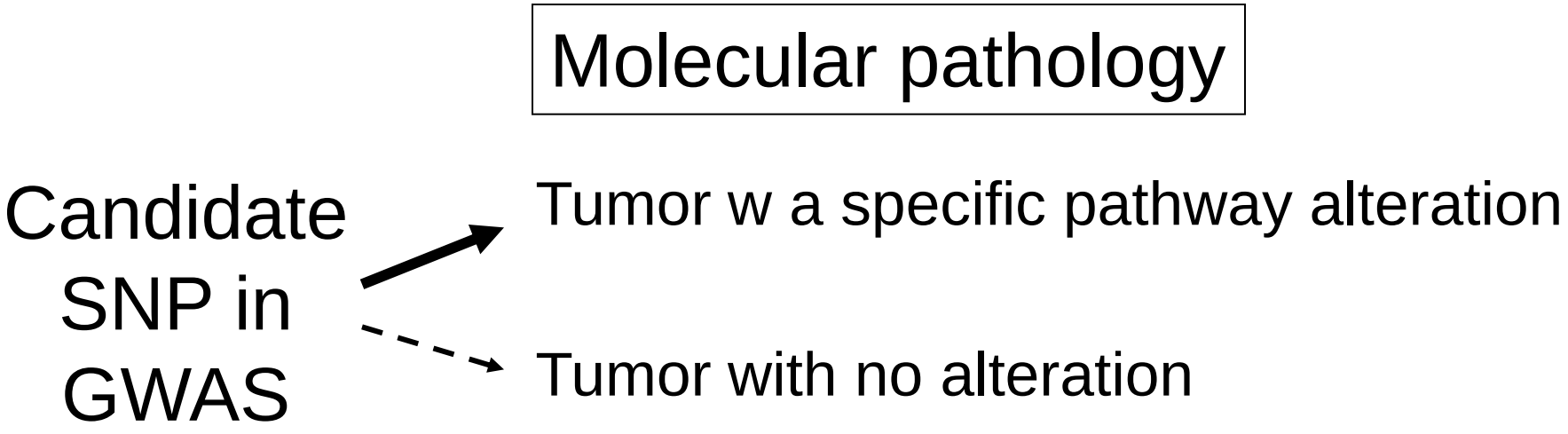


Cancer

MPE - next step of GWAS (genome-wide association studies)

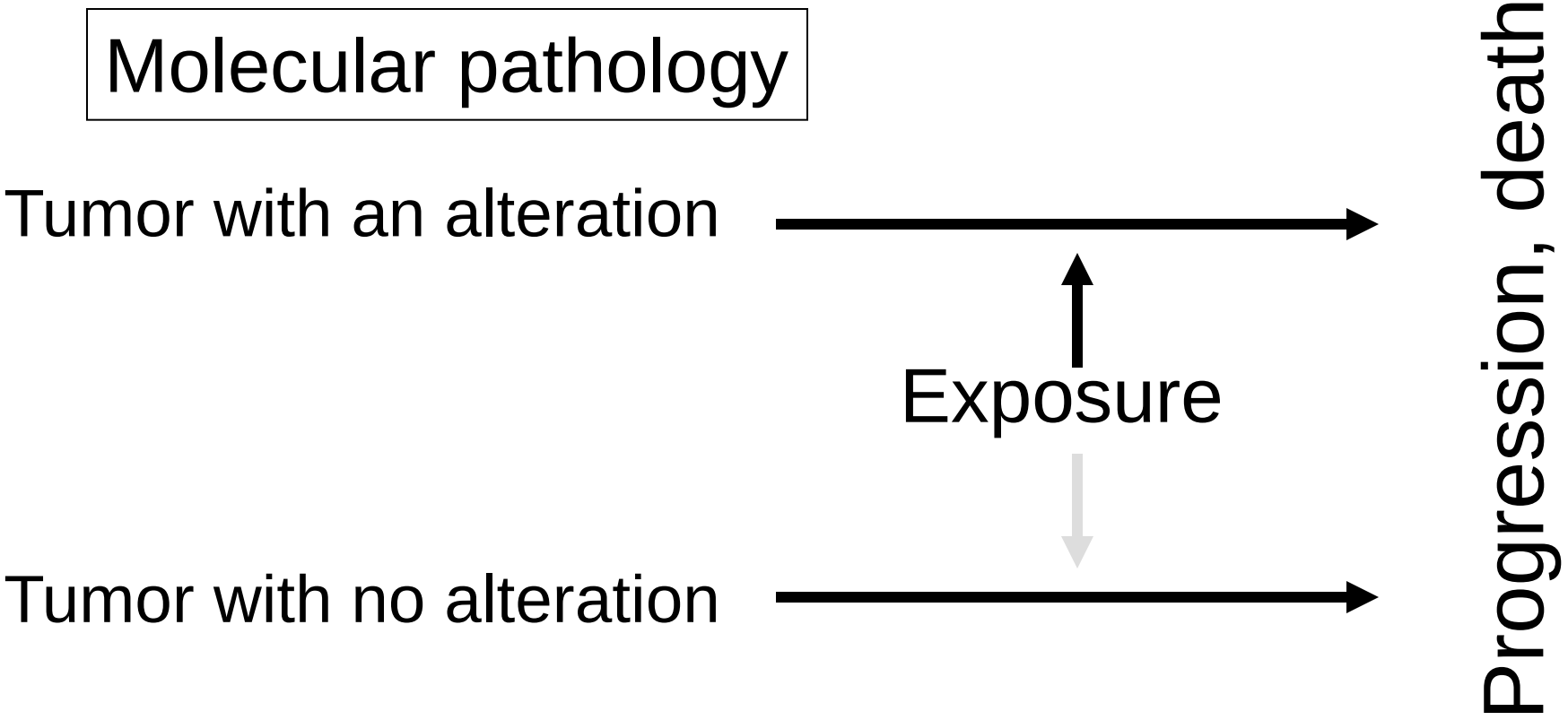
- Heterogeneous subtypes were lumped together into 1 disease category
- Functional significance of most variants is uncertain
- Effect size is very small (eg, OR=1.1)

"GWAS-MPE Approach"



Ogino et al. Gut 2011

MPE study design

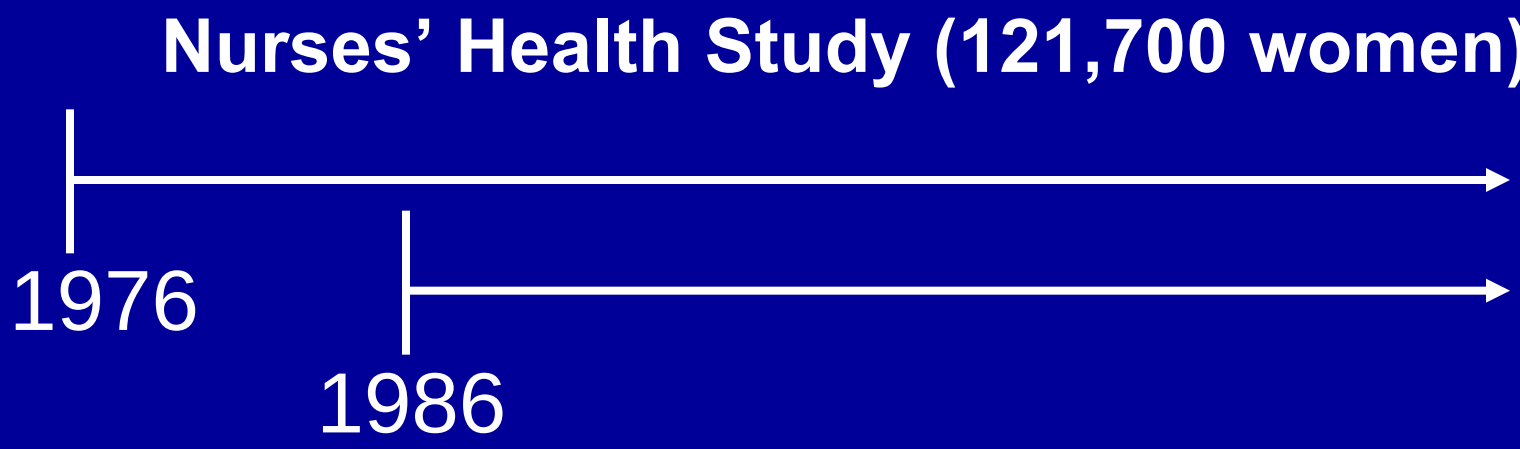


Most recently, *N Engl J Med 2012: in press*

First done by Ogino et al. J Clin Oncol 2008 (FASN x BMI)

An Overview of Our MPE Database

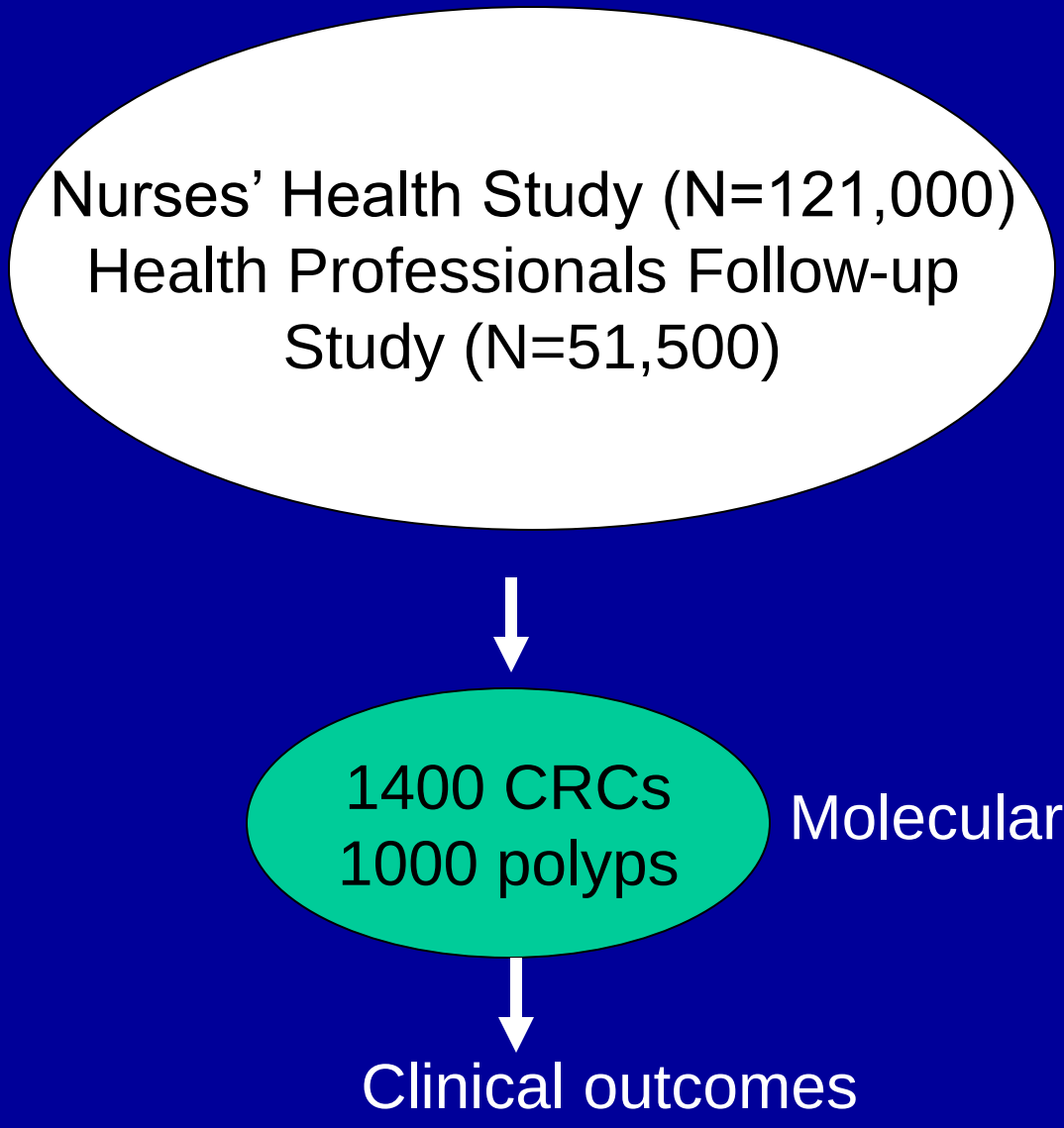
Prospective Cohort Studies



Nurses' Health Study (N=121,000)
Health Professionals Follow-up Study (N=51,500)

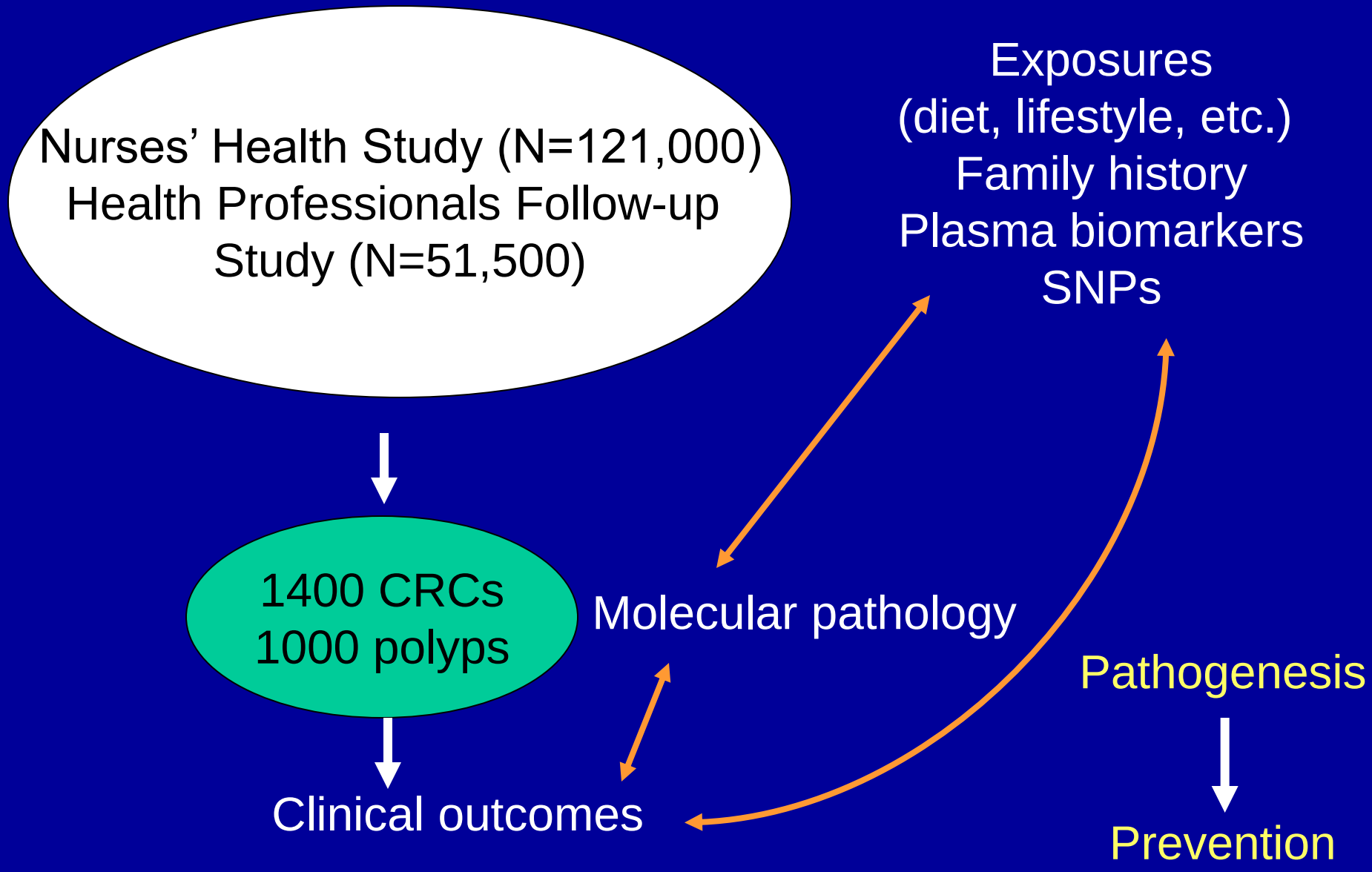
Exposures
(diet, lifestyle, etc.)
Family history
Plasma biomarkers
SNPs

Information was collected before tumor developed, to avoid recall bias



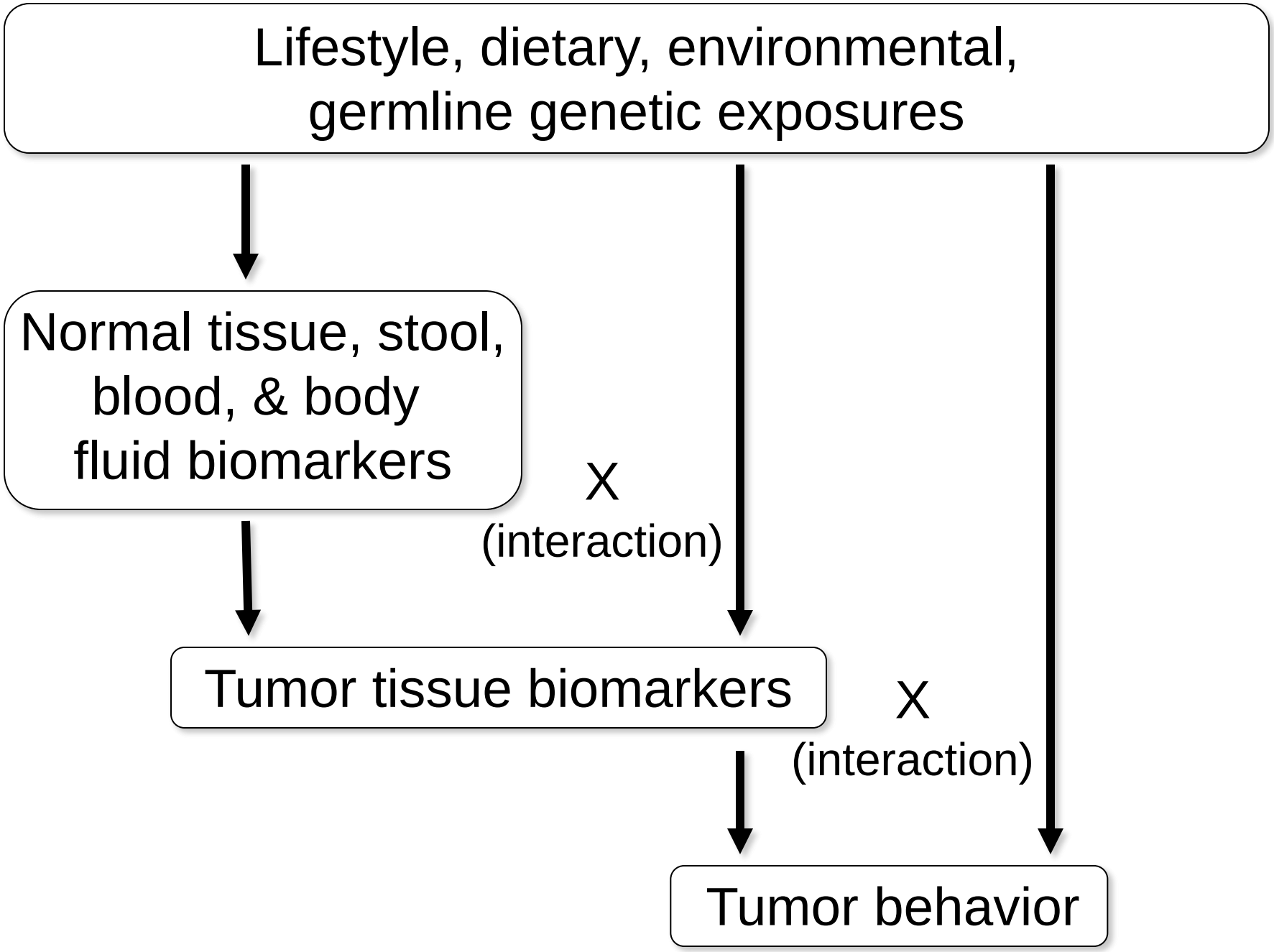
Exposures
(diet, lifestyle, etc.)
Family history
Plasma biomarkers
SNPs

Molecular pathology


Nurses' Health Study (N=121,000)
Health Professionals Follow-up Study (N=51,500)
Exposures
(diet, lifestyle, etc.)
Family history
Plasma biomarkers
SNPs
1400 CRCs
1000 polyps
Molecular pathology
Pathogenesis
Clinical outcomes
Prevention

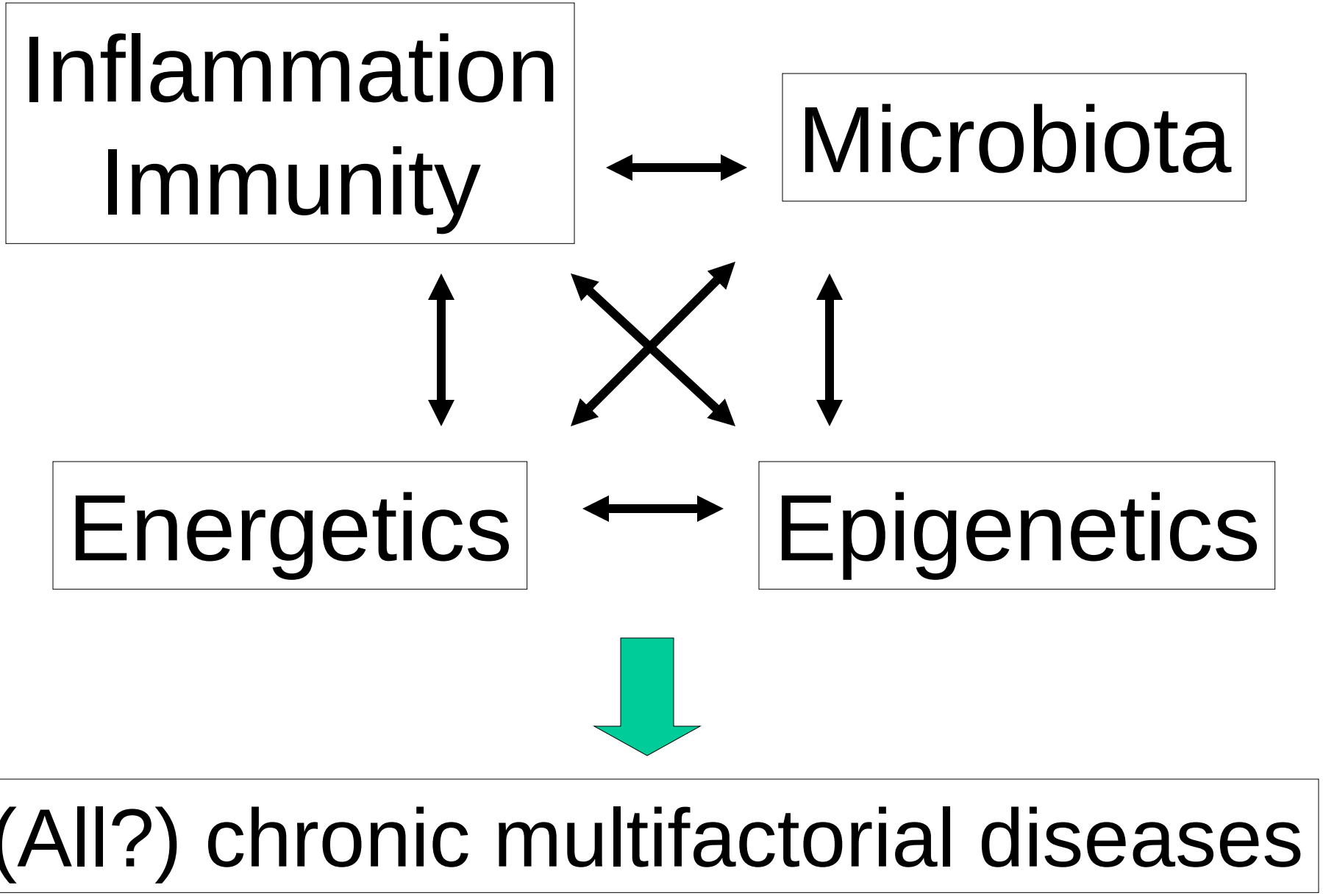
Omics analyses (FFPE)

- Tumor FFPE tissue
  - Genome-wide mRNA expression
    - *Waldron et al. Clin Cancer Res in press*
  - Whole exome sequencing
  - Copy number assay
  - Methylome sequencing
  - Microbiome (FFPE tumor and stool)
- Blood
  - Whole genome SNPs (for GWAS)


Lifestyle, dietary, environmental, germline genetic exposures
Normal tissue, stool, blood, & body fluid biomarkers
X
(interaction)
Tumor tissue biomarkers
X
(interaction)
Tumor behavior

Building great database = big science
Why is “data-omics” important?

- Point: Hypotheses First
  - *R Weinberg. Nature 2010*
- Counterpoint: Data First
  - *T Golub. Nature 2010*


Inflammation
Immunity
Microbiota
Energetics
Epigenetics
(All?) chronic multifactorial diseases

Molecular pathological epidemiology (MPE) data

- Just several examples

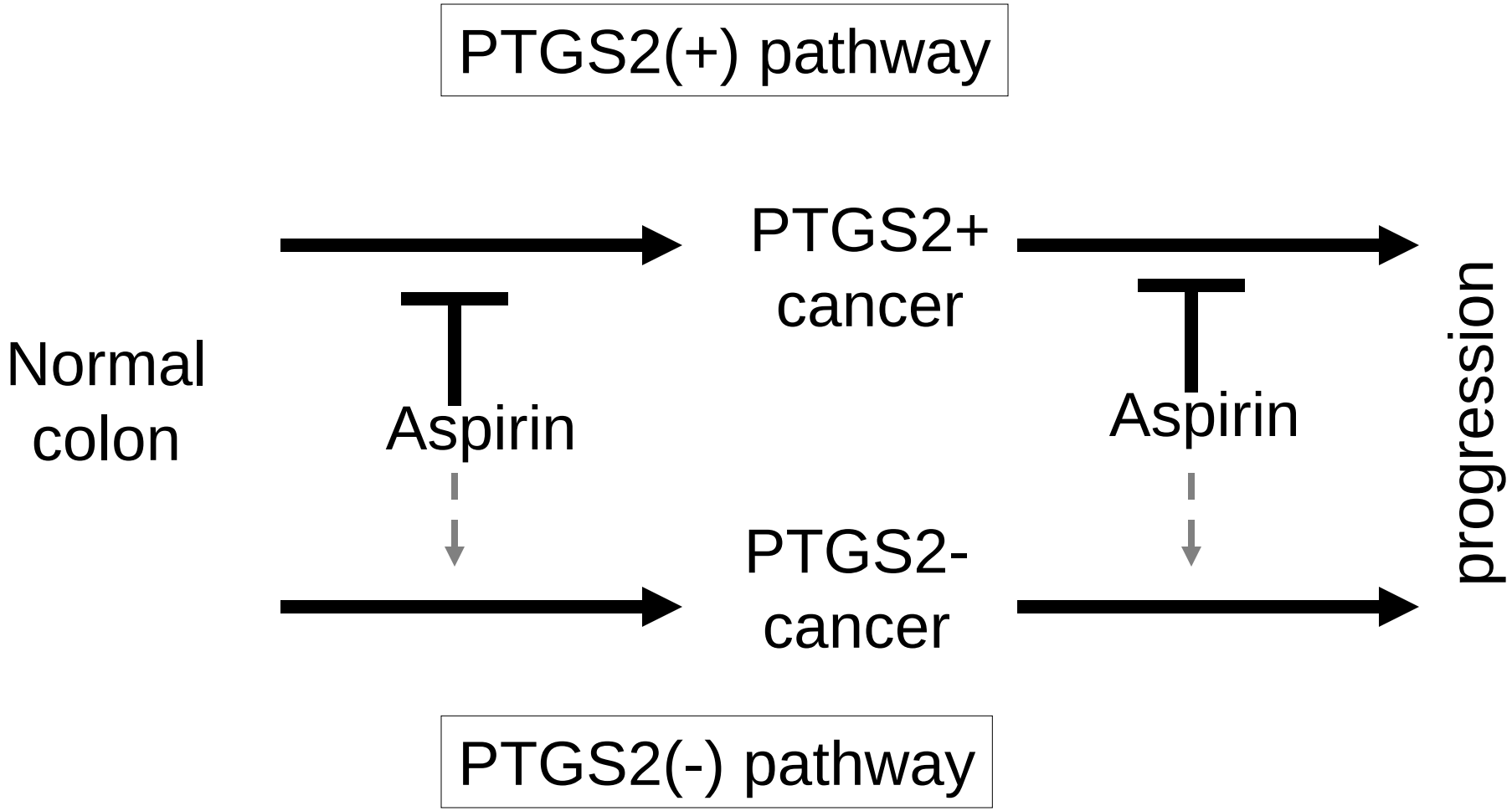
- Aspirin use -> PTGS2 (cyclooxygenase-2) in CRC
  - *Chan et al. N Engl J Med 2007*
- Aspirin use x PTGS2 -> CRC survival
  - *Chan et al. JAMA 2009*
- Folate and alcohol intake -> TP53 in CRC
  - *Schernhammer et al. Gastroenterology 2008*
- Folate and alcohol -> LINE-1 methylation in CRC
  - *Schernhammer et al. Gut 2010*
- Obesity -> FASN in CRC
  - *Kuchiba et al. J Natl Cancer Inst 2012*
- Obesity x FASN -> CRC survival
  - *Ogino et al. J Clin Oncol 2008*
- Obesity and exercise x CTNNB1 -> CRC survival
  - *Morikawa et al. JAMA 2011*

Inflammation

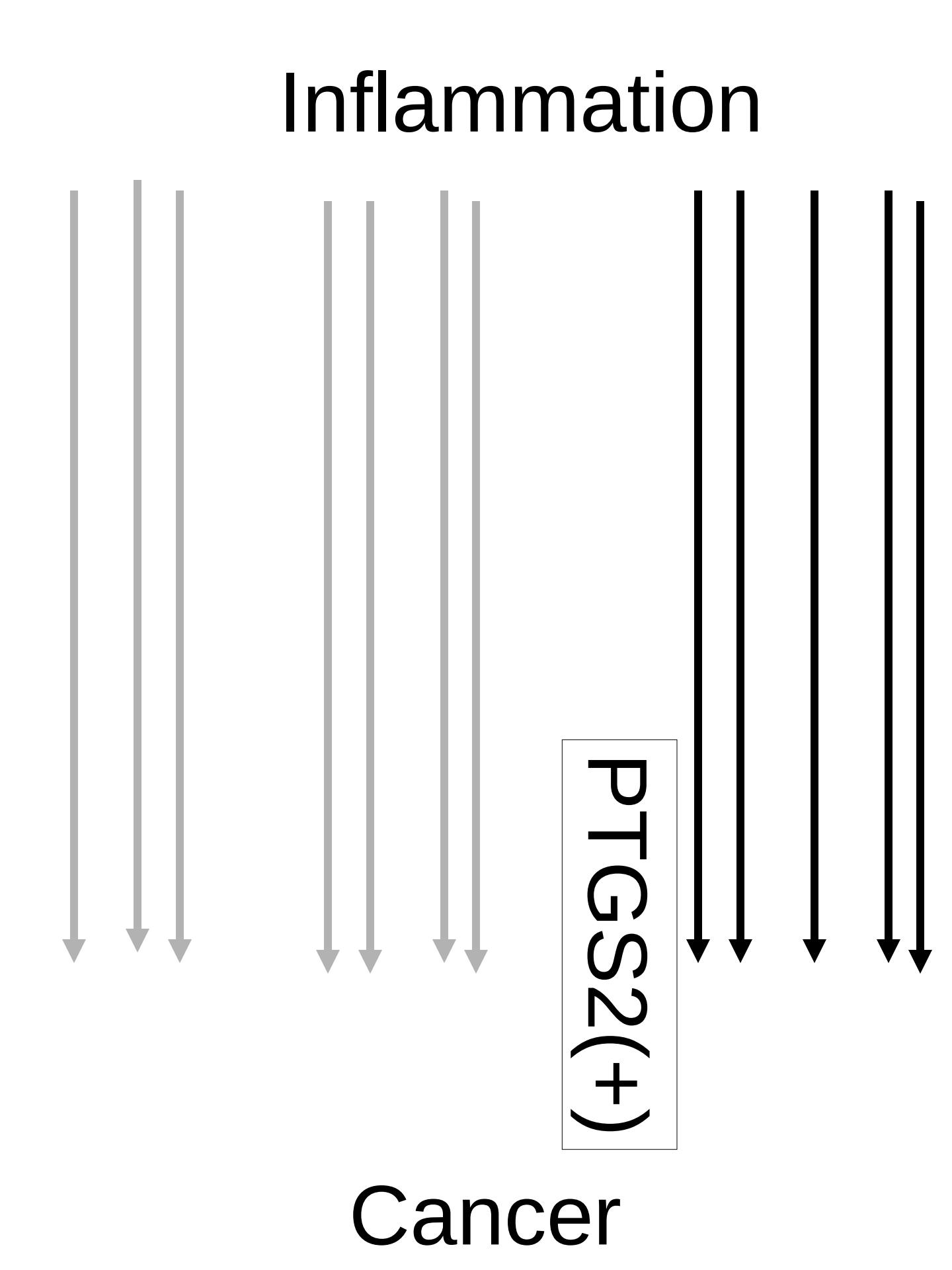
Aspirin

- Aspirin decreases colorectal cancer risk
  - Aspirin inhibits PTGS2 (cyclooxygenase-2)

- Hypothesis: Aspirin prevents PTGS2+ cancer



Chan et al. NEJM 2007; JAMA 2009


Inflammation
PTGS2(+)
Cancer

Aspirin and PTGS2

- Aspirin inhibits PTGS2(+) tumor progression
- PTGS2 is a predictive biomarker for response to aspirin
- Provide information for clinical decision making!
  - You don't want to give aspirin to all patients

Chan et al. JAMA 2009

Epigenetics

Global DNA (LINE-1) hypomethylation

- Activation of oncogenes
- Chromosomal instability (CIN)
  - LINE-1 is a transposable element

LINE-1

(Long Interspersed Nucleotide Element-1)

Repetitive elements, ~400,000 copies

Occupies 20% of the human genome

LINE-1 hypomethylation: Uniform alterations across cancers

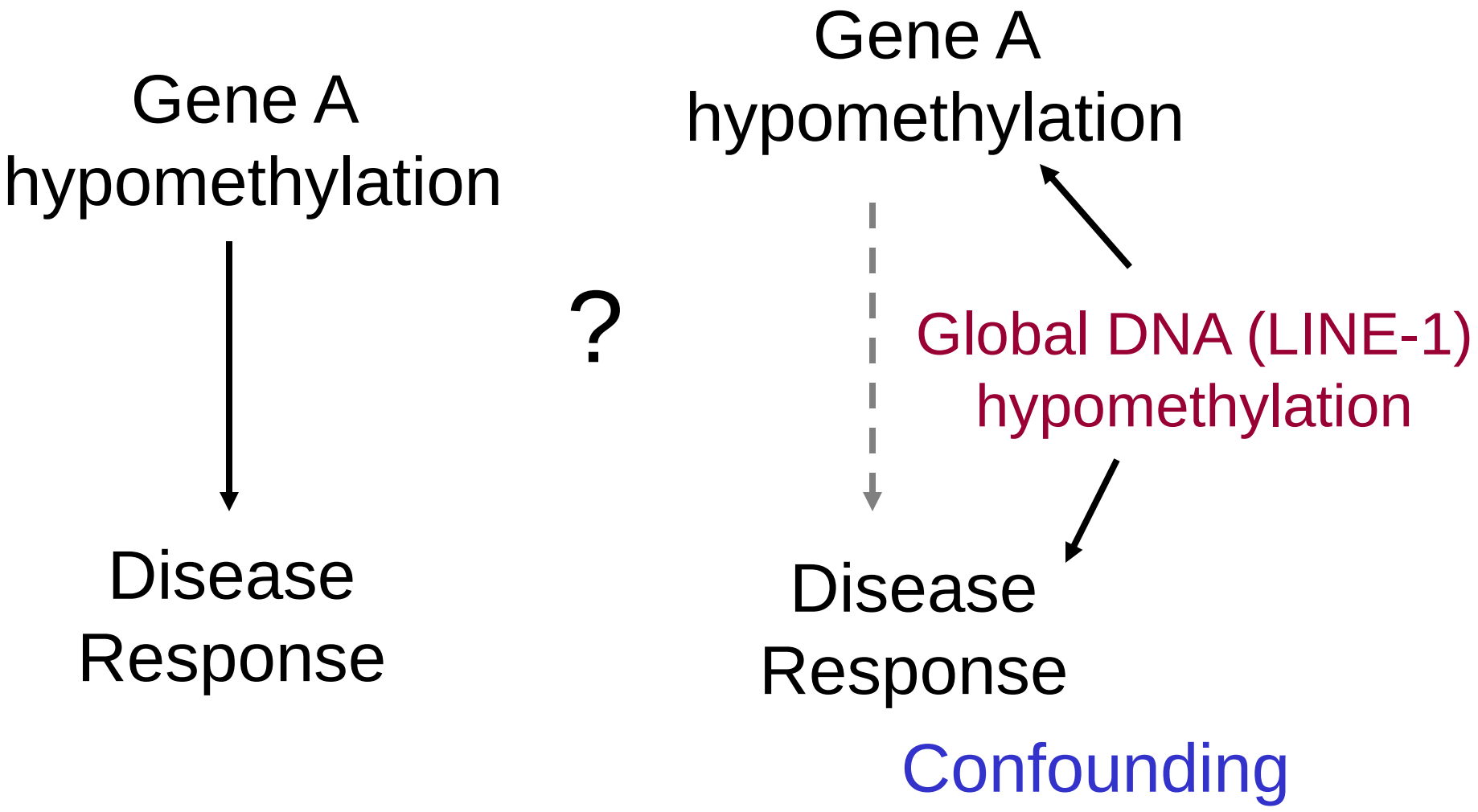
- Colorectal cancer
- Prostate cancer
  - *Yegnasubramanian et al. Cancer Res 2008*
- Ovarian cancer
  - *Pattamadilok et al. Int J Gynecol Ca 2008*
- Leukemia (CML)
  - *Roman-Gomez et al. Oncogene 2005*
- Pancreatic endocrine tumors / carcinoid
  - *Choi et al. Mod Pathol 2007*
- GIST (gastrointenstinal stromal tumor)
  - *Igarashi et al. Clin Cancer Res 2010*

Why study global change?

Gene A
hypomethylation

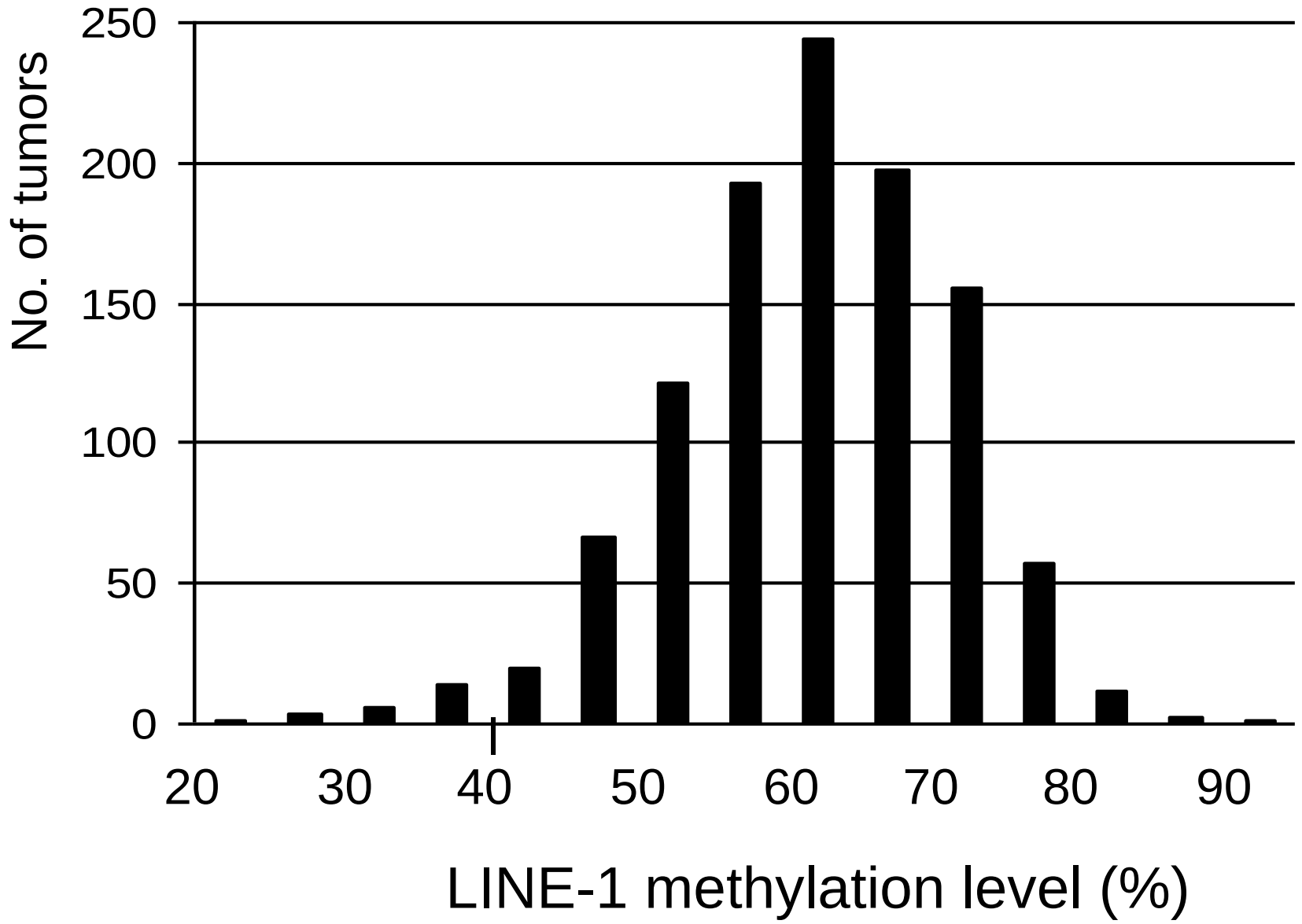
Disease
Response

Why study global change?

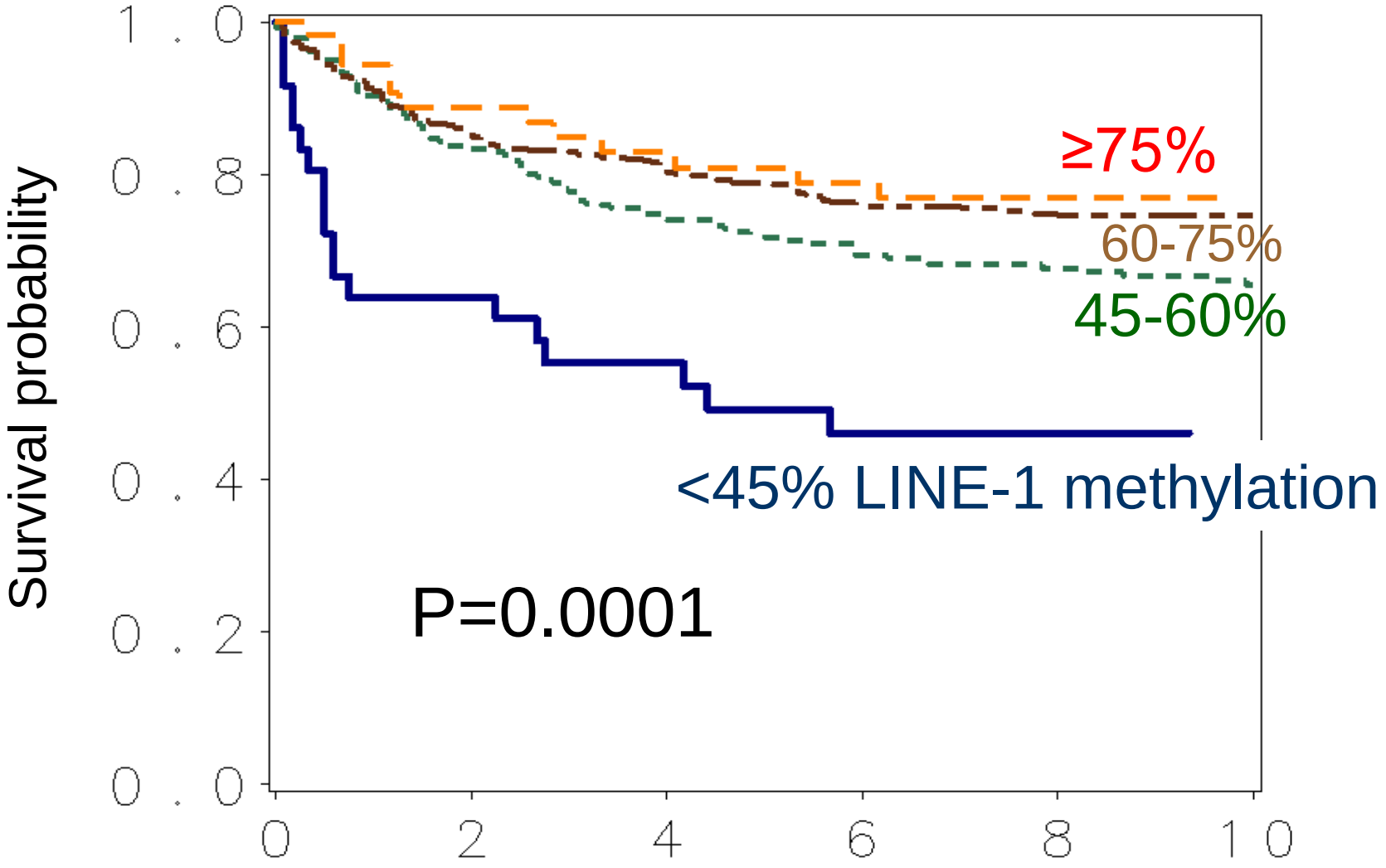


Global hypoM - conductor
Locus-specific changes - players

LINE-1 methylation in 1200 tumors shows ~normal distribution



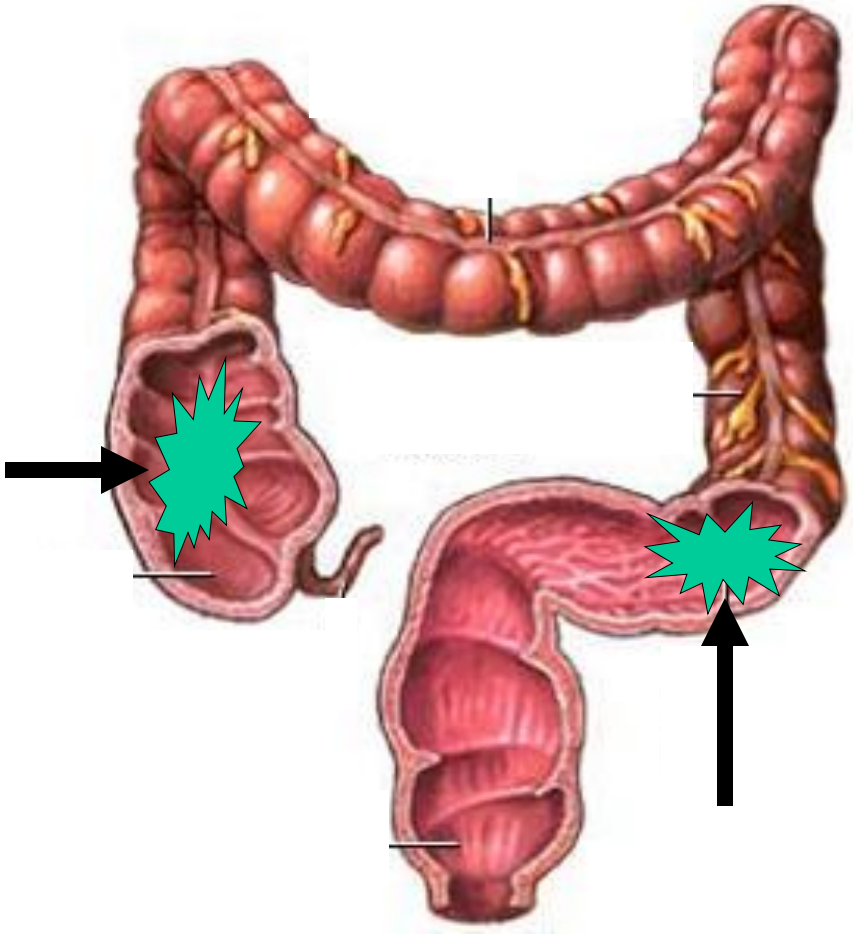
LINE-1 Hypomethylation is the Best Molecular Prognosticator



Ogino et al. J Natl Cancer Inst 2008

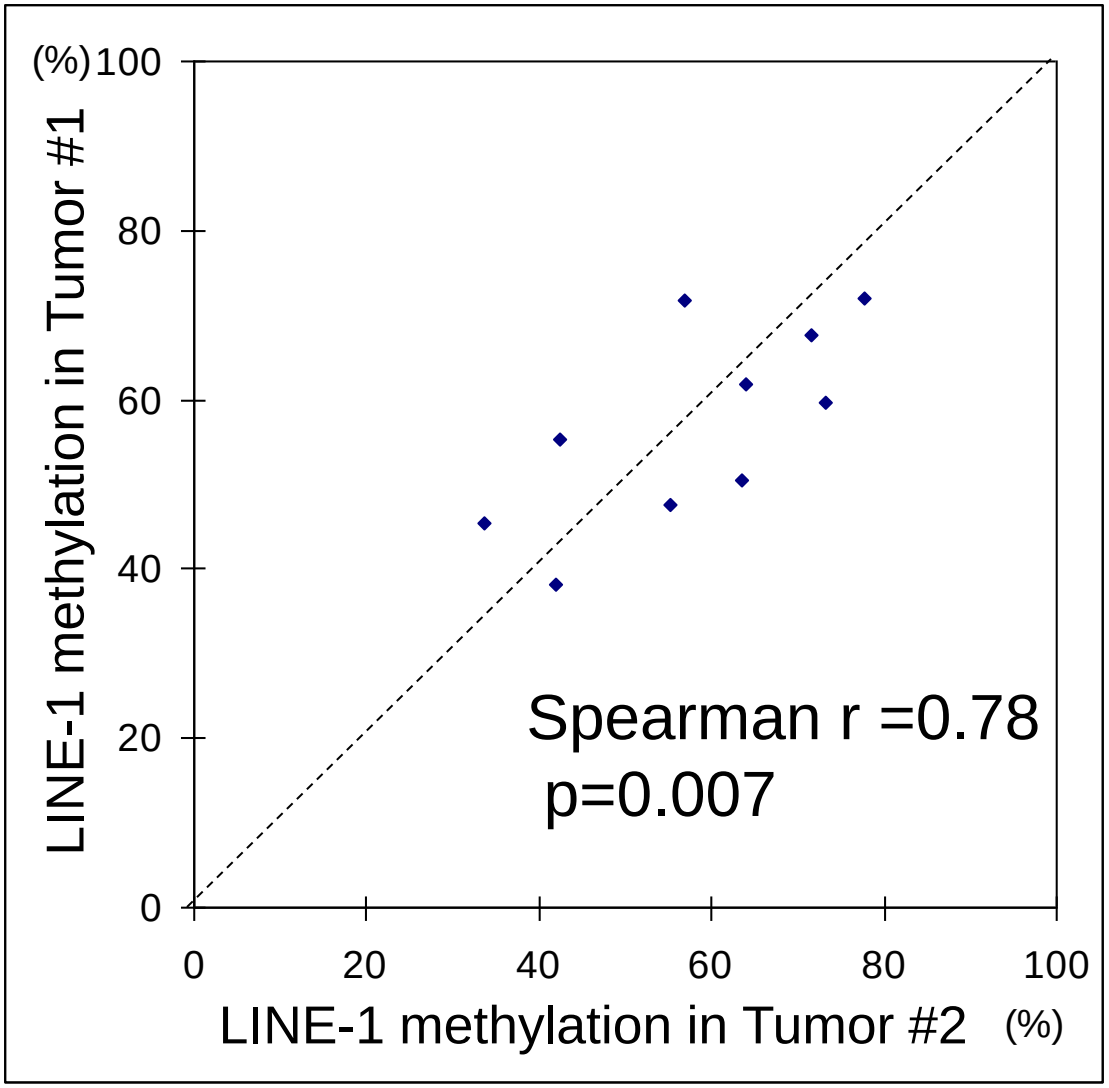
Is LINE-1 hypomethylation non-random or stochastic?

Synchronous colorectal cancers provide a unique model to examine carcinogenic process



Nosho et al. Gastroenterology 2009

LINE-1 methylation levels in synchronous cancer pairs

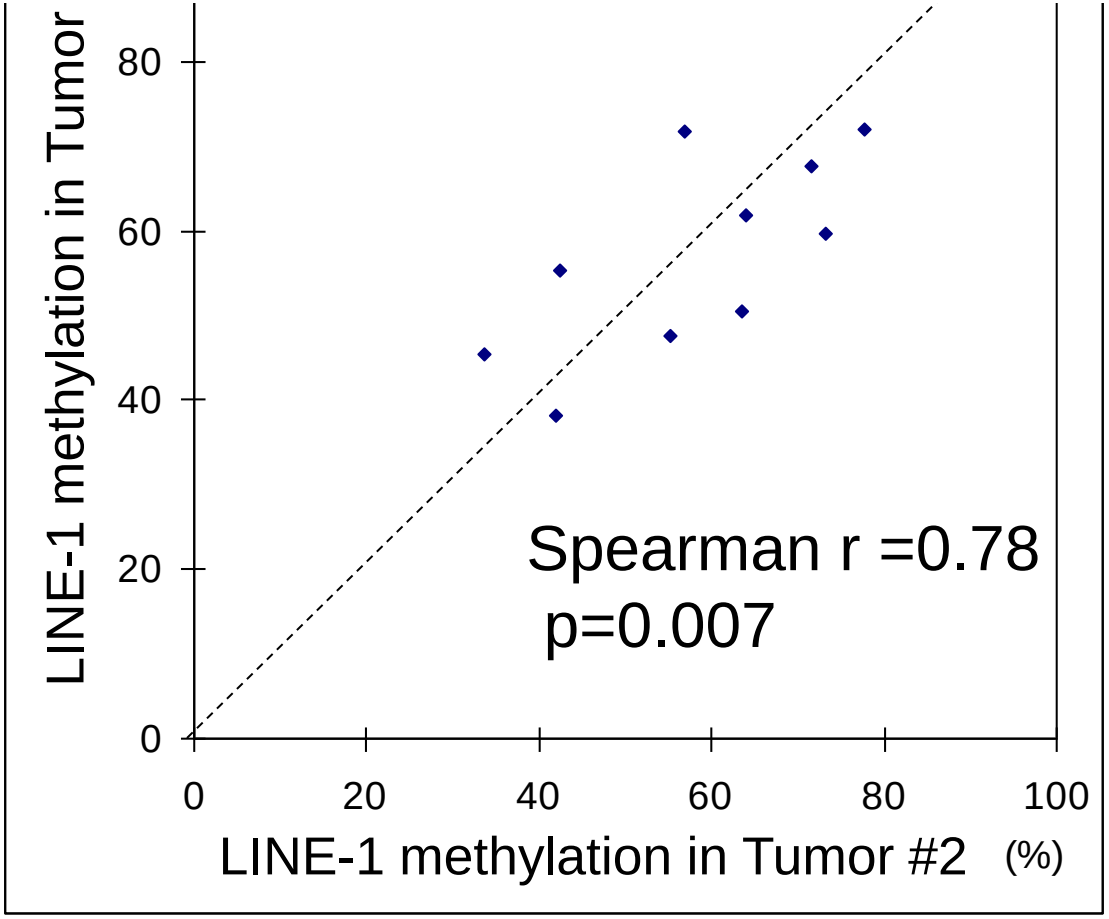


Nosho et al. Gastroenterology 2009

LINE-1 hypoM is not stochastic

Field effect vs.

Common genetic/environmental etiologies



Nosho et al. Gastroenterology 2009

What causes LINE-1 hypomethylation?

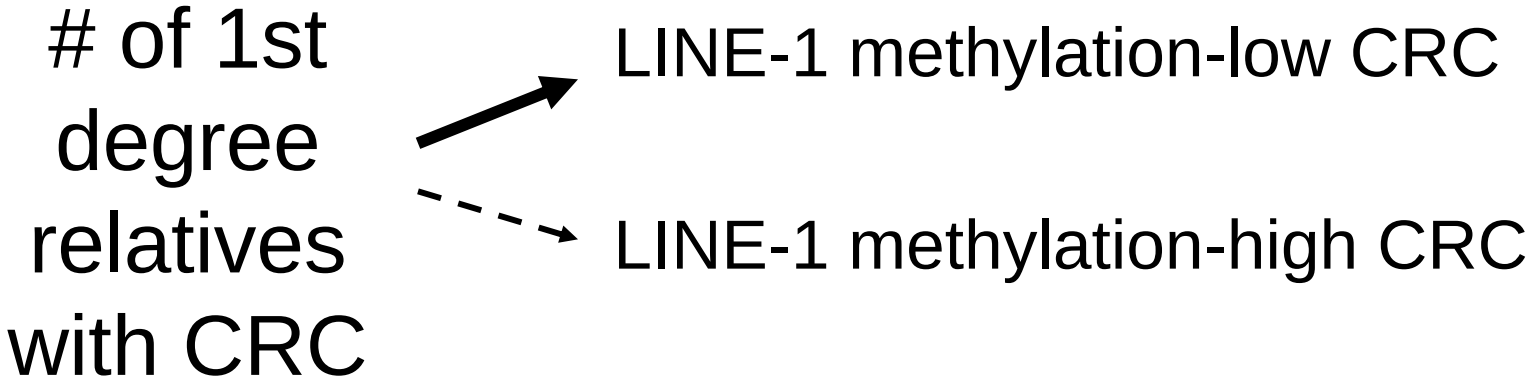
Genetics?

Familial history of colorectal cancer (CRC)

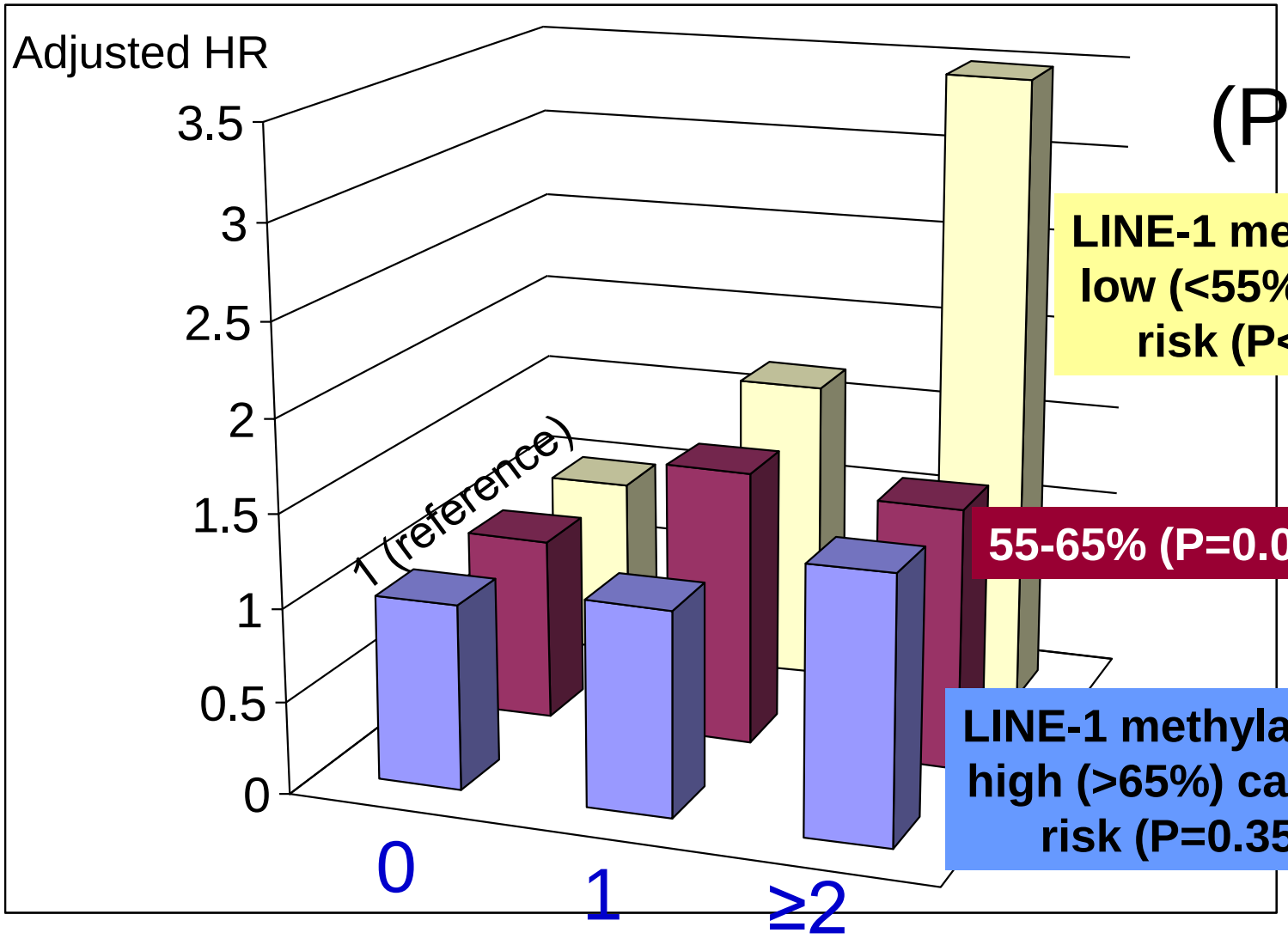
- Lynch syndrome - microsatellite instability (MSI)-high CRC

- Some MSS (microsatellite stable) CRC in familial cases showed LINE-1 hypomethylation
  - *Goel et al. Gastroenterology 2010*

Family history of colorectal cancer (CRC)



Family history of CRC → Risk of MSS cancer (by LINE-1 methylation level)



(P_{trend})

LINE-1 methylation low (<55%) cancer risk (P<0.001)

55-65% (P=0.002)

LINE-1 methylation high (>65%) cancer risk (P=0.35)

$P_{heterogeneity} = 0.02$

LINE-1 hypomethylation

- LINE-1 methylation test can be a routine test for all CRC (just as MSI testing)
  - Poor prognosis
  - Familial cancer risk

CpG Island Methylator Phenotype (CIMP)

- Epigenomic phenomenon
- Widespread CpG island methylation
  - *Toyota et al. Proc Natl Acad Sci 1999*

CIMP -> *MLH1* promoter methylation -> microsatellite instability (MSI)

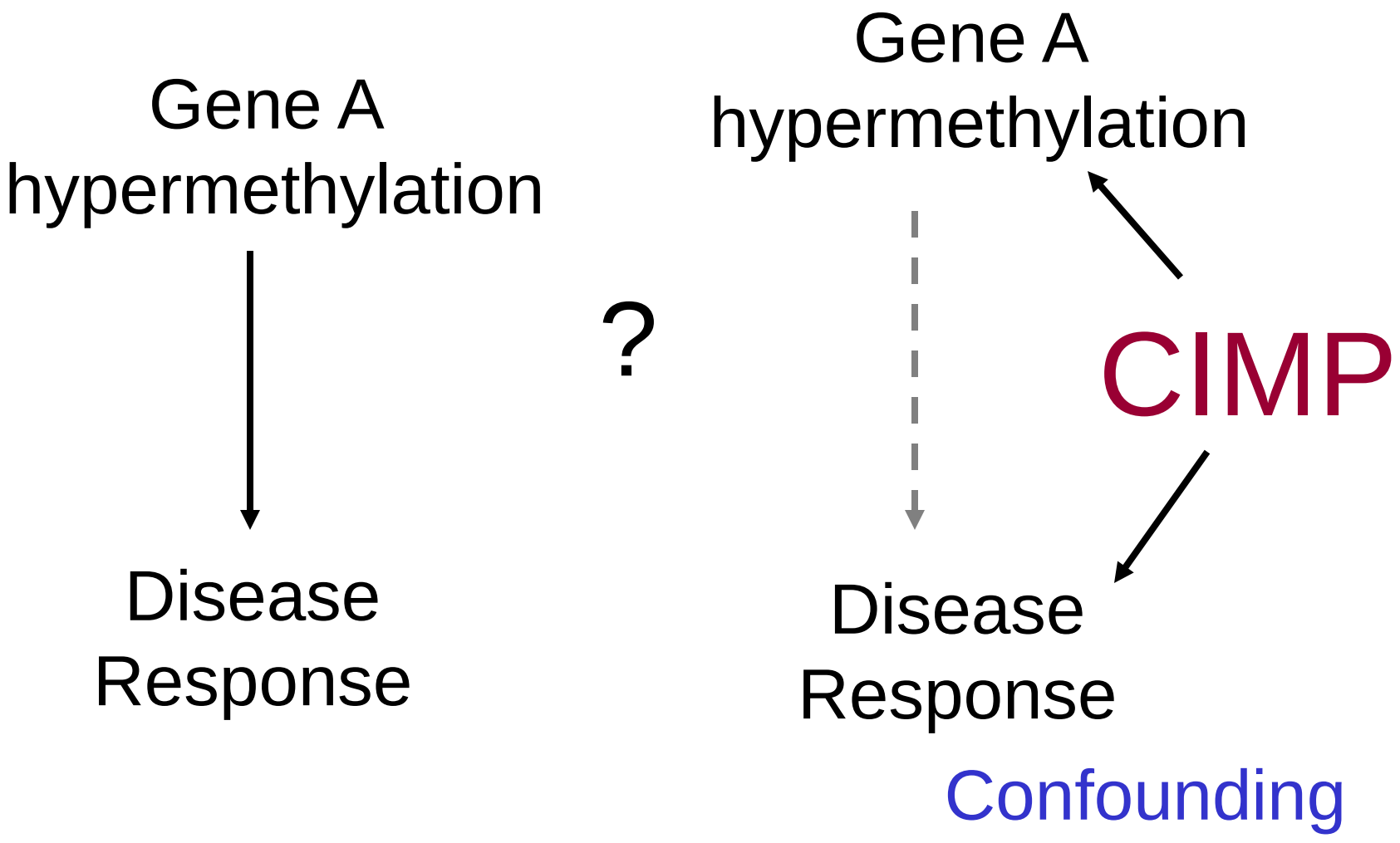
BRAF mutation

Why study global change?

Gene A
hypermethylation

Disease
Response

Why study global change?



CIMP - conductor
Locus-specific changes - players

Colorectal Continuum Theory (2012)

Yamauchi, et al. Gut 2012

ORIGINAL ARTICLE

Assessment of colorectal cancer molecular features along bowel subsites challenges the conception of distinct dichotomy of proximal versus distal colorectum

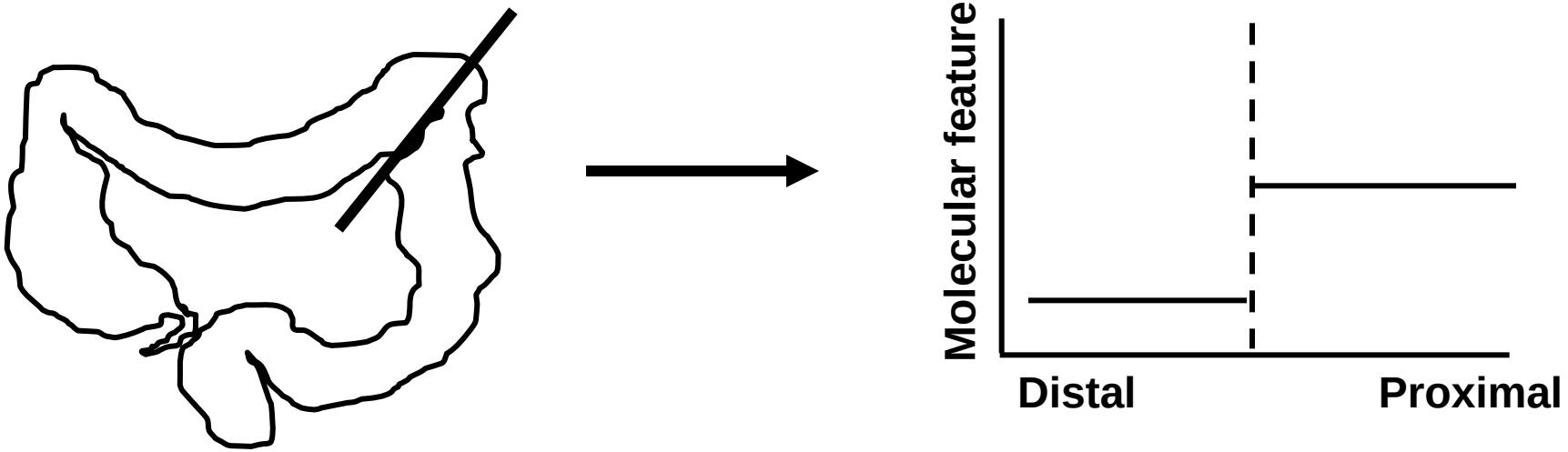
Mai Yamauchi,[1] Teppei Morikawa,[1] Aya Kuchiba,[1] Yu Imamura,[1] Zhi Rong Qian,[1] Reiko Nishihara,[1] Xiaoyun Liao,[1] Levi Waldron,[2,3] Yujin Hoshida,[4] Curtis Huttenhower,[2] Andrew T Chan,[5,6] Edward Giovannucci,[6,7,8] Charles Fuchs,[1,6] Shuji Ogino[1,9]

Colorectal cancer: a tale of two sides or a continuum?

Mai Yamauchi,[1] Paul Lochhead,[1] Teppei Morikawa,[1] Curtis Huttenhower,[2] Andrew T Chan,[3,4] Edward Giovannucci,[4,5] Charles Fuchs,[1,4] Shuji Ogino[1,6]

Both Gut 2012

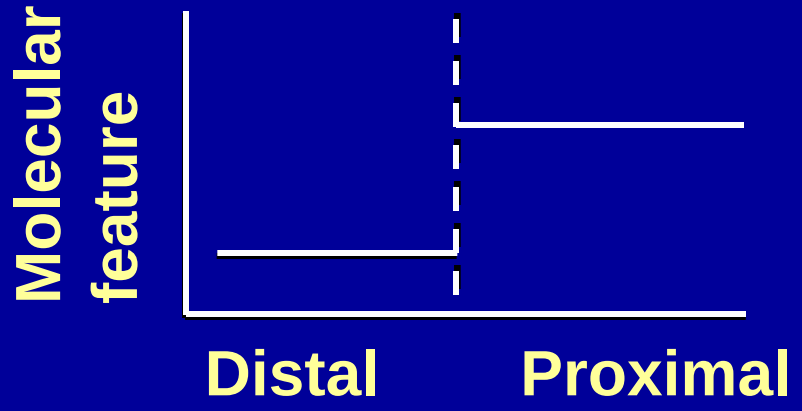
Two Colon Dogma



Two Colon Dogma

- Proximal colon cancers show higher frequencies of CIMP, MSI and *BRAF* mutation than distal cancers

- "Distinct genetics and epigenetics in proximal vs. distal cancers …"



Two Colon Dogma

- On Saturday at Nobel Museum, I found interesting words:

- Scientific truth undergoes 3 steps

Two Colon Dogma

- On Saturday at Nobel Museum, I found interesting words:

- Scientific truth undergoes 3 steps
- People say it conflicts with Bible

Two Colon Dogma

- On Saturday at Nobel Museum, I found interesting words:

- Scientific truth undergoes 3 steps
- People say it conflicts with Bible
- People say it was discovered by someone else

Two Colon Dogma

- On Saturday at Nobel Museum, I found interesting words:

- Scientific truth undergoes 3 steps
- People say it conflicts with Bible
- People say it was discovered by someone else
- People say we always believed it

Colon is a continuous tube

Gut contents probably change gradually (not abruptly)

Luminal contents (and host-tumor-microbe interaction) are important in carcinogenesis

Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma

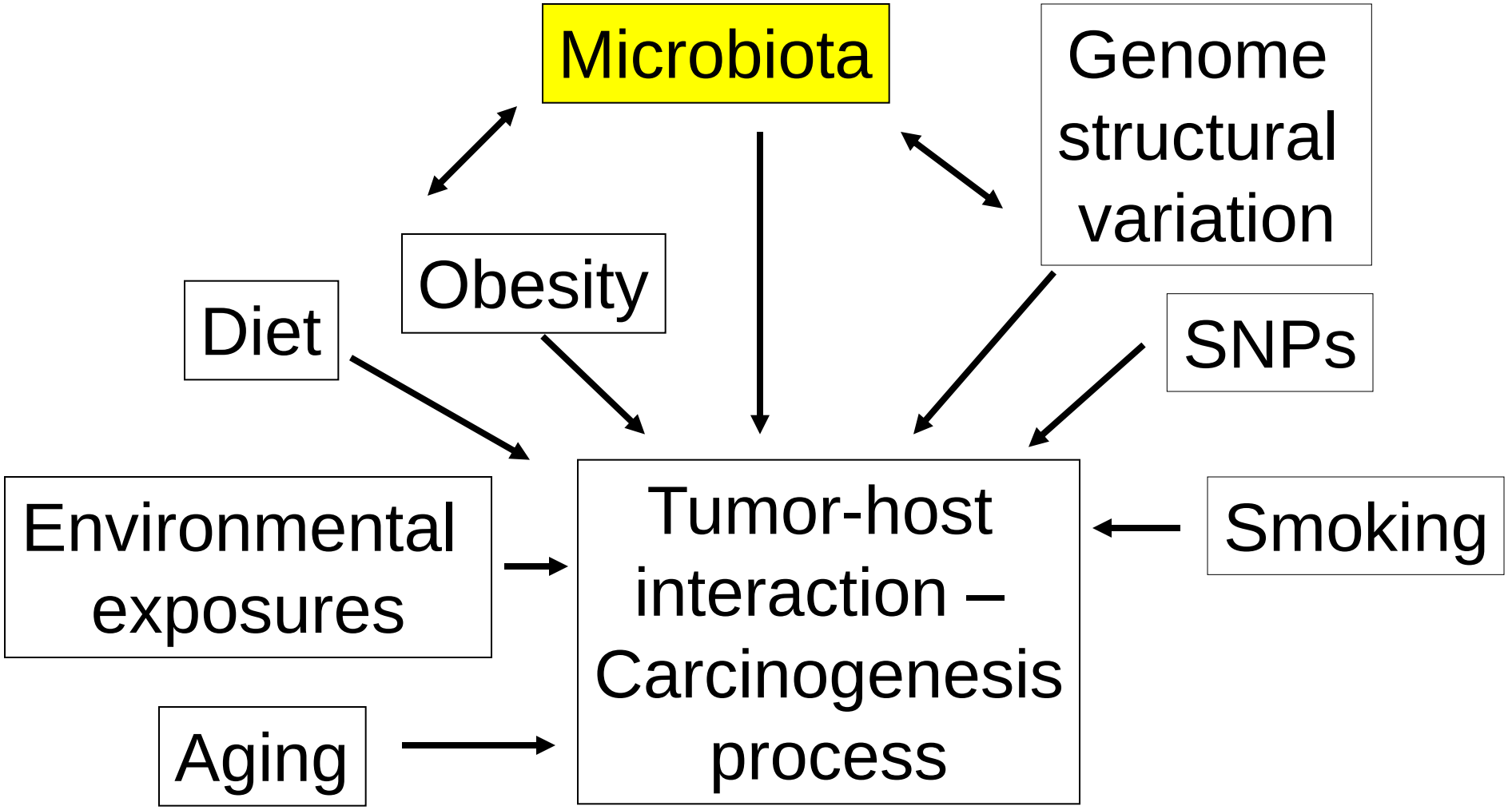
Aleksandar D. Kostic,[1,2] Dirk Gevers,[1] Chandra Sekhar Pedamallu,[1,3] Monia Michaud,[4] Fujiko Duke,[1,3] Ashlee M. Earl,[1] Akinyemi I. Ojesina,[1,3] Joonil Jung,[1] Adam J. Bass,[1,3] Josep Tabernero,[5] José Baselga,[5] Chen Liu,[6] Ramesh A. Shivdasani,[3] Shuji Ogino,[2,3,7] Bruce W. Birren,[1] Curtis Huttenhower,[1,8] Wendy S. Garrett,[1,3,4] and Matthew Meyerson[1,2,3,9]

Fusobacterium nucleatum infection is prevalent in human colorectal carcinoma

Mauro Castellarin,[1,2,6] René L. Warren,[1,6] J. Douglas Freeman,[1] Lisa Dreolini,[1] Martin Krzywinski,[1] Jaclyn Strauss,[3] Rebecca Barnes,[4] Peter Watson,[4] Emma Allen-Vercoe,[3] Richard A. Moore,[1,5] and Robert A. Holt[1,2,7]

Genome Res 2012

(Significant medical breakthrough.
Time's 2011 Top 10 Story)

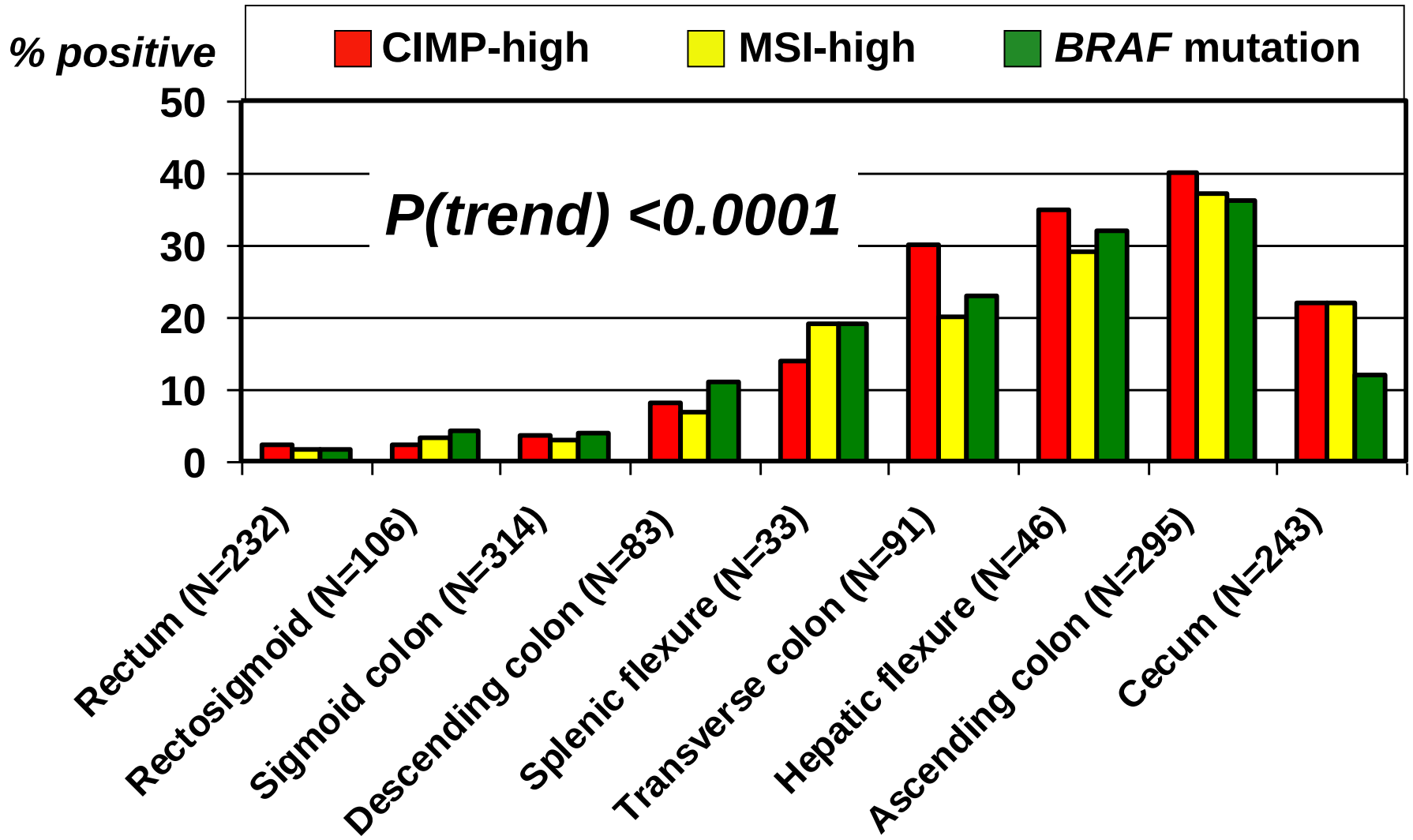

Microbiota
Genome structural variation
Obesity
Diet
SNPs
Environmental exposures
Tumor-host interaction – Carcinogenesis process
Smoking
Aging

Colon Continuum Hypothesis:

The frequency of CIMP increases continuously to proximal segments

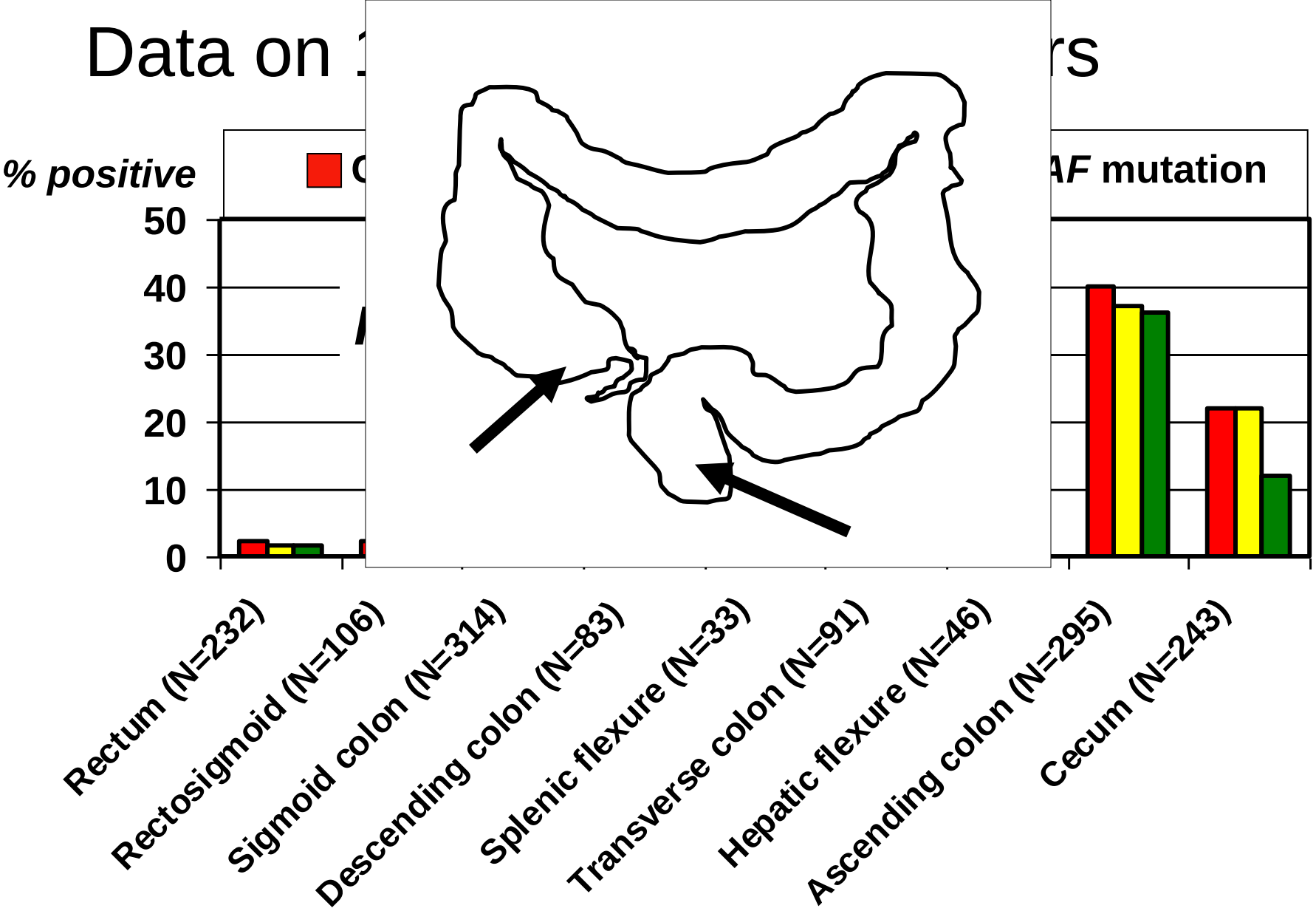
Yamauchi et al. Gut 2012

Data on 1443 colorectal cancers



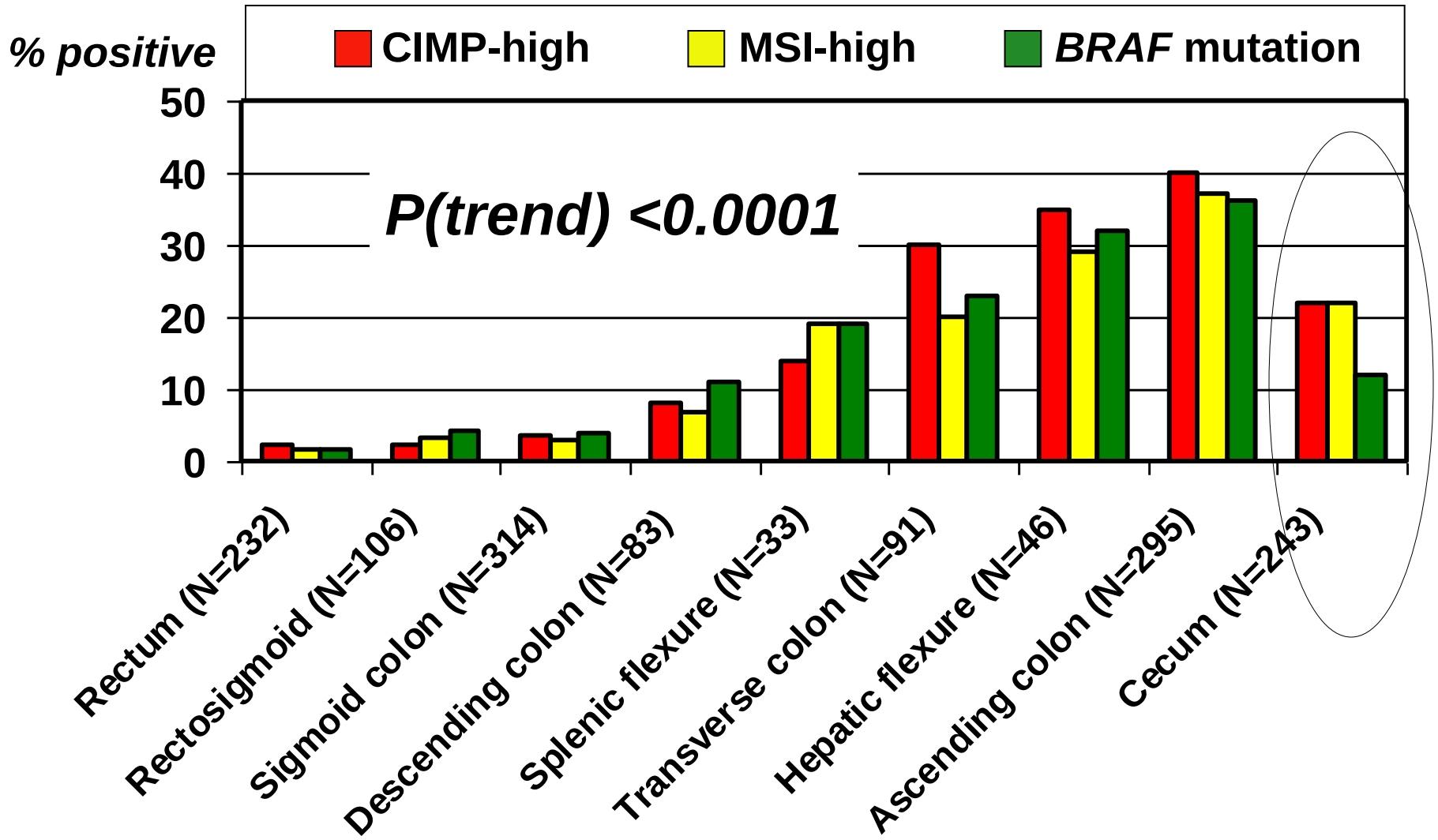
Yamauchi et al. Gut 2012

Data on 1 rs

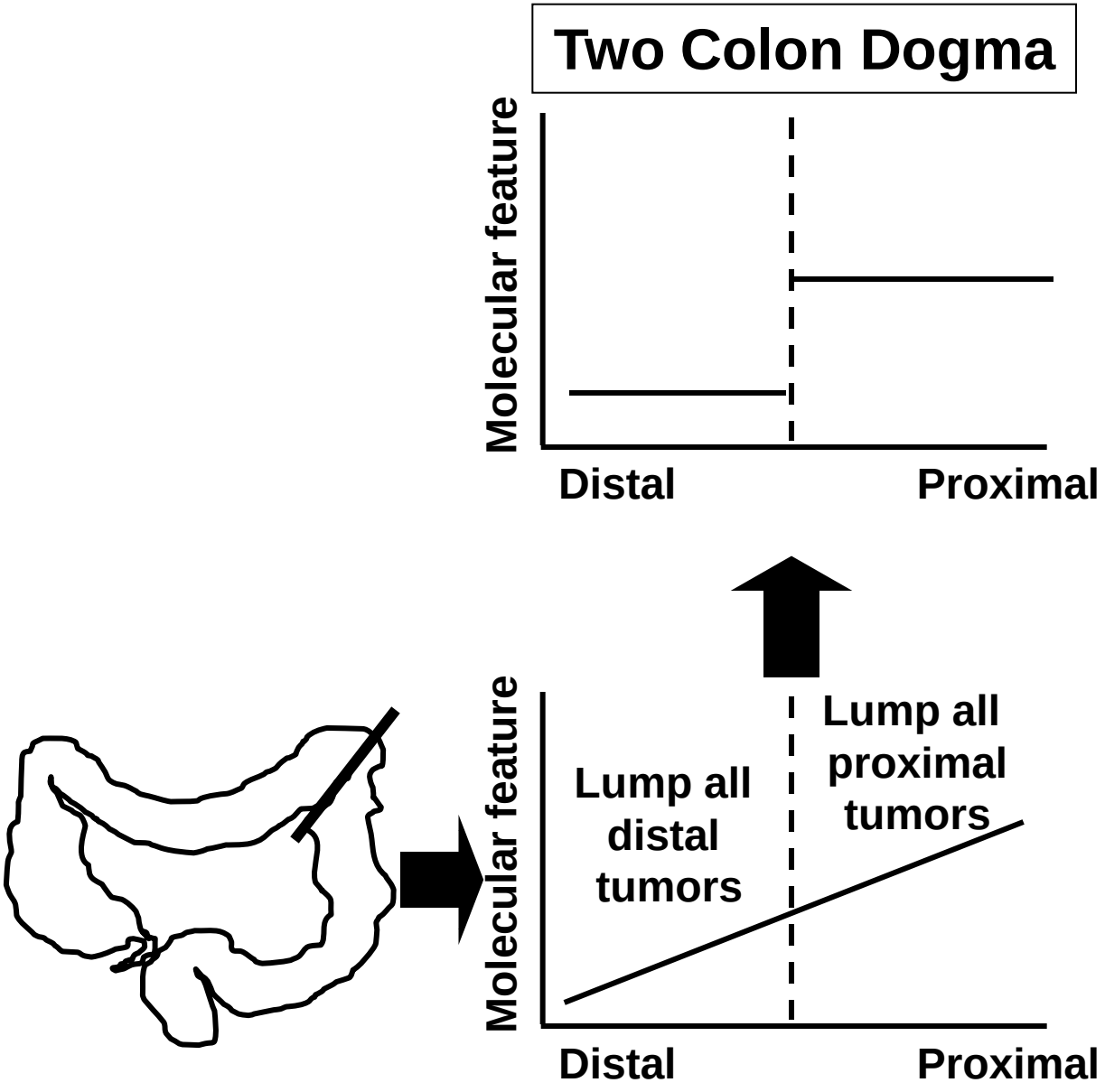


Yamauchi et al. Gut 2012

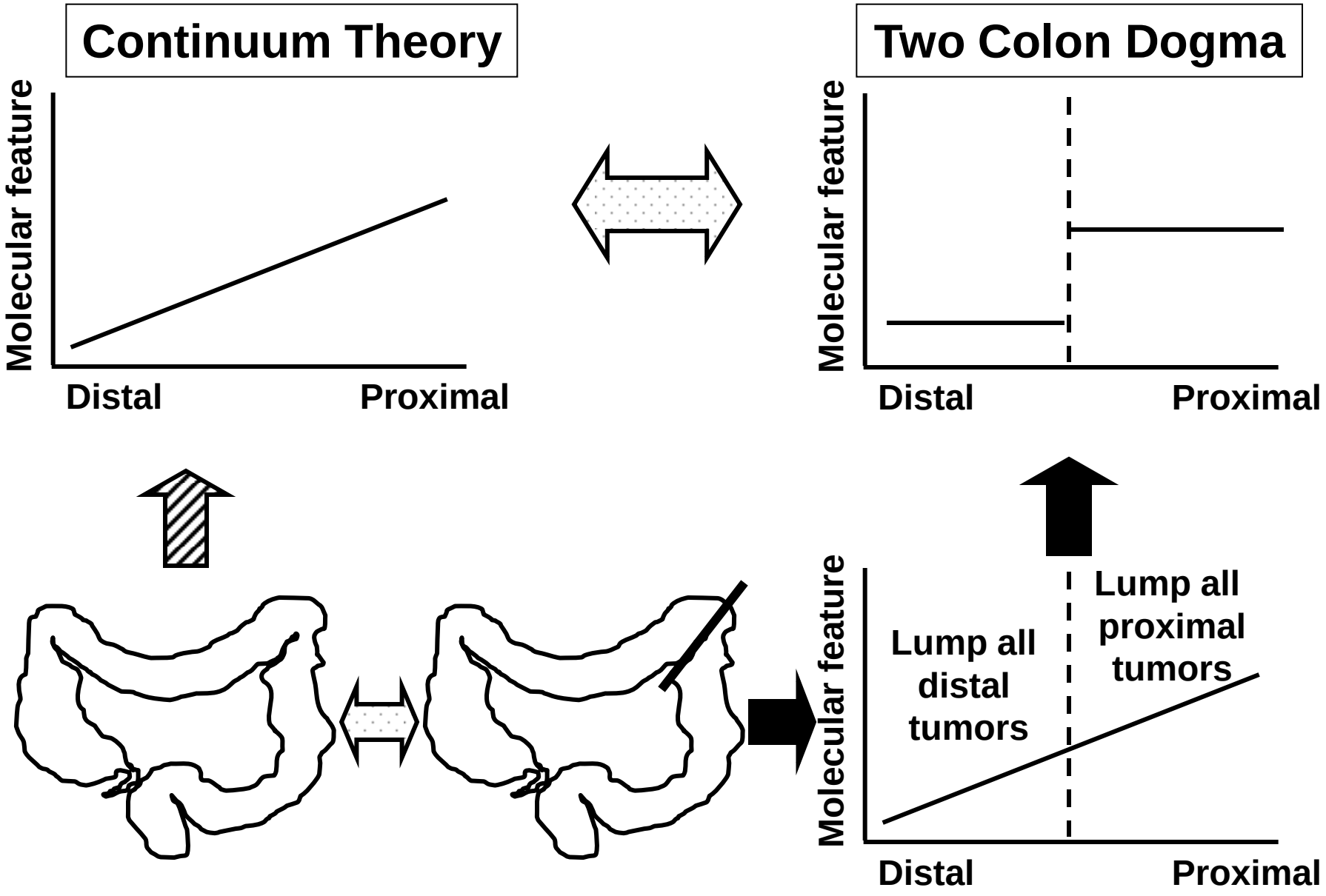
Data on 1443 colorectal cancers



Yamauchi et al. Gut 2012

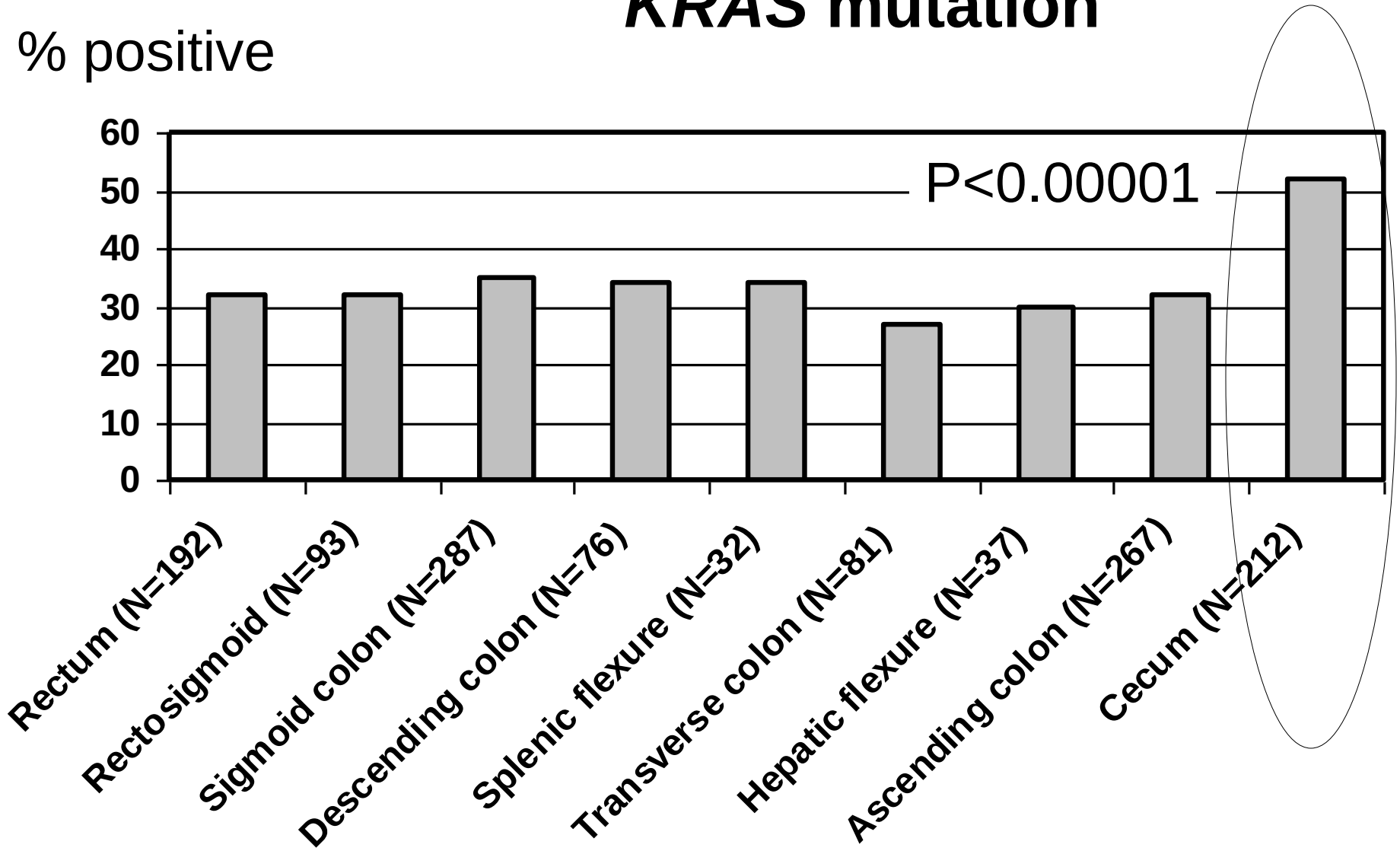


Yamauchi et al. Gut 2012



Yamauchi et al. Gut 2012

KRAS mutation



Yamauchi et al. Gut 2012

Implications of colorectal continuum

- Doubt dogmas!
- Gut microbiota and microenvironment
- We need a big sample size to examine gut biogeography
  - More collaborations are needed!

- For other diseases, consider biogeography
  - Lung cancer, GYN cancer, skin cancer, etc.

Summary 1

- Each individual has unique disease process
- Molecular disease classification is essential

Summary 2

- “Molecular Pathological Epidemiology (MPE)”
  - High dimensional data
  - MPE will become a mainstream of epidemiology
- MPE can decipher diseases at both the molecular and population levels
- Validated molecular markers can be used for personalized medicine

Summary 3

- Pathologists have increasing roles in broader areas of science
- Pathologists and epidemiologists can teach each other
- Pathologists should learn epidemiology
  - Proper study design
  - Proper statistical analysis and inference (what is a P value?)

Novel Integrative Science of Molecular Pathological Epidemiology (MPE) of Cancer

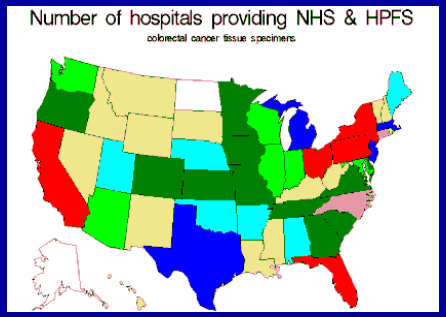
Register now for the live webinar
on November 13, 2012
at 10:00 a.m. EST (USA)
/ 4:00 p.m. CET

Sponsored by QIAGEN

I will be around
Please come to me
Let’s discuss!

Acknowledgements #2

- NHS (n=121,700) and HPFS (n=51,500) prospective cohort participants
- Various US hospitals/pathology departments for proving medical records and tissue specimens



Funding

- NIH/NCI
- Bennett Family Fund
- Entertainment Industry Foundation National Colorectal Cancer Research Alliance (NCCRA)
- Frank Knox Memorial Foundation
- Japan Society for Promotion of Science
- Japanese Society for Multidisciplinary Treatment of Cancer
- Uehara Memorial Foundation
- DFCI Friends

- The Ogino Lab
  - Yu Imamura
  - Aya Kuchiba
  - Xiaoyun Liao
  - Paul Lochhead
  - Reiko Nishihara
  - Zhi Rong Qian
  - Ruifang Sun
  - Mai Yamauchi
  - Akihiro Nishi
  - Many past lab members

Acknowledgements #3

- DFCI
  - Charles Fuchs
  - Jeffrey Meyerhardt
  - Levi Garraway
  - Glenn Dranoff
  - John Quackenbush
  - Giovanni Parmigiani
  - Ramesh Shivdasani
  - Kimmie Ng
  - Nadine Jackson McCleary
  - David Frank
  - Matthew Kulke
  - Matthew Freedman
  - Matthew Meyerson
  - William Hahn
  - Adam Bass
- Massachusetts General Hospital
  - Andrew Chan
  - Mari Mino-Kenudson
  - Umar Mahmood
- BWH Pathology
  - Jason Hornick
  - Amitabh Srivastava
  - Danny Milner
  - Emily King
- The Broad Institute
  - Todd Golub
  - Yujin Hoshida
  - Alexander Meissner
- Harvard School of Public Health/BWH Channing Lab
  - Edward Giovannucci
  - Frank Speizer
  - Walter Willett
  - Meir Stampfer
  - Donna Spiegelman
  - Molin Wang
  - Kana Wu
  - Cutis Huttenhower
  - Levi Waldron
  - Eva Schernhammer
  - Peter Kraft
  - Aditi Hazra
  - Xuehong Zhang
  - Cindy Wu Chen
  - Wendy Garrett
- BWH Preventive Medicine
  - Jennifer Lin
- Beth Israel Deaconess Medical Center
  - Andrew Beck
- UMASS
  - Michael Green
- Case Western Reserve University
  - Sanford Markowitz
- Queensland Institute for Medical Research
  - Barbara Leggett
  - Vicki Whitehall
- Sookmyung Women's University
  - Jung Eun Lee