National Cancer Institute

Finding genetic susceptibility to radiation effects in humans—Will GWA (or NGS) studies help?

Alice Sigurdson, Ph.D.

Radiation Epidemiology Branch

Division of Cancer Epidemiology and Genetics

sigurdsa@mail.nih.gov

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

National Institutes of Health

Outline

- There are radiation sensitive (and resistant) individuals
  - Who and where are they?
- Concept of interaction to detect genetic susceptibility
- Genome-wide association (GWA) studies have uncovered complex disease genetic variants
  - Most with unknown function in non-coding regions
- Overview of Next Generation Sequencing (NGS) studies
  - Tumours, germline, compare tumour with normal tissue
  - Enrich case sets with radiation-related tumours
- Analytic issues
  - Data avalanche; need “pattern recognition” for grouping
- Future is sequencing

What do we think we know?

- Inherently radiation-sensitive (and resistant) individuals exist
  - For eg. Defects in DNA double strand break repair
    - AT, NBS, Riddle, Ligase IV, XLF, DNA-PKcs deficiency
      - Many reviews; see Jeggo P, Radiation Research, 2011
  - Relatives of RB and AT patients
    - Clinically normal but show increased radiosensitivity by phenotypic assay
      - Review in Kato TA et al, 2009 Health Physics
- Increasing cancer genetic information available
  - Some GWA study results in radiation-related cancers
    - More to come from WECARE and CCSS
  - GWA study results for “radiation–sensitive” to radiotherapy from the Radiogenomics Consortium to come

What is radiation "sensitive" in humans?

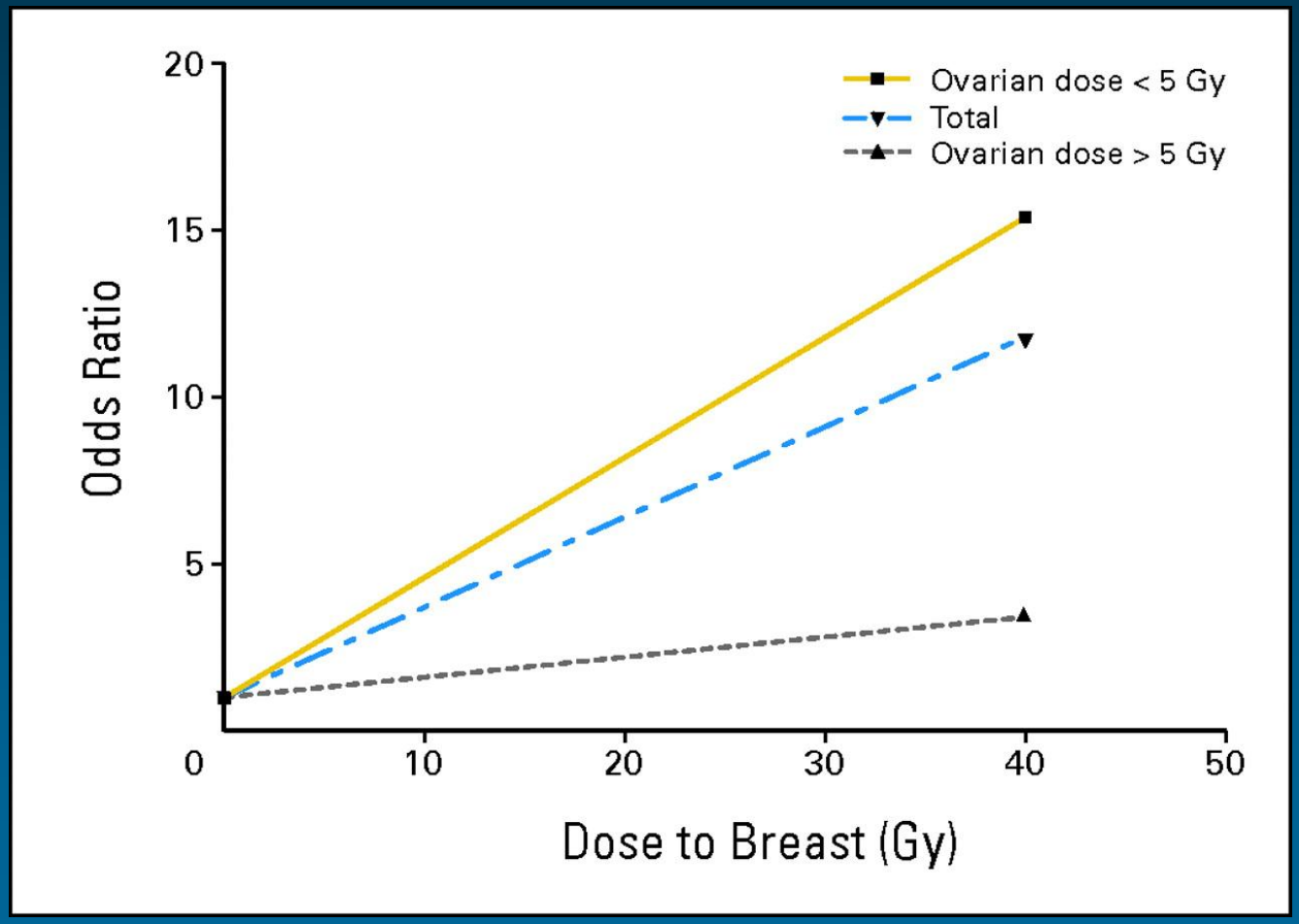
- Increase over "baseline" in a cancer risk biomarker assay
  - Number of chromosome aberrations, chromatid breaks or micronucleii
    - With or without a radiation "challenge"
- How common is radiation sensitivity (resistance)?
  - May depend on cut-off used
  - Mild hypersensitivity up to 30%? **(Kato, Rad Res 2009)**
- Does radiation sensitivity "predict" increased radiation-associated cancer risk?
  - Unknown; may be associated with cancer risk
    - "Radiation sensitivity" may be non-specific (increased cancer risk in absence of radiation exposure)
  - At present such tests lack good predictive abilities
- Need other ways to find susceptible individuals

General Concept

Genetic susceptibility implies detecting statistical evidence of interaction or effect modification of risk over strata of another factor.

This means the radiation dose response differs (higher or lower) depending on the genetic background.

Radiation-gene interaction (CCSS breast cancer example)



From Inskip et al. Radiation Dose and Breast Cancer Risk in the Childhood Cancer Survivor Study J Clin Oncol 27:24:3901-7, 2009

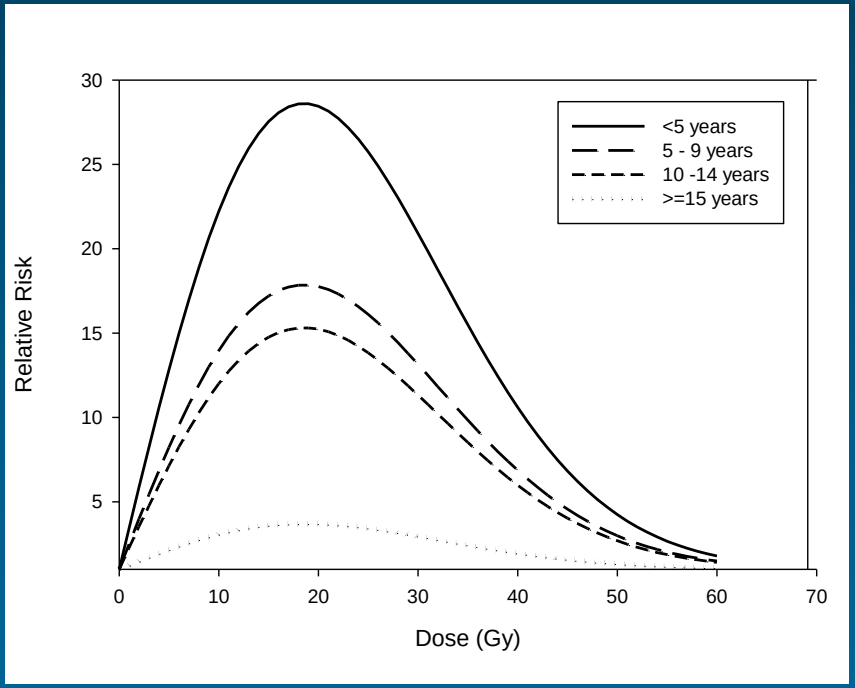
a)

b)

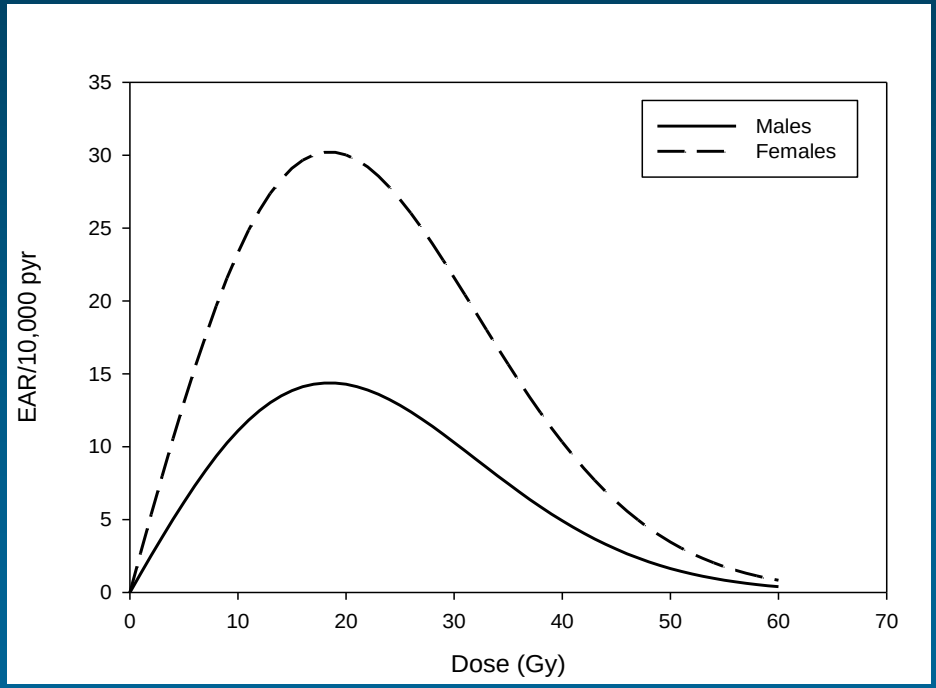
Second thyroid cancer: Cohort analysis in CCSS

Effect modification of fitted relative risk and excess absolute risk dose-responses:

Relative risk by age at radiation exposure



Excess absolute risk by sex

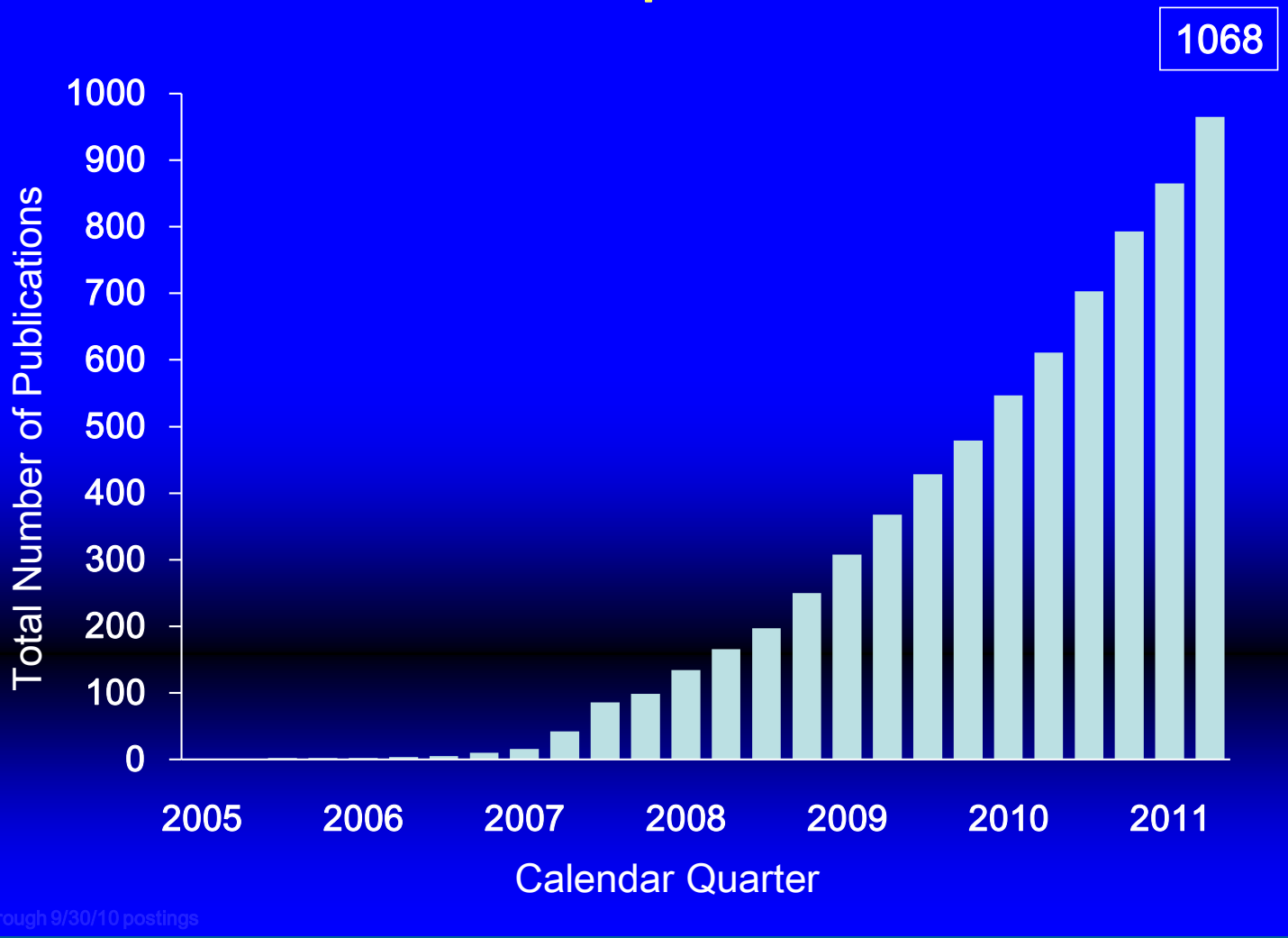


119 cases among 12,547 5-yr survivors--Bhatti et al, Radiation Research 2010 174(6): 741–752.

How might genome-wide association studies “fit” to find “susceptible” persons?

There have been a lot of them…

Published GWA Reports, 2005 – 9/2011



(Likely to be supplanted with next-generation sequencing studies)

Information from GWA studies

Published Genome-Wide Associations through 09/2011, 1,449 published GWA at p≤5x10-8 for 237 traits NHGRI GWA Catalog www.genome.gov/GWAStudies

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Diseases evaluated with GWA studies

Published Genome-Wide Associations through 09/2011, 1,449 published GWA at p≤5x10-8 for 237 traits NHGRI GWA Catalog www.genome.gov/GWAStudies

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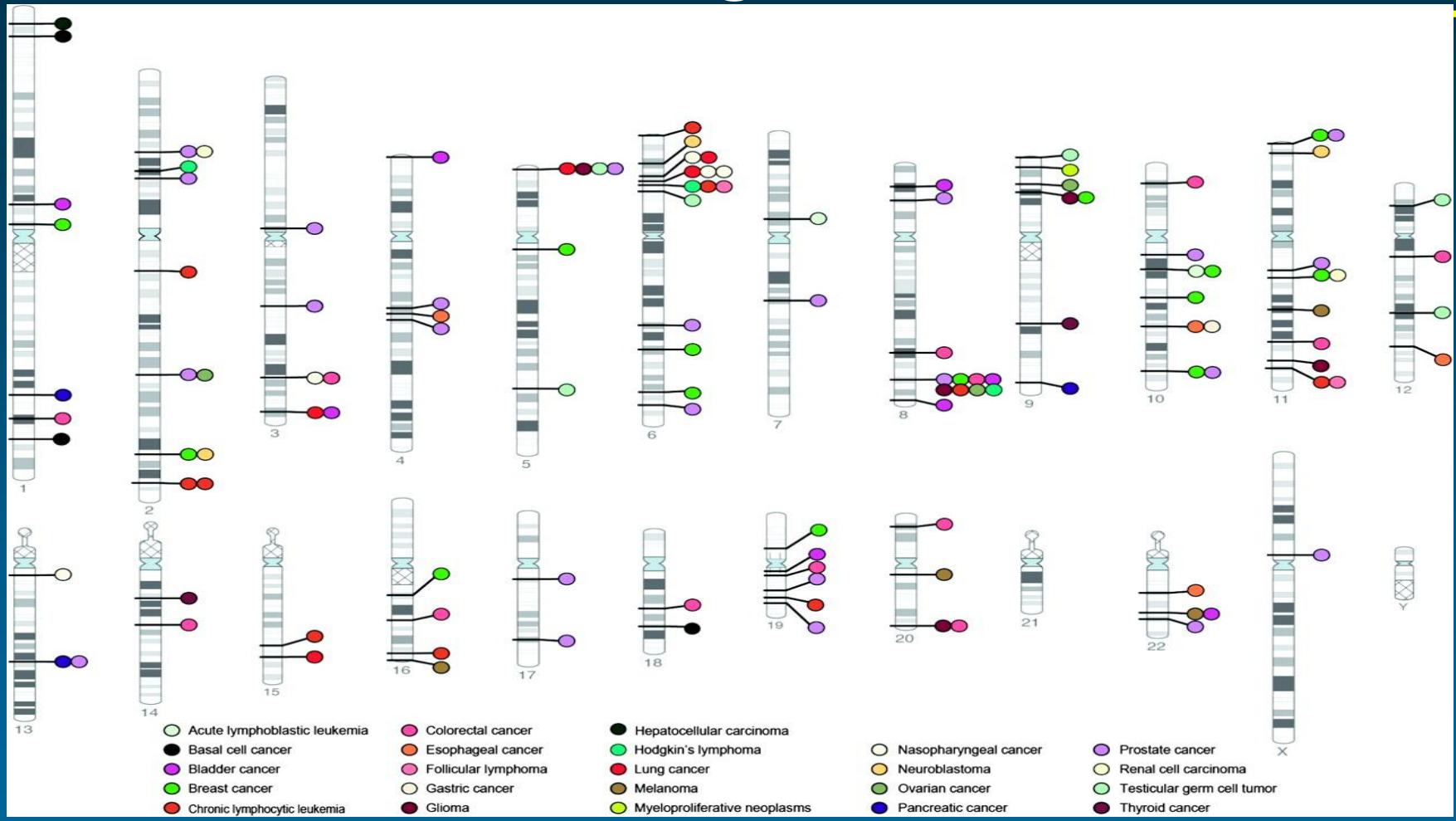
Cancer-associated genetic variants identified through GWA studies.



Genetic variants were identified from the NHGRI Genome-wide Association Study catalog (www.genome.gov/gwastudies) and include all cancer associations at $P < 5 \times 10^{-8}$ through 2010.

Hindorff L A et al. Carcinogenesis 2011;32:945-954

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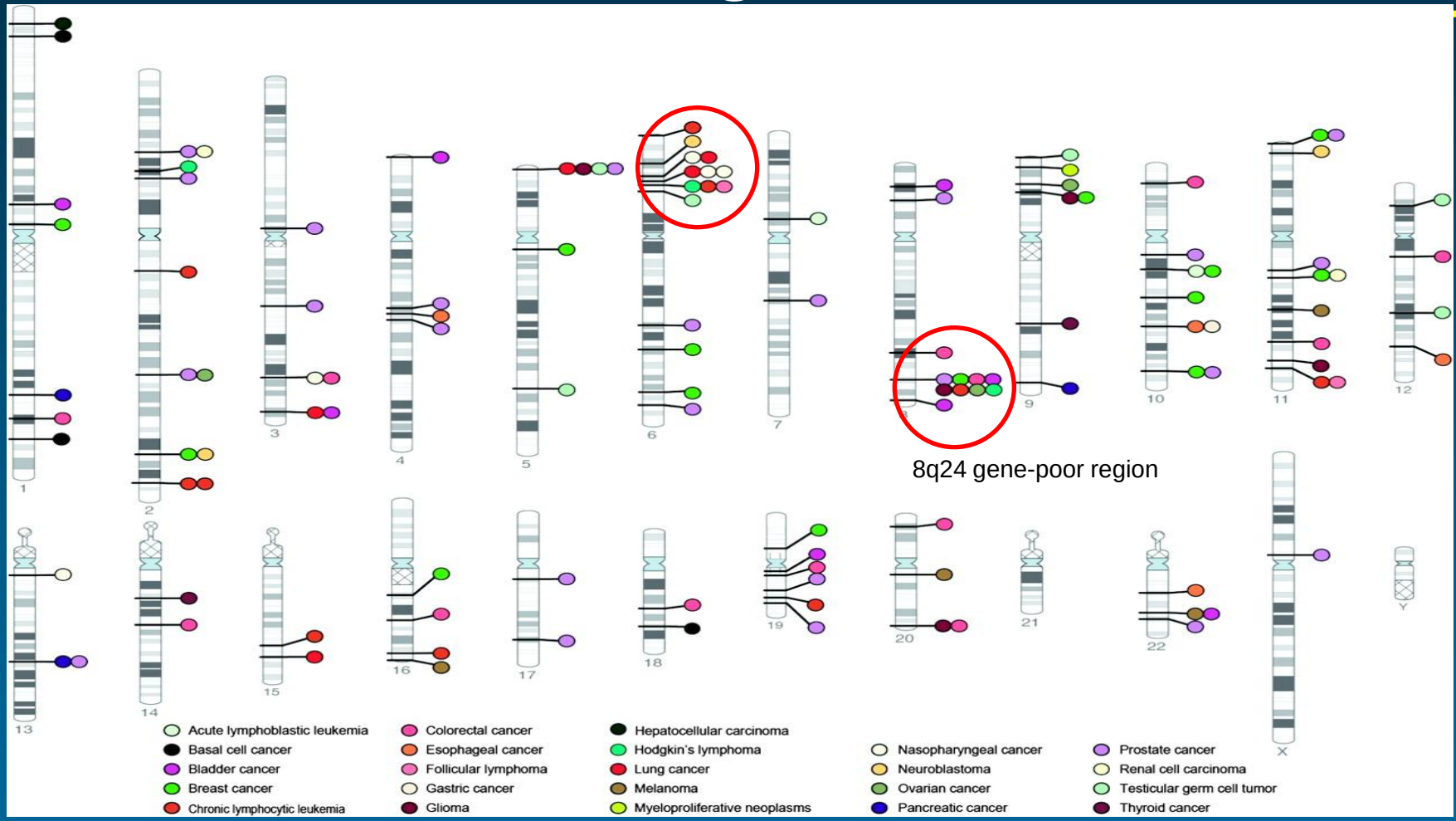

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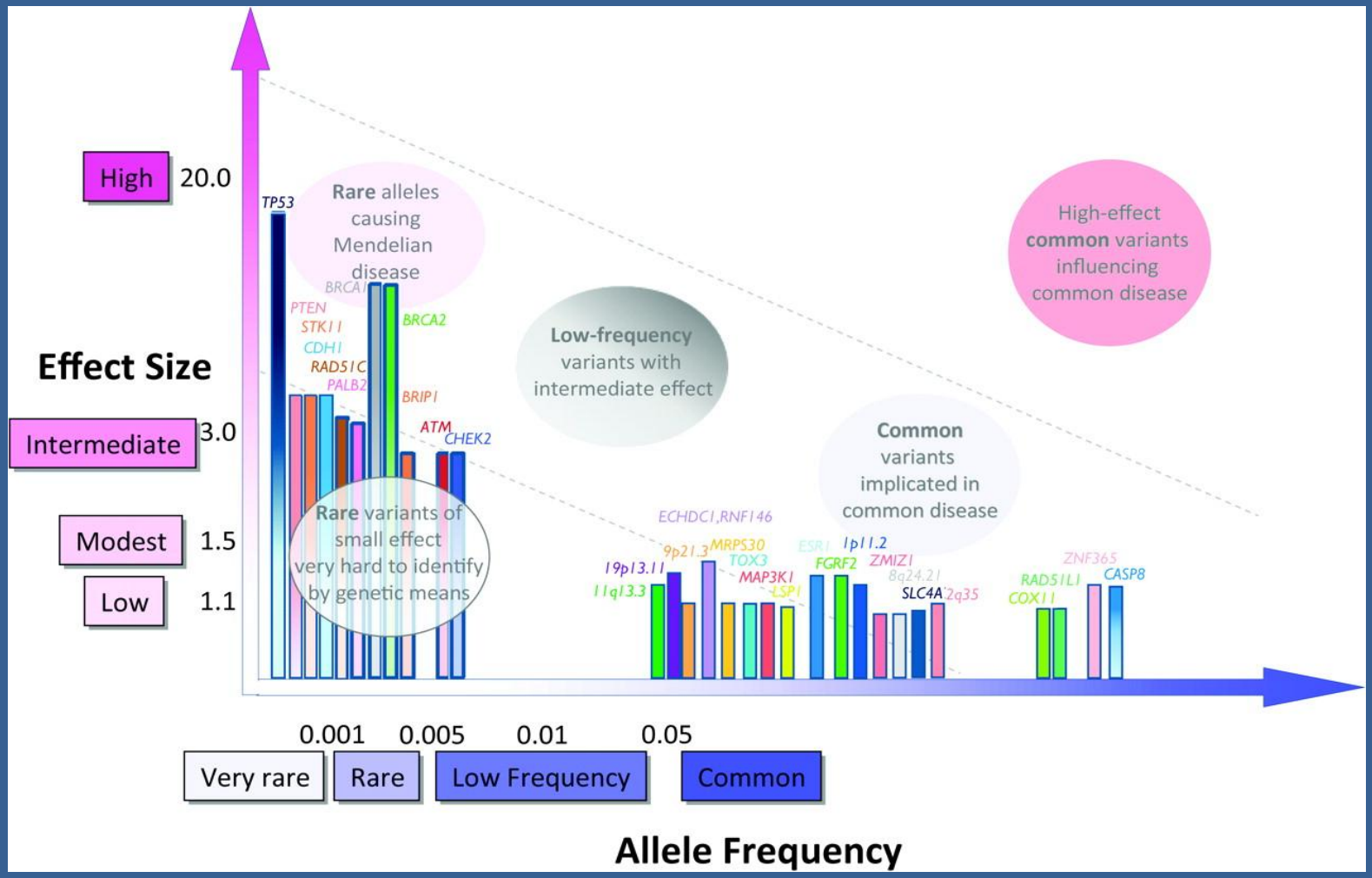
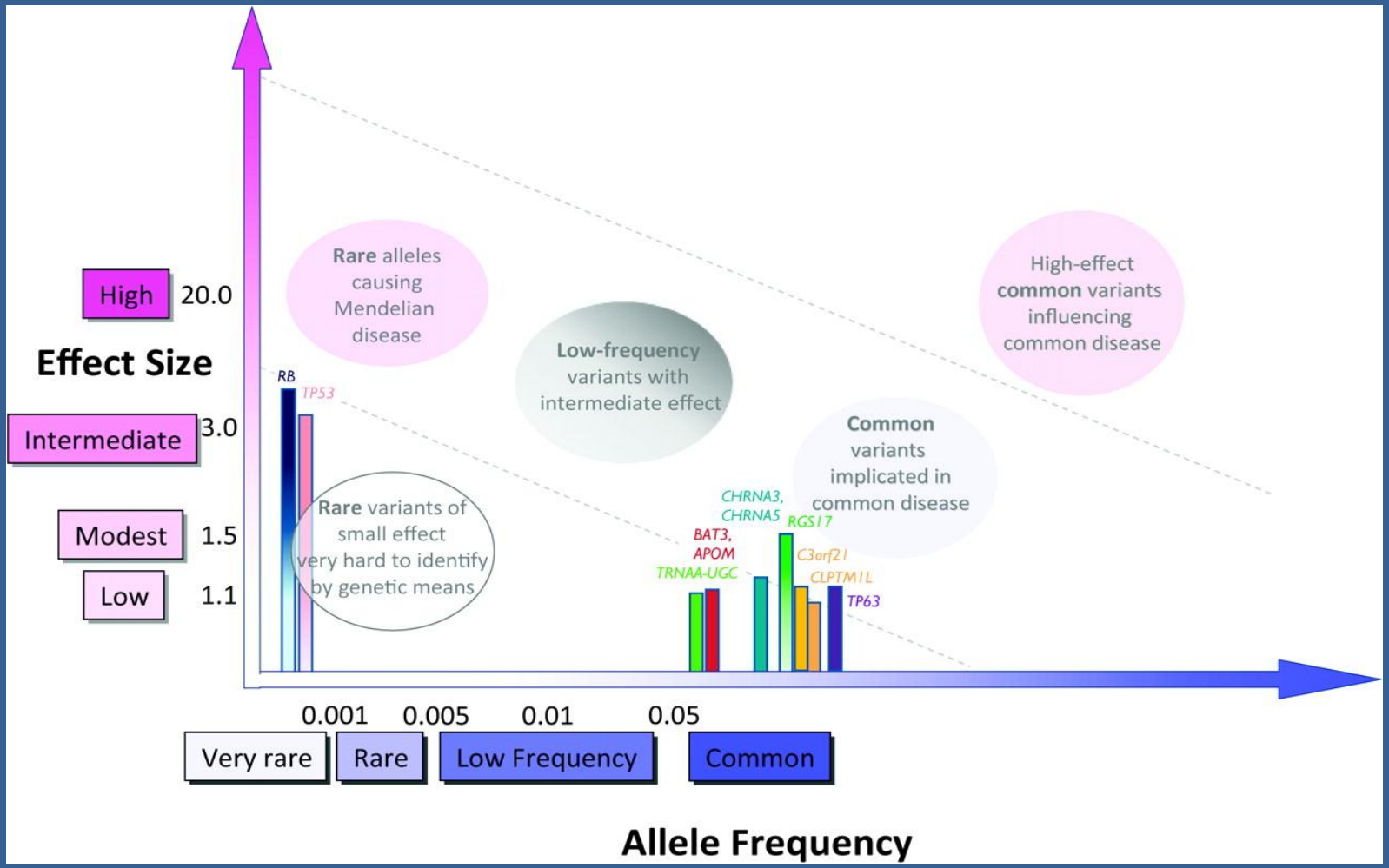
Allele frequency and effect sizes for genetic variants associated with breast cancer.



Associations identified through GWA or GWA follow-up studies are shown with solid colored bars; all others are shaded from dark (top) to light (bottom

Hindorff L A et al. Carcinogenesis 2011;32:945-954

Allele frequency and effect sizes for genetic variants associated with lung cancer.



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GWA studies of radiation-associated cancers

- **Rather limited**
  - *FOXE1,* **previously reported for sporadic thyroid cancer, also found in a GWA study of thyroid cancers from Chernobyl** **(Takahashi M et al, Hum Mol Genet 2010 (12):2516-23.)**
  - *PRDM1* reported among second cancers after XRT for Hodgkins Disease (Best et al,)

Characteristics of 465 unique trait related SNPs from GWA studies

- 43% located in intergenic regions
  - Such as 8q24, so called “gene desert”
- 45% intronic
- 9% nonsynonomous
- 2% in 5’ or 3’untranslated region
- 2% synonomous
- ORs (median) 1.3 (range) 1.04-29.4

Hindorf et al, PNAS, October, 2009

Encyclopedia of DNA elements (ENCODE) sheds light on non-coding genetic variation

"Genome-wide association studies have identified many noncoding variants associated with common diseases and traits. These variants are concentrated in regulatory DNA marked by deoxyribonuclease I (DNase I) hypersensitive sites (DHSs). Distant gene targets for hundreds of variant-containing DHSs may explain phenotype associations. Disease-associated variants systematically perturb transcription factor recognition sequences, frequently alter allelic chromatin states, and form regulatory networks."

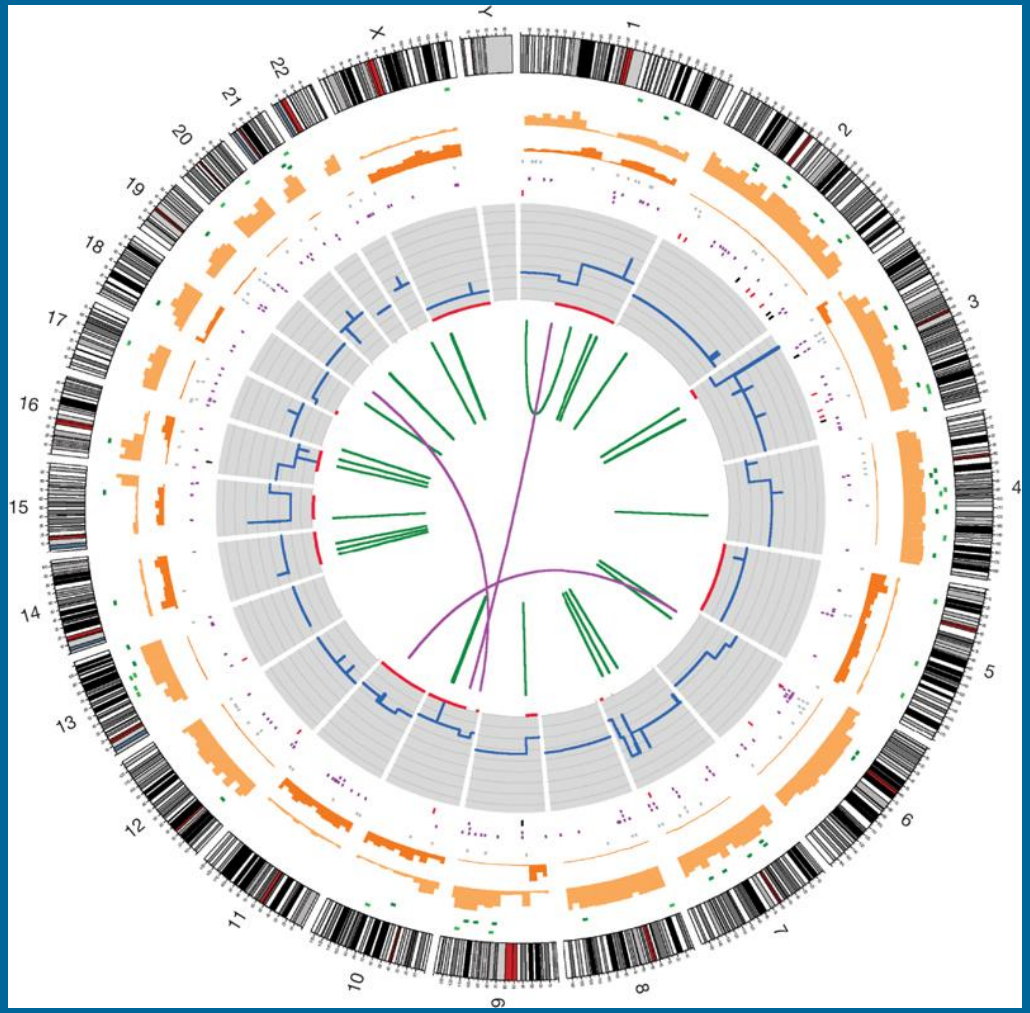
Systematic Localization of Common Disease-Associated Variation in Regulatory DNA. Maurano MT et al. Science, Sept 7, 2012.

Next generation sequencing (NGS) of cancer tumors

- Increasing
  - Analytic and data reduction hurdles lessened
- Costly ("nicknamed" the $1K genome, $100K analysis due to massive data storage)
  - Sequence many times over same area
    - 10 to 30 to 100 to 1000 times (depends)
- Wider genetic diversity than SNPS or CNVs
  - Example of "Circos Plots"
- Hints of exposure "signatures"
    - UV and cigarette smoking

Next generation sequencing of cancer tumors (2)

- Trace cancer history back to tumor genesis
  - Concepts from evolutionary biology
  - “Driver” mutations (few)
    - Provide some selective advantage
  - “Passenger” mutations (lots)
    - Acquired along the way
- Possible to use NGS on single cell nucleii
- May be possible to use FFPE tumours



The catalogue of somatic mutations in COLO-829.
(Immortal pre-treatment cancer cell line from a melanoma metastasis)

Most substitutions were C>T/G>A transitions and CC>TT/GG>AA, suggesting UV exposure

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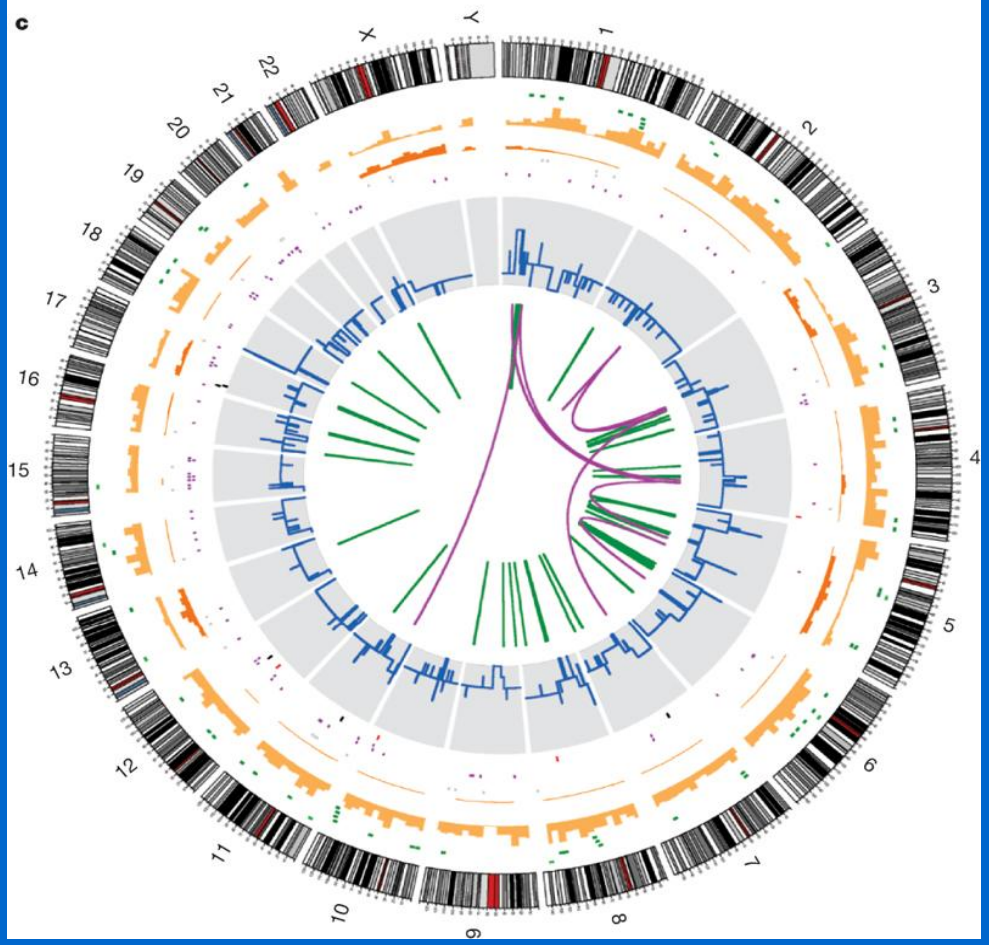
A comprehensive catalogue of somatic mutations from a human cancer genome
Pleasance ED et al.
Nature Jan 2010

Chromosome ideograms are shown around the outer ring and are oriented pter–qter in a clockwise direction with centromeres indicated in red. Other tracks contain somatic alterations (from outside to inside): validated insertions (light-green rectangles); validated deletions (dark-green rectangles); heterozygous (light-orange bars) and homozygous (dark-orange bars) substitutions shown by density per 10 megabases; coding substitutions (coloured squares: silent in grey, missense in purple, nonsense in red and splice site in black); copy number (blue lines); regions of LOH (red lines); validated intrachromosomal rearrangements (green lines); validated interchromosomal rearrangements (purple lines).

A small-cell lung cancer genome with complex signatures of tobacco exposure

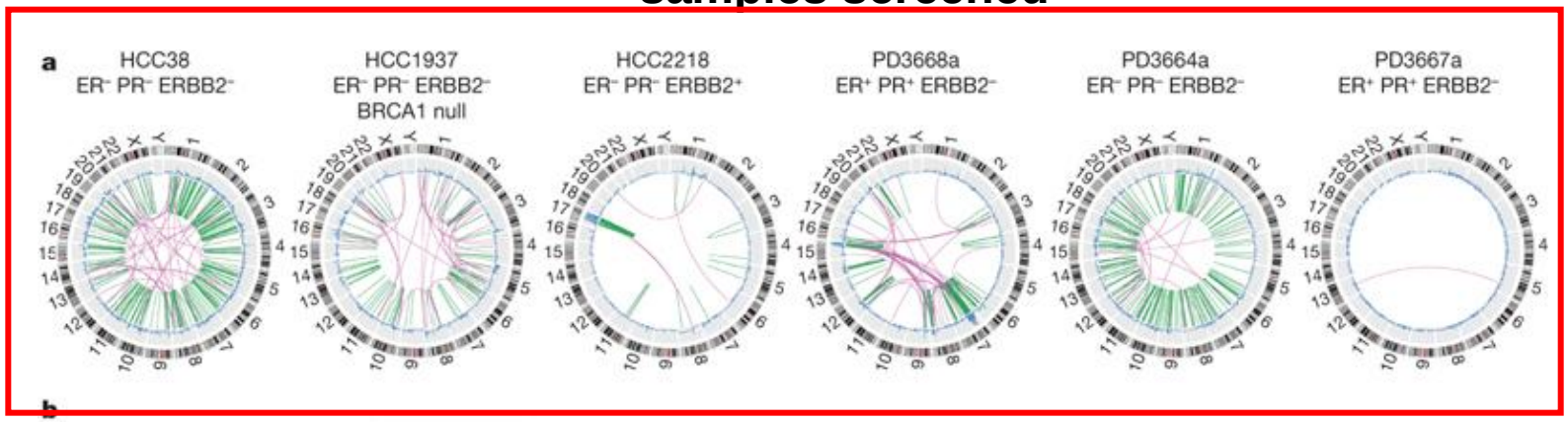
(G>T/C>A transversions and others related to *TP53* mutation spectra and smoking seen previously in SCLC)



Figurative representation of the catalogue of somatic mutations in the genome of NCI-H209. Chromosome ideograms are shown around the outer ring and are oriented pter–qter in a clockwise direction with centromeres indicated in red. Other tracks contain somatic alterations (from outside to inside): validated insertions (light-green rectangles); validated deletions (dark-green rectangles); heterozygous (light-orange bars) and homozygous (dark-orange bars) substitutions shown by density per 10 megabases; coding substitutions (coloured squares; silent in grey, mis-sense in purple, nonsense in red and splice site in black); copy number (blue lines); validated intrachromosomal rearrangements (green lines); and validated interchromosomal rearrangements (purple lines).

Pleasance ED et al.
Nature 463, 184-190(14 January 2010)
doi:10.1038/nature08629

Somatic rearrangements observed in six of twenty-four breast cancer samples screened

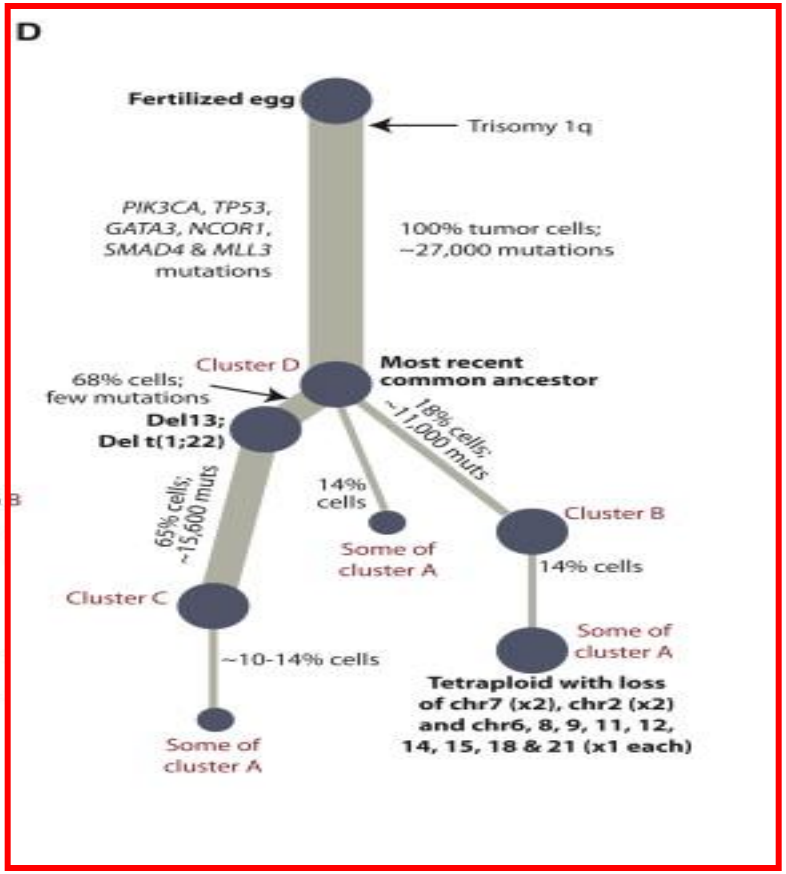


PJ Stephens *et al. Nature* **462**, 1005-1010 (2009)

Genome-wide Circos plots of somatic rearrangements. An ideogram of a normal karyotype is shown in the outer ring. A copy number plot is represented by the blue line shown inner to the chromosome ideogram. Within the inner ring each green line denotes an intrachromosomal rearrangement and each purple line an interchromosomal rearrangement. ER, oestrogen receptor; PR, progesterone receptor. **b**, The prevalence of rearrangement architectures in individual cancers: deletion (dark blue), tandem duplication (red), inverted orientation (green), interchromosomal rearrangements (light blue), rearrangements within amplified regions (orange). **c**, Extent of overlapping microhomology at rearrangement breakpoints. The number of base pairs of microhomology is plotted on the horizontal axis.

Reconstructing the Evolution of breast tumor PD4120a

Serena Nik-Zainal, Peter Van Loo, David C. Wedge, Ludmil B. Alexandrov , Christopher D. Greenman, et al. The Life History of 21 Breast Cancers Cell Volume 149, Issue 5 2012 994 - 1007



(D) Reconstruction of the phylogenetic tree. The thickness of the branches reflects the proportion of tumor cells comprising that lineage. The length of the branches reflects the number of mutations specific to that lineage.

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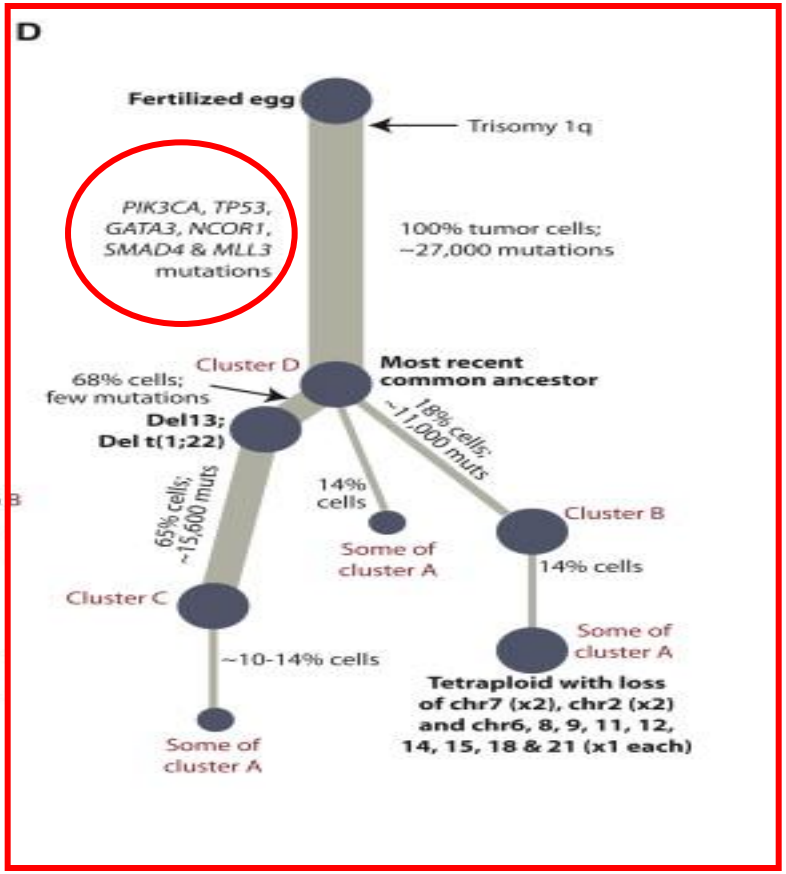

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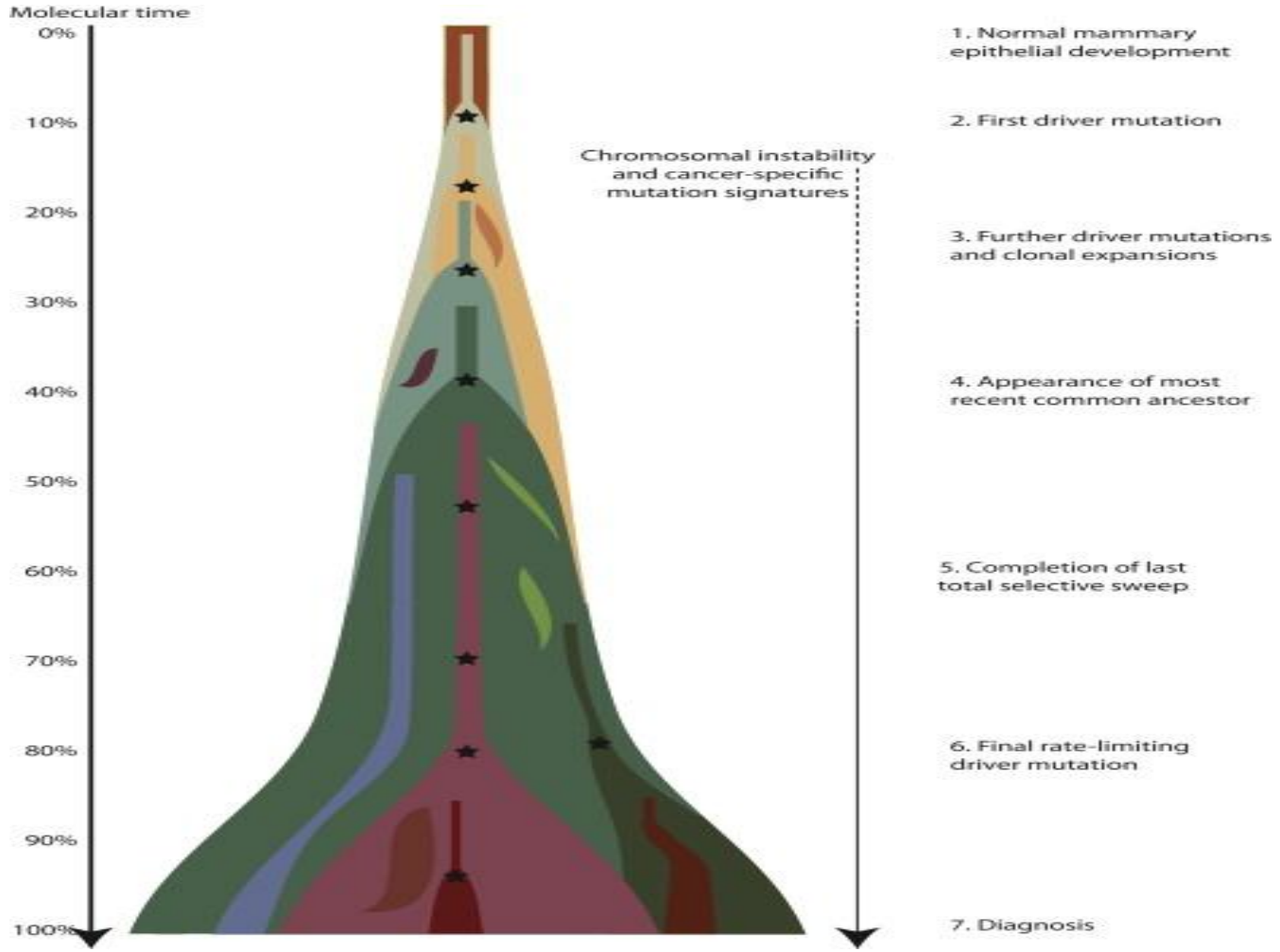

A Model for Breast Cancer Development over Molecular Time. **The cancer evolves through acquisition of driver mutations (black stars), which produce clonal expansions. These driver mutations occur only infrequently in long-lived lineages of cells, which passively accumulated many mutations without expansion (passengers).**

Serena Nik-Zainal, Peter Van Loo, David C. Wedge, Ludmil B. Alexandrov, Christopher D. Greenman, et al. The Life History of 21 Breast Cancers Cell Volume 149, Issue 5 2012 994 - 1007

Finding genetic susceptibility to radiation effects in humans—Will NGS studies help?

Qualified YES.
Assuming dose-response analyses can be integrated with genetic “patterns” of variation

The End

(or another beginning?)

The Future is Now

- Enrich for radiation-related cancers
  - Thyroid cancer good candidate
- NGS on tumor and germline DNA
- Compare tumor “life history” for early events and pattern characteristics with sporadic tumors (use The Cancer Genome Atlas (TCGA) resource)
- Use pattern similarities to define radiation-related (RR) tumors
- Compare germline NGS of RR cases to germline NGS of non-RR cases to germline NGS of controls (all with varying radiation exposure)
  - Assess interaction with radiation using “NGS pattern”