National Cancer Institute

The Aging Genome and Its Relationship to Cancer

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

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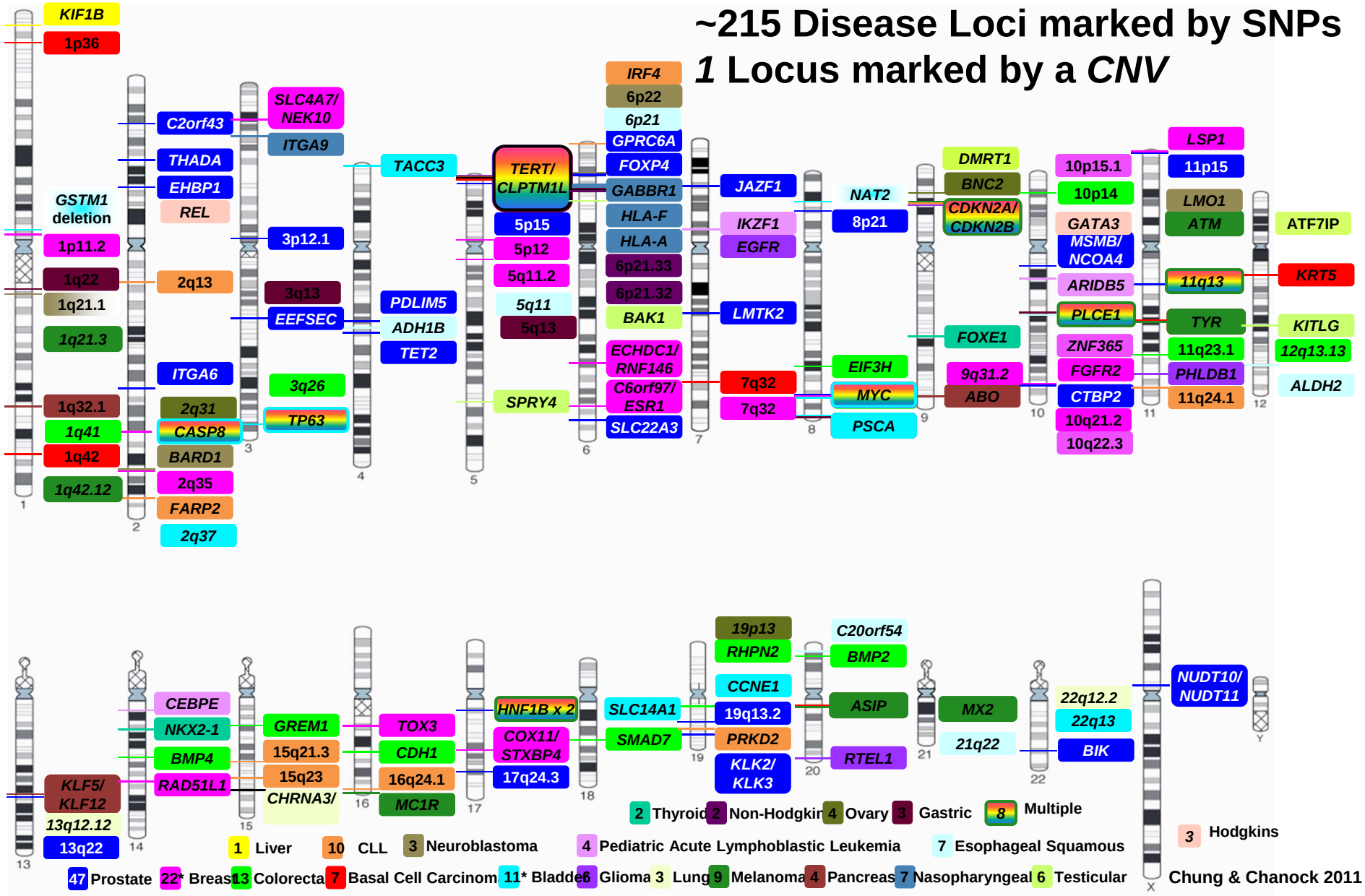
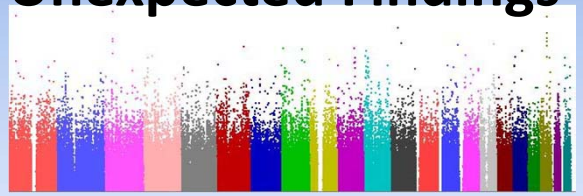
Status of Genome-Wide Association Studies

1. **Discovery of New Regions in the Genome Associated with Diseases/Traits**
   - ***New "Candidate Genes"***
2. **Clues for Mechanistic Insights Using Common Variants**
   - ***Etiology***
   - ***Gene-Environment/Lifestyle Interactions***
   - ***Outcomes & Pharmacogenomics***
3. **Challenge of Genetic Markers for Risk Prediction for Individual or Public Health Decisions**
   - ***Common Variants Represent a Fraction of the Genetic Contribution to Risk***
   - ***Integration of Lifestyle/Environment***

Published Cancer GWAS Etiology Hits: 12.1.11

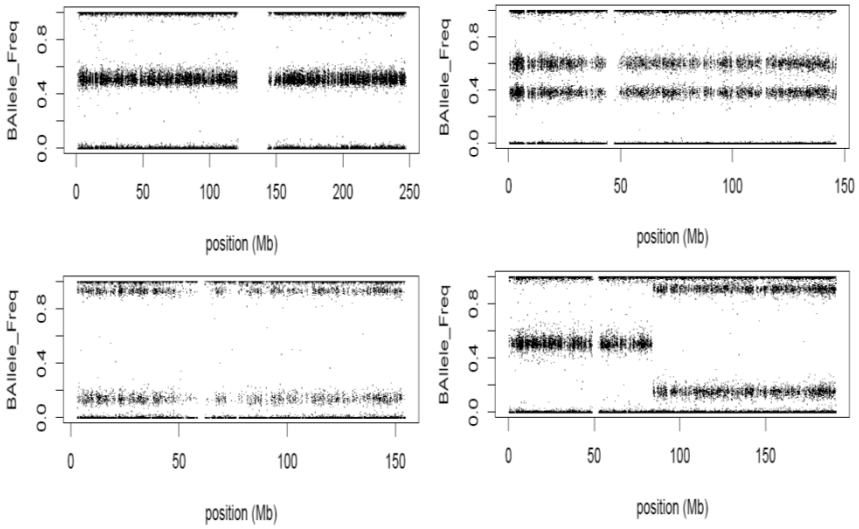


Unexpected Findings



Genome-wide association studies

Large chromosomal abnormalities, structural variation, aneuploidy in Germline DNA



Rodriguez-Santiago AJHG 2010

In 6 years of GWAS @ DCEG:

Scanned 80,000 samples in 13 GWAS

Generated >76 trillion genotypes

Discovered many SNPs related to cancer susceptibility

Observed instances of complex chromosomal abnormalities

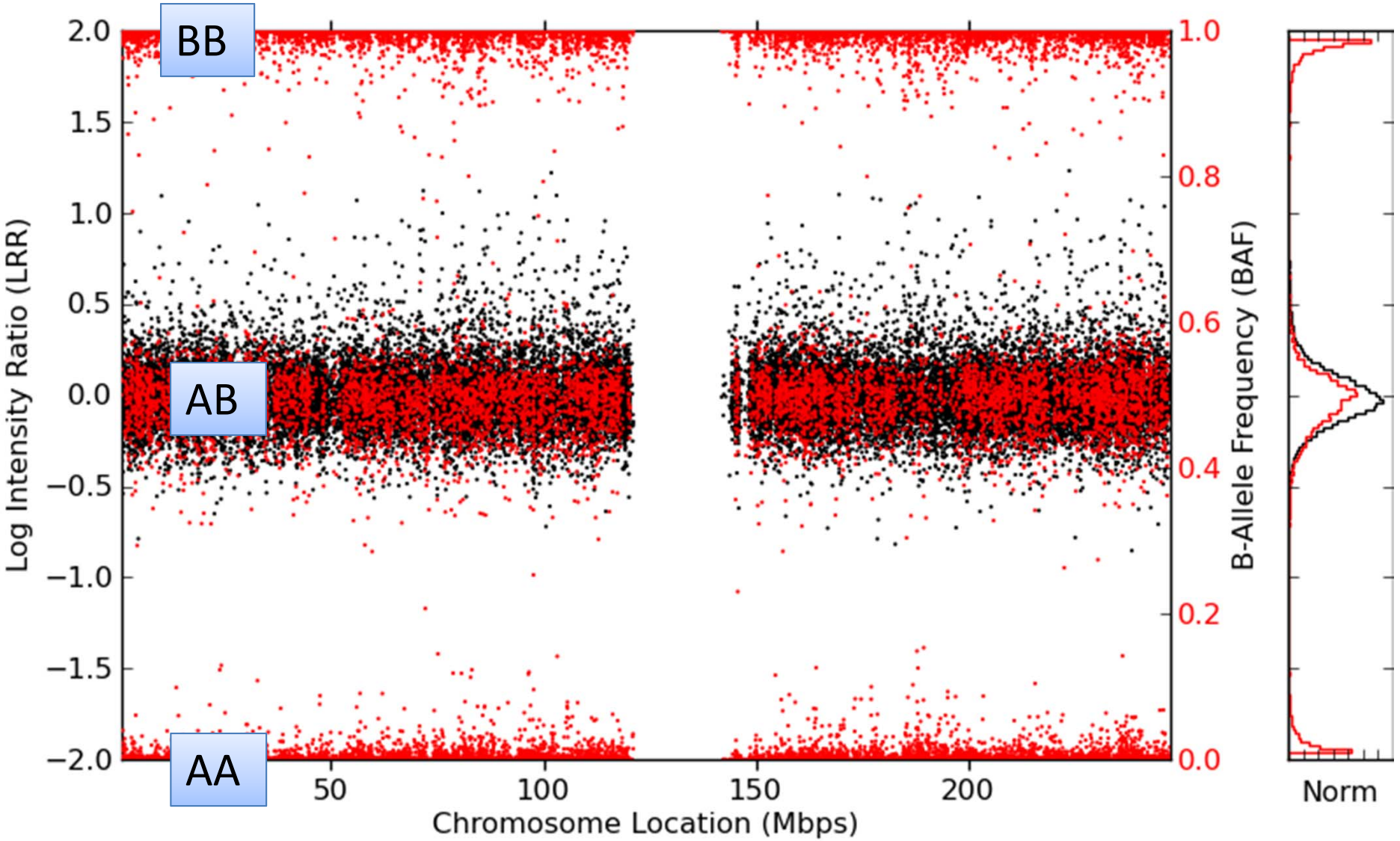
However, these abnormalities were rare and not considered germane to the primary analyses

So what did we do?

We threw them out as Good Quality Control

We did make a note to revisit them……

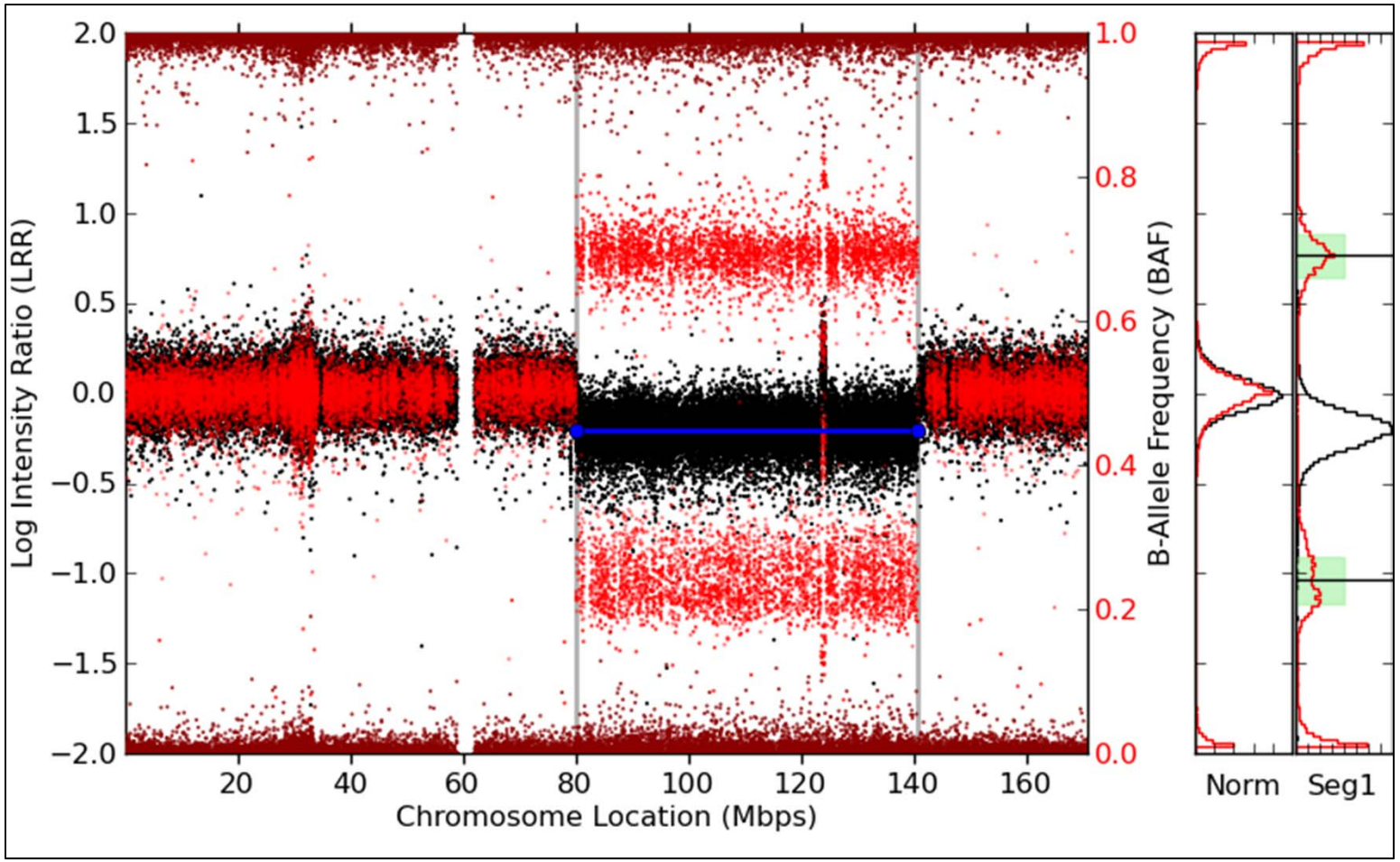
A Normal Chromosome 1



BAF= B Allele Frequency

LRR=log2 relative probe intensity ratio

Example of Mosaicism



Deletion of Part of Chromosome in Subpopulation of Cells

Definition of Genetic Mosaicism

Co-existence of distinct subpopulations of cells regardless of the clonal or developmental origin

Presence of large structural genomic events (> 2 Mb)

Resulting in alteration of

- Copy number (gain or loss)
- Loss of heterozygosity

Why Study Genetic Mosaicism in Germline?

Genetic instability and somatic alterations have been implicated in cancer etiology

But in GWAS we've been looking at germline DNA, right?

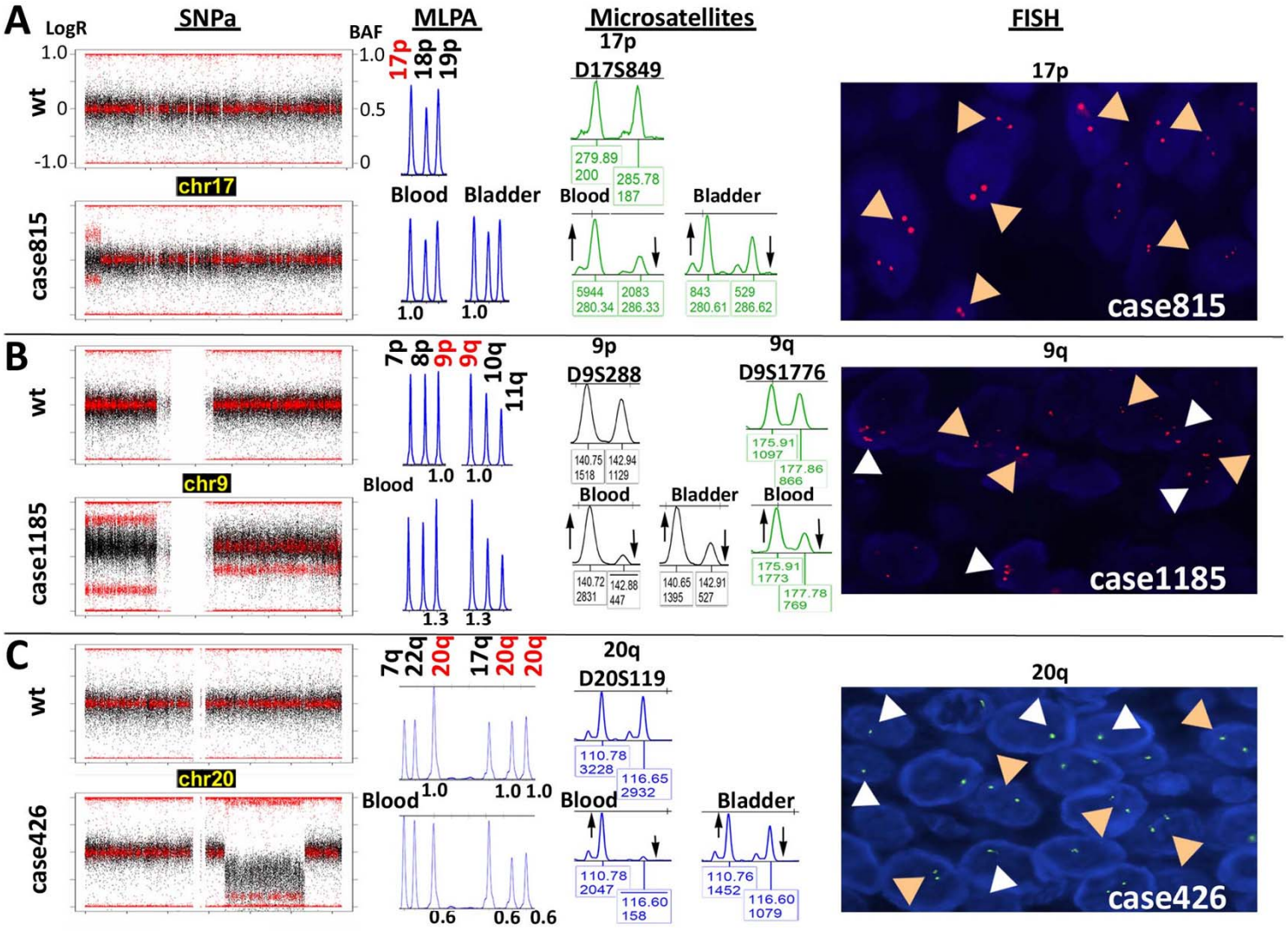
Is DNA extracted from blood and buccal swabs a good exemplar of germline DNA?

Could genetic instability be related to age, sex, smoking, DNA source, or cancer?

Mosaicism in the Extreme

- Age-old explanation for developmental disorders and catastrophic diseases (NF)
- Rare, Highly Penetrant Mutations
  - *BUB1B*
  - *CEP57*
- Complex Syndromes
  - Proteus Syndrome & *AKT1* (*NEJM* 2011)
  - Ollier Disease & *IDH1/IDH2* (*Nature Genetics* 2011)
  - *HRAS*- Skin/Cancer (*NEJM* 2011)

Validation for 42 events: 100% Validation



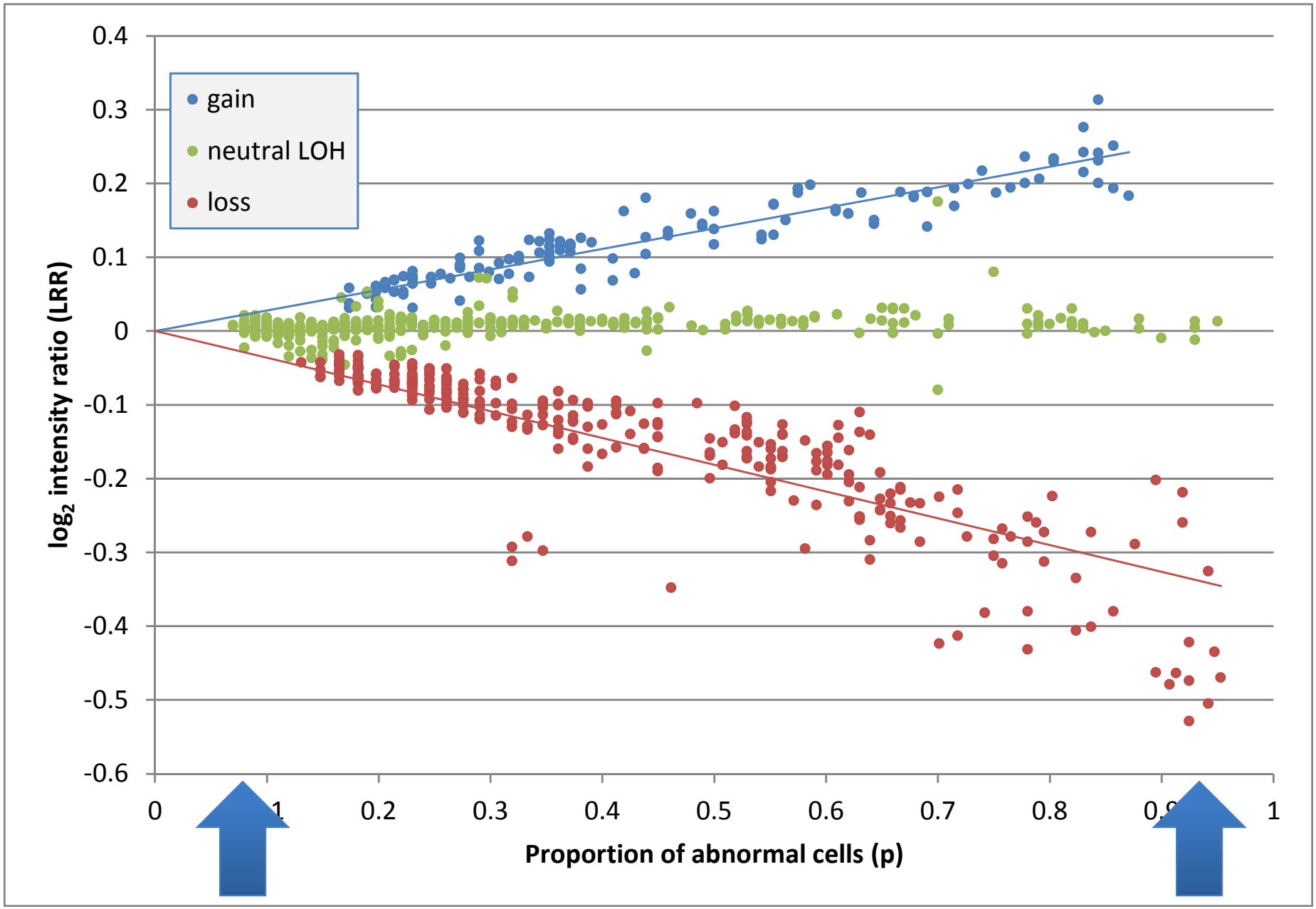
Rodríguez-Santiago et al. *Am J Hum Genet*. 2010;87:129-38

Results of Genetic Mosaicism Analysis

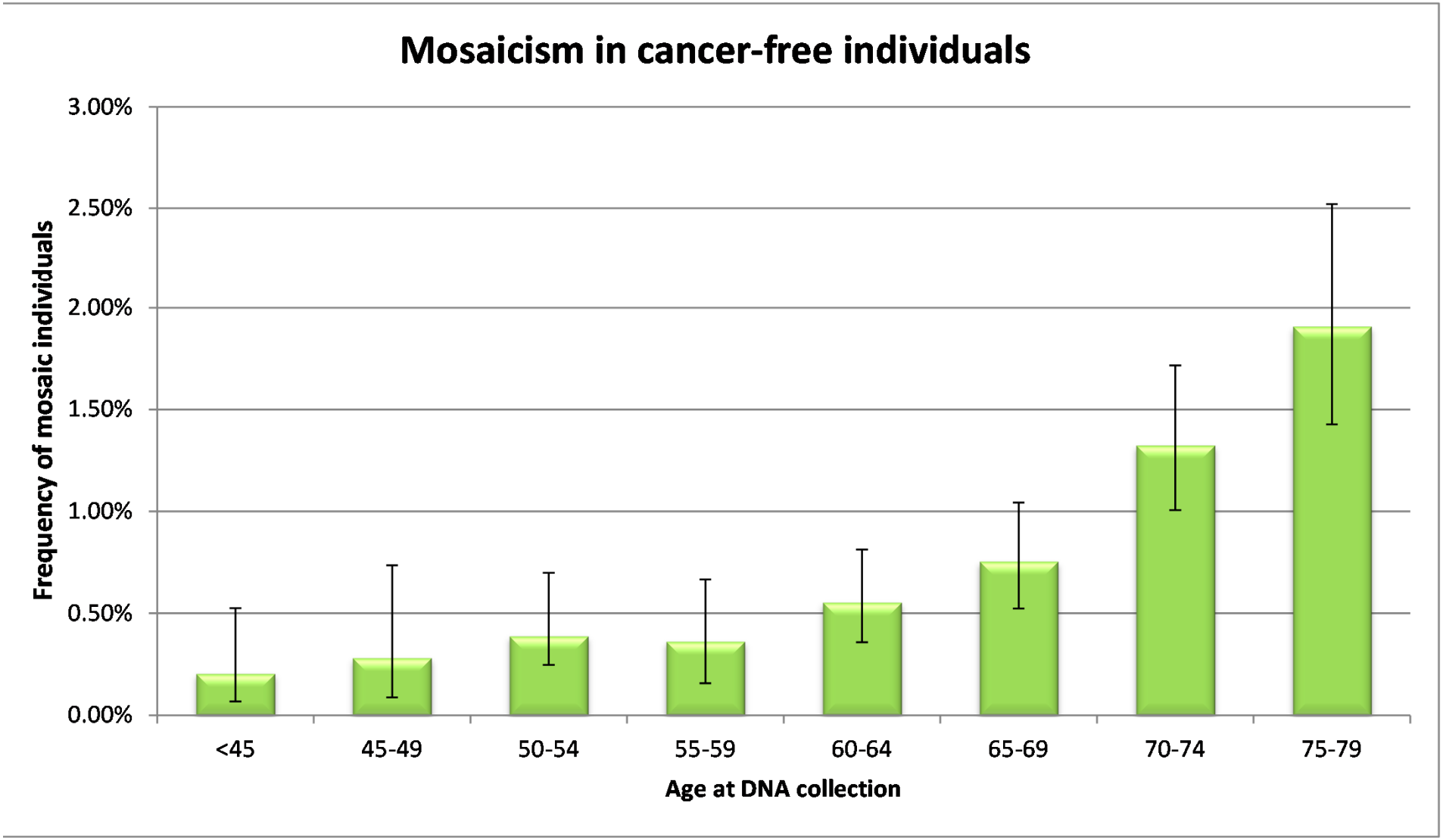
- Analysis of 13 GWAS 57,853
  - Cancer cases 31,717
  - Cancer-free controls 26,136

- Mosaic events detected 681
- Autosomal chromosomes 641
- Individuals 517
- Individuals with multiple events 69

Genetic Mosaic Events



Age at DNA Collection is the Strongest Predictor of Genetic Mosaicism



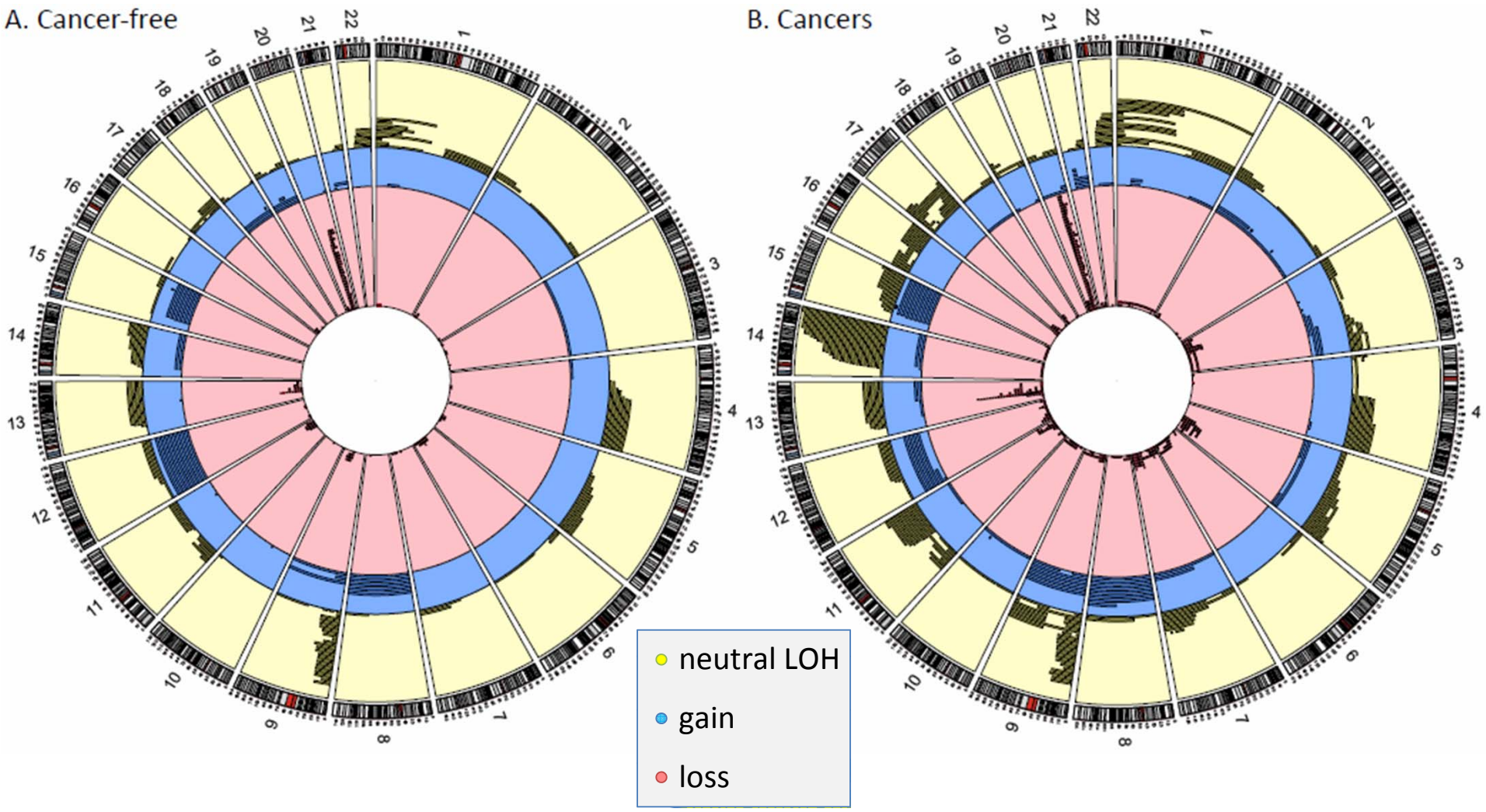
Higher Frequency in Men Compared to Women

	Females	Males
Cancer Free	0.56%	0.87%
Cancer	0.79%	1.21%
Overall	0.65%	1.04%

No Difference by

- Ancestry
- Smoking
- Source of DNA
  - Blood vs Buccal

Circos Plot of mosaic events in 57,583 individuals (681 events)



Recurrent Events

- 4 regions were altered >20 times
  - 9p copy-neutral LOH
  - 13q deletion
  - 14q copy-neutral LOH
  - 20q deletion

Adjusted Analysis of Association Between Genetic Mosaicism and Cancer in 49 studies

	All Cancer Cases			Likely Untreated			Possibly Treated		
	OR	95% CI	P value	OR	95% CI	P value	OR	95% CI	P Value
Non-heme Cancers	1.27	1.05-1.52	0.012	1.45	1.18-1.80	5.4E-04	1.03	0.81-1.30	0.804

Adjusted Analysis of Association Between Genetic Mosaicism & Cancer

	All cancer cases			Likely Untreated			Possibly Treated		
Site of first cancer	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**	**OR**	**95% CI**	**p value**
non-hematologic cancer	**1.27**	**(1.05-1.52)**	**0.012**	**1.45**	**(1.18-1.80)**	**5.4E-04**	**1.03**	**(0.81-1.30)**	**0.804**
bladder	1.30	(0.90-1.89)	0.164	1.50	(1.01-2.23)	0.043	0.73	(0.32-1.68)	0.455
breast	0.72	(0.41-1.27)	0.256	0.49	(0.18-1.32)	0.159	0.90	(0.46-1.79)	0.770
endometrium	1.27	(0.64-2.50)	0.494	1.35	(0.42-4.30)	0.611	1.24	(0.54-2.82)	0.610
esophagus	0.86	(0.34-2.18)	0.751	3.51	(0.45-27.58)	0.232	0.76	(0.29-2.03)	0.590
glioma	0.88	(0.45-1.74)	0.717	0.95	(0.44-2.05)	0.892	0.70	(0.17-2.86)	0.622
kidney	1.98	(1.27-3.06)	2.3E-03	2.32	(1.46-3.69)	3.6E-04	0.95	(0.30-3.03)	0.931
lung	1.56	(1.18-2.08)	2.0E-03	1.69	(1.23-2.33)	1.3E-03	1.27	(0.81-1.96)	0.295
osteosarcoma	1.34	(0.39-4.59)	0.637				1.34	(0.39-4.59)	0.637
ovary	1.18	(0.48-2.93)	0.718	1.09	(0.27-4.47)	0.903	1.27	(0.40-4.04)	0.690
pancreas	0.89	(0.60-1.33)	0.574	0.55	(0.14-2.24)	0.406	0.93	(0.62-1.41)	0.735
prostate	1.14	(0.79-1.64)	0.485	1.28	(0.85-1.92)	0.243	0.92	(0.51-1.66)	0.781
stomach	1.43	(0.68-3.03)	0.345	3.35	(0.74-15.13)	0.116	1.32	(0.61-2.88)	0.481
testis	3.29	(0.59-18.46)	0.176	3.29	(0.59-18.46)	0.176			
other sites	1.49	(0.55-4.05)	0.438				1.49	(0.55-4.05)	0.438

Early Detection of Hematological Cancers as Genetic Mosaicism

	Mosaic Counts			Non-Mosaic Counts			Mosaic Frequency (%)		
	Untreated	Possibly Treated	Total	Untreated	Possibly Treated	Total	Untreated	Possibly Treated	Overall
hematologic cancer	**9**	**9**	**18**	**34**	**62**	**96**	**20.93**	**12.68**	**15.79**
leukemia	**9**	**8**	**17**	**34**	**11**	**45**	**20.93**	**42.11**	27.42
lymphocytic	5	4	9	14	5	19	26.32	44.44	32.14
myeloid	3	4	7	16	5	21	15.79	44.44	25.00
other/nos	1	0	1	4	1	5	20.00	0.00	16.67
lymphoma	0	1	1	0	42	42		2.33	2.33
multiple myeloma	0	0	0	0	9	9		0.00	0.00

- For untreated leukemia vs. cancer-free controls
  - DNA collected at least one year prior to diagnosis
  - OR=35.4 (14.7-76.6 95% CI), $p=3.8 \times 10^{-11}$
- DNA was obtained >5 years prior to diagnosis for 6 mosaic individuals, with the longest interval being 14 years

Hematological Cancers & Genetic Mosaicism

- Detection of genetic mosaicism as harbinger of precursor states or hematological cancers
- Incidental cancers in controls and as second cancers
  - 13q14 abnormalities
  - 20q deletions
- Detection years before diagnosis

Two Hypotheses for Mosaicism in the Aging Genome

Early Event

Embryonic Progenitors with Somatic Alterations Are Below Threshold of Detection

Unknown Events Trigger Survival Bottleneck

LEADS TO

Positive Selection with Rapid Expansion of Second Clonal Population

Late Event

Increase in Somatic Alterations with Age

PLUS

Decreased Genomic Stability due to Telomere Attrition

LEADS TO

Proliferation of Suppressed Populations of Somatically Altered Clones

Decreased Cellular Diversity with Aging and Cell Populations Become Increasingly Oligoclonal

Co-existence of Two Clonal Populations

Ongoing Studies

- Longitudinal GWAS Analysis in NCI cohorts (PLCO)
  Do mosaic proportions vary?
  Can we garner insights into the mechanisms?
- Do ‘germ-line’ mosaic events correlate with somatic events in tumor tissue?
- Why might Genetic Mosaicism be associated with subsets of epithelial cancers?
  Lung and Kidney Cancers
- Currently conducting NHL scan with 9000 cases
  Case-control and cohort studies

Conclusions

- Detection of large-scale Genetic Mosaicism increases with age
- Genetic Mosaicism may be a risk factor for Adult Epithelial Cancers
- GWAS data can detect early or pre-leukemic states
- Large-scale Genetic Mosaicism represents the tip of the "*Iceberg of Genomic Instability*"
  - Hard to detect smaller events with current technologies
  - Next Generation Sequencing will be informative

The Aging Genome: Implications for Cancer Studies

- Importance of thorough characterization of ‘germline’ DNA in parallel with somatic analyses
- Insights into Genomic Instability

  Early vs. Late Events
- GWAS as a biomarker for early detection of hematological cancers

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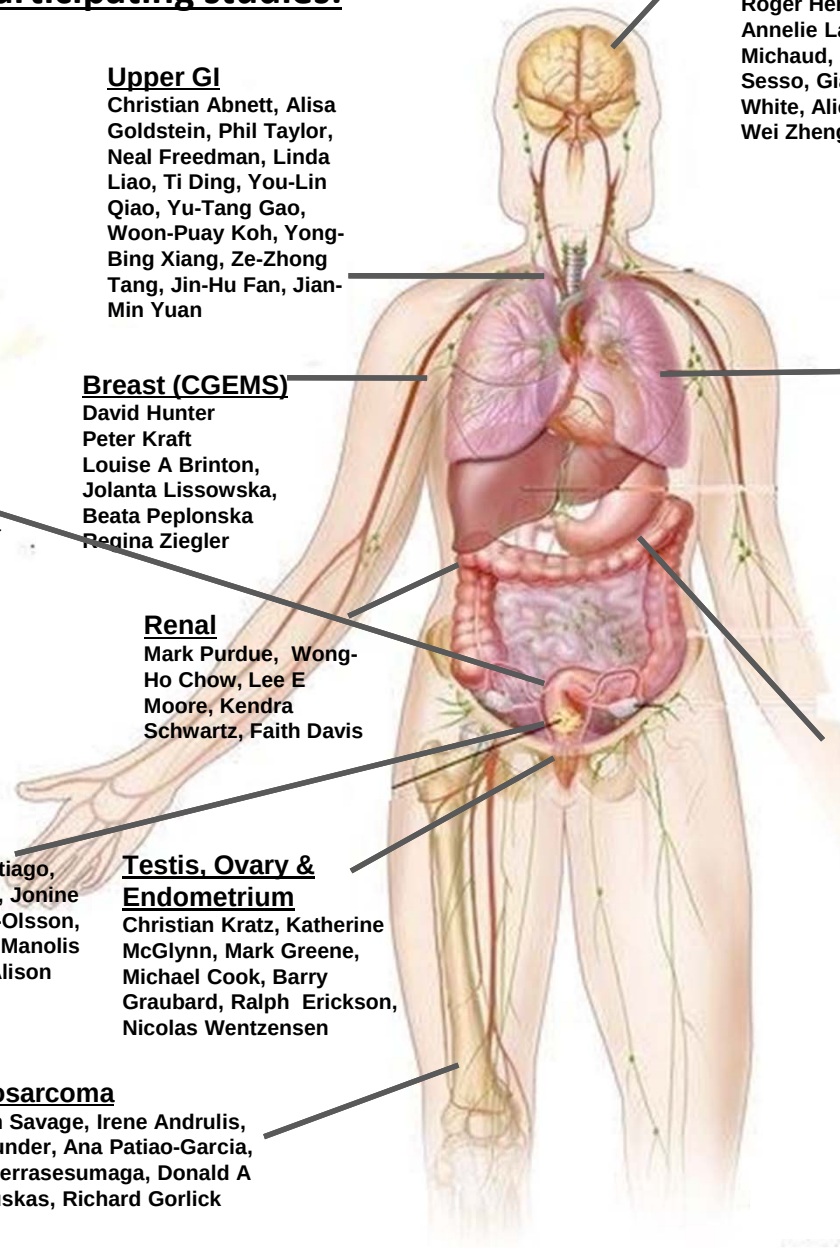
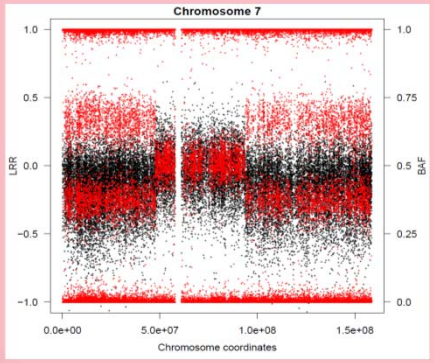


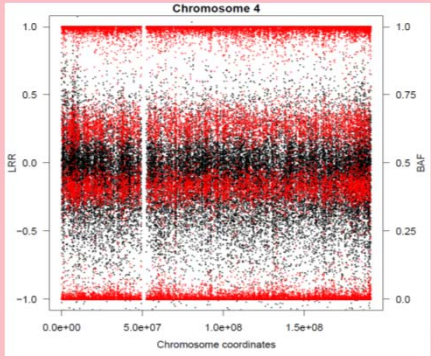
	Mosaic Counts					Mosaic Frequency (%)				
First cancer site	13q (del)	20q (del)	9p (cnloh)	14q (cnloh)	other	13q (del)	20q (del)	9p (cnloh)	14q (cnloh)	other
Cancer Free	10	30	22	6	126	5	15	12	3	64
Cancer DX *	22	46	18	26	206	7	14	6	8	65
non-hematologic										
bladder	2	4	1	7	29	5	9	2	16	67
breast	1	1	0	1	10	8	8		8	77
endometrium	0	1	2	1	5		11	22	11	56
esophagus	0	1	1	1	4		14	14	14	57
glioma	0	2	2	0	5		22	22	0	56
kidney	1	3	0	3	17	4	13	0	13	71
lung	8	13	5	7	66	8	13	5	7	67
osteosarcoma	0	0	1	0	2			33		67
ovary	0	2	0	0	3		40			60
pancreas	1	6	1	1	22	3	19	3	3	71
prostate	5	9	4	1	27	11	20	9	2	59
stomach	0	1	1	1	12		7	7	7	80
testis	0	0	0	0	2					100
other sites	0	1	0	1	2		25		25	50
hematologic										0
leukemia	4	2	0	2	5	33	17		17	31
lymphoma	0	0	0	0	1					100
Overall	32	76	40	32	332	6	15	8	6	65

How Stable is a ‘Germline’ Genome?
Observed Variation in Intensity Plots for SNP Calling

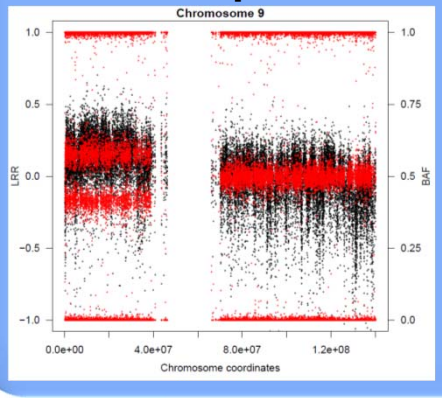
Types of Large Structural Events Detected
> 2 Mb in DCEG/NCI GWAS of > 57,000 Subjects

Deletion/Mosaic Deletion

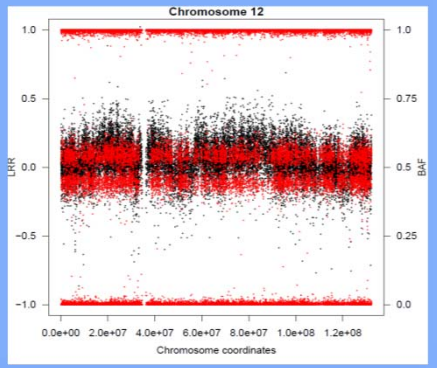




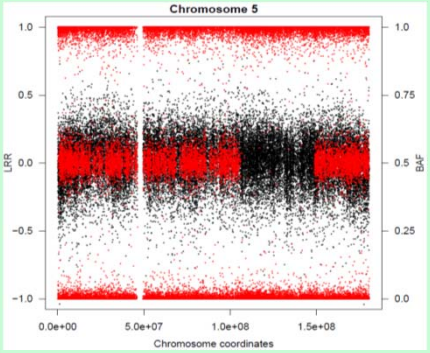
Mosaic Duplication



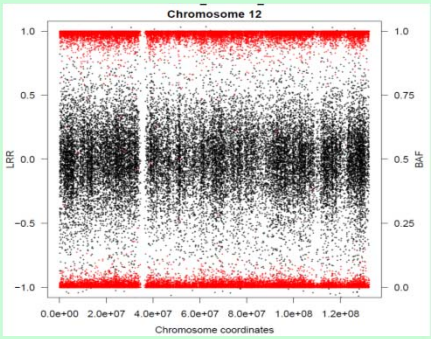
Mosaic Trisomy



Copy neutral LOH



Uniparental Disomy



Mosaic Copy Neutral Loss of Heterozygosity