Prevalence of clonal hematopoiesis of indeterminate potential (CHIP) measured by an ultra-sensitive sequencing assay: exploratory analysis of the Circulating Cell-free Genome Atlas (CCGA) study

Charles Swanton, MBPhD FRCP FRS[1]; Oliver Venn, PhD[2]; Alex Aravanis, MD, PhD[2]; Earl Hubbell, PhD[2]; Tara Maddala, PhD[2]; John F. Beausang, PhD[2]; Darya Filippova, PhD[2]; Samuel Gross, PhD[2]; Arash Jamshidi, PhD[2]; Ling Shen, PhD[2]; Anton Valouev, PhD[2]; Nan Zhang, PhD[2]; Kelly L. Bolton, MD[3]; Timothy J. Yeatman, MD, FACS[4]; Michael Seiden, MD, PhD[5], Geoffrey Oxnard, MD[6], Minetta Liu, MD[7], Richard T. Williams, MBBS, PhD[2*], Anne-Renee Hartman, MD[2], Jose Baselga, MD, PhD[3]

Affiliations: [1]Francis Crick Institute, London, UK. [2]GRAIL, Inc. Menlo Park, CA. [3]Memorial Sloan Kettering Cancer Center, New York, NY. [4]Gibbs Cancer Center and Research Institute, Spartanburg, SC. [5]US Oncology Research, The Woodlands, TX. [6]Dana Farber Cancer Institute, Boston, MA. [7]Mayo Clinic, Rochester, MN. *Current affiliation: WuXi NextCODE.

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Disclosures

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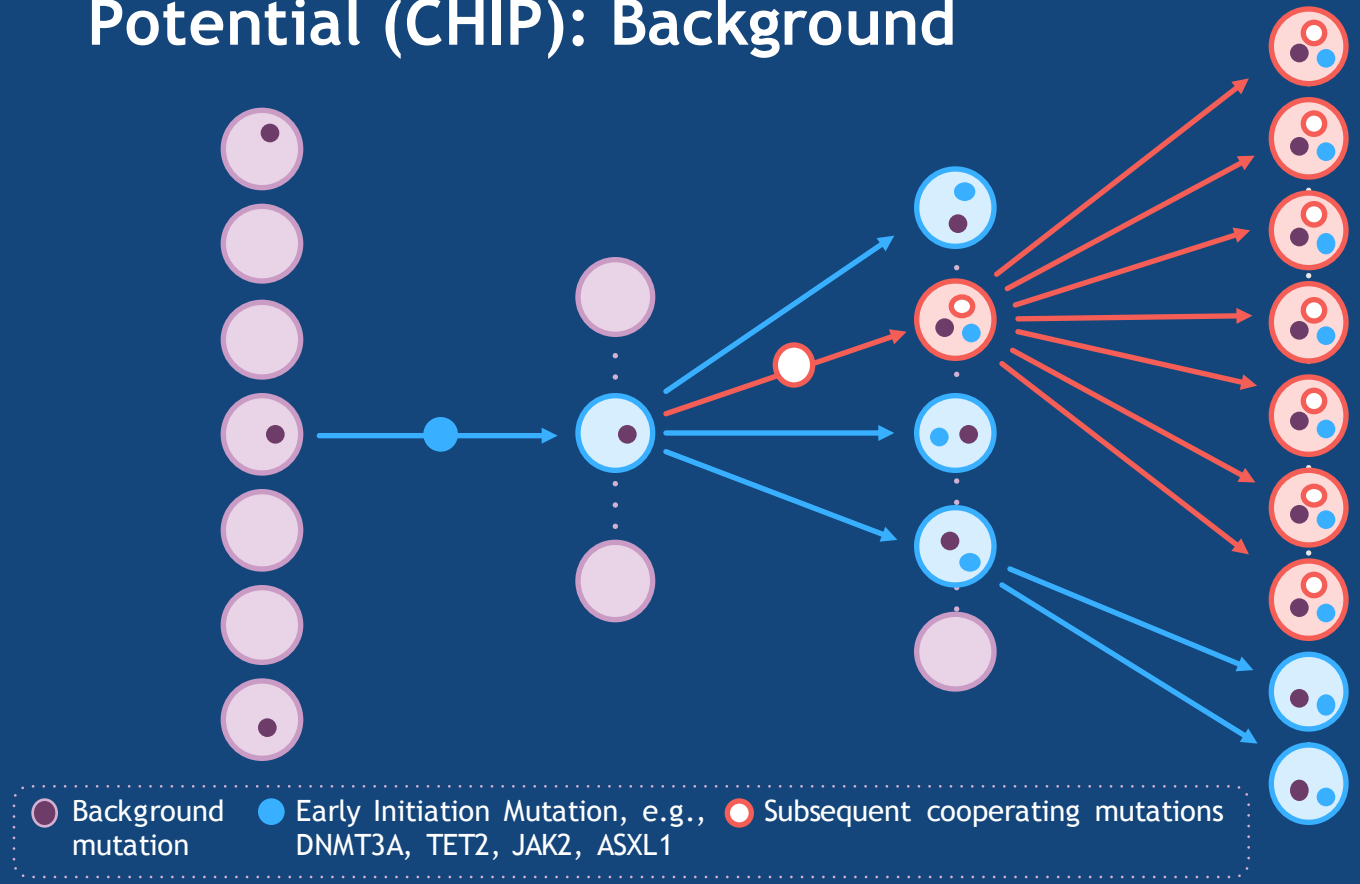
Clonal Hematopoiesis of Indeterminate Potential (CHIP): Background



- Somatic mutations represent normal aging process
- May also represent premalignant, initiating events causing clonal hematopoietic expansion
- Subsequent cooperating mutations may contribute to transformation to a malignant state

Adapted from Xie et al. *Nat Med* 2014;20:1472-1478.

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Clonal Hematopoiesis of Indeterminate Potential (CHIP)

- Originally defined by presence of a hematologic malignancy-associated somatic mutation in blood or marrow but without other diagnostic criteria for a hematologic malignancy[1]
- Prevalence ranges from 2% to 33% in recently-published studies[2-7]
- Prevalence increases with age and with deeper sequencing approaches
- Associated with increased risk for hematologic malignancy, cardiovascular disease, and overall mortality[2,8]
- Prevalence increased in patients with solid tumors[9]
- Because WBCs contribute to the cfDNA fraction, CHIP may confound cfDNA-based assays[10,11]
  - WBCs can also confound circulating tumor cell or tumor tissue sequencing assays
- Important to understand prevalence to more fully define impact on outcomes, and to understand how CHIP may confound cfDNA-based assays

[1]Steensma DP et al. *Blood* 2015;126:9-16. [2]Jasiwal S et al. *N Engl J Med* 2014. [3]Xie M et al. *Nat Med* 2014; 20:1472-1478.[4]Genovese G et al. *N Engl J Med* 2014;371:2477-2487.[5]Phallen J et al. *Sci Transl Med* 2017;9:403. [6]Coombs CC et al. *Cell Stem Cell* 2017;21:374-382.[7]Gillis NK et al. *Lancet Oncol* 2017;18:112-121. [8]Jaiswal S et al. *N Engl J Med* 2017;377:111-121. [9]Coombs CC et al. *Cell Stem Cell* 2017;21:374-382. [10]Kammesheidt A et al. *Int J Mol Epidemiol Genet* 2018;9:1-12.[11]Hu Y et al. *Clin Canc Res* 2018;doi: 10.1158/1078-0432.CCR-18-0143.

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CCGA is a Prospective Longitudinal Cohort Study Designed for Early Cancer Detection

- Sequencing of white blood cells identifies potentially confounding signal
- Allows identification of CHIP prevalence in CCGA participant population

15,000+ participants:
70% with cancer
30% without

142 Active Sites

Blood samples
(from all participants)

Targeted sequencing cfDNA, WBCs

Whole-genome sequencing cfDNA, WBCs

Targeted & whole-genome bisulfite sequencing cfDNA

Whole transcriptome sequencing cfRNA

Tissue samples
(cancer only)

Whole genome sequencing of tumor tissue

Follow up for 5 years
All participants

Participants with cancer: Data on treatment, recurrence, mortality

Participants without cancer: Remain cancer free or develop new cancer diagnosis, cancer status & treatment, mortality

FPI: 08/2016; 12,292 enrolled. Target: Complete enrollment of all 15,000 participants in 2018

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CCGA Participant Demographics

- Cancer and non-cancer groups were comparable with respect to age, race, sex, and body mass index (not shown).
- Comparable age is critical for:
  - Developing a classifier
  - Identifying CHIP in cancer versus non-cancer samples (as CHIP increases with age)
- **All patients were treatment-naive at the time of blood draw.**

	Cancer					Non-Cancer
	Breast	**Lung**	**Prostate**	**Colorectal**	**Other***	
Total	410	127	74	51	322	580
Age, Mean ± SD	58 ± 13	67 ± 9	64 ± 8	60 ± 11	62 ± 12	60 ± 13
Sex (%)						
Female	100%	54%	0%	53%	59%	78%
Race/Ethnicity (%)						
White, Non-Hispanic	86%	88%	82%	92%	85%	84%
African American	8%	5%	12%	<1%	6%	8%
Hispanic, Asian, Other	6%	7%	6%	7%	9%	8%
Smoking Status (%)						
Never-smoker	60%	15%	50%	63%	47%	57%

*Other includes renal, uterine, pancreas, esophageal, lymphoma, head & neck, ovarian, hepatobiliary, melanoma, cervical, multiple myeloma, leukemia, thyroid, bladder, gastric, anorectal, unknown primary/other.

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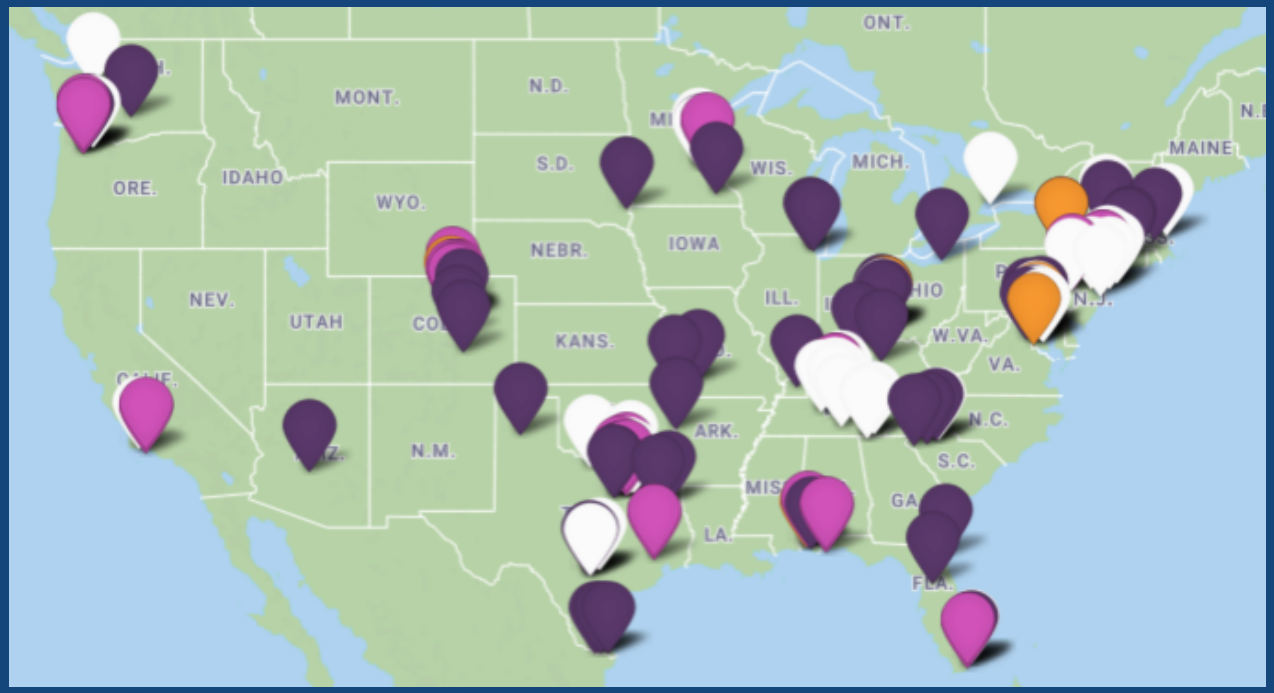
CCGA is Geographically Diverse with Enrollment Representative of United States Population

142 active sites representing 24 states in the U.S. and one site in Canada



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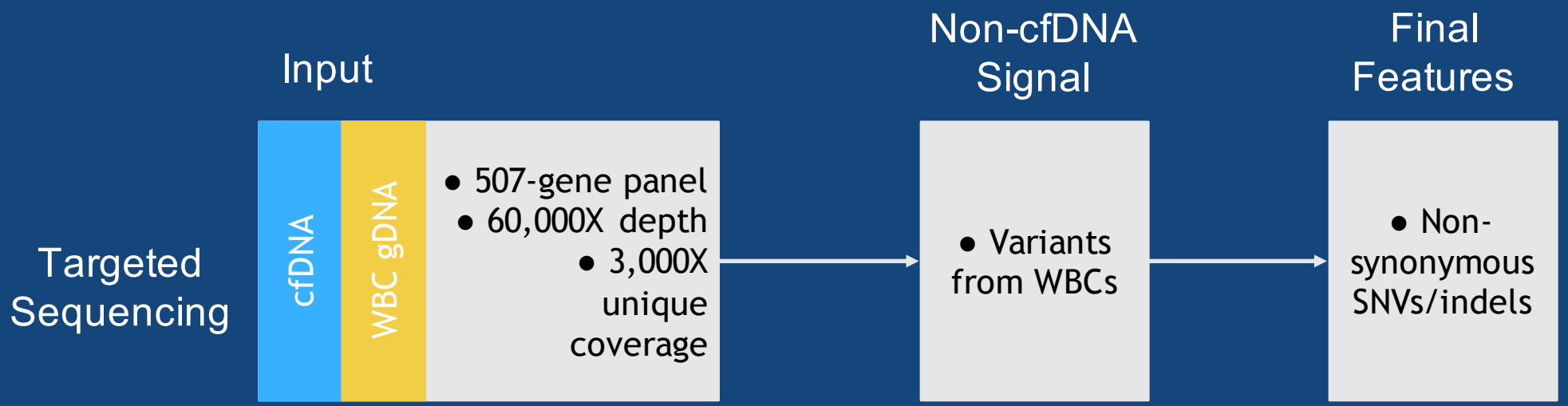
CCGA Prototype Assays Consider White Blood Cell Signal



- **Sequencing to exhaustive depth allowed comprehensive quantification of CHIP prevalence in CCGA participants**

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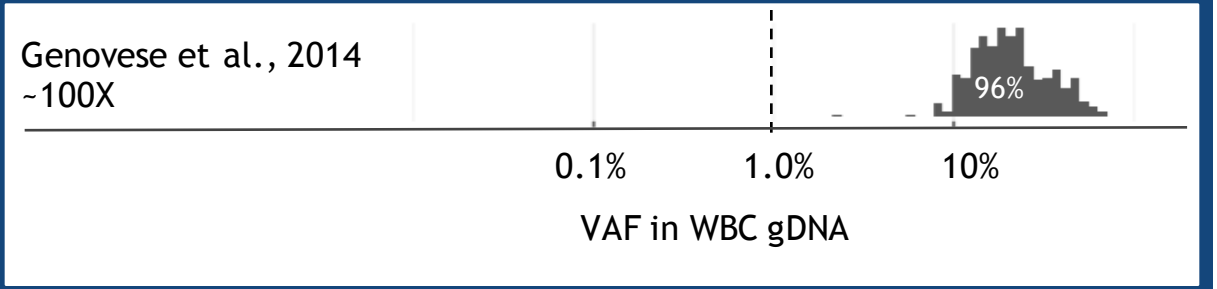
Assessing CHIP Through Matched WBC and cfDNA Sequencing

- Previous study sequencing at ~100X: 96% of variants at >1% variant allele frequencies (VAF)[1]



1. Genovese et al. *N Engl J Med* 2014;371:2477-2487.

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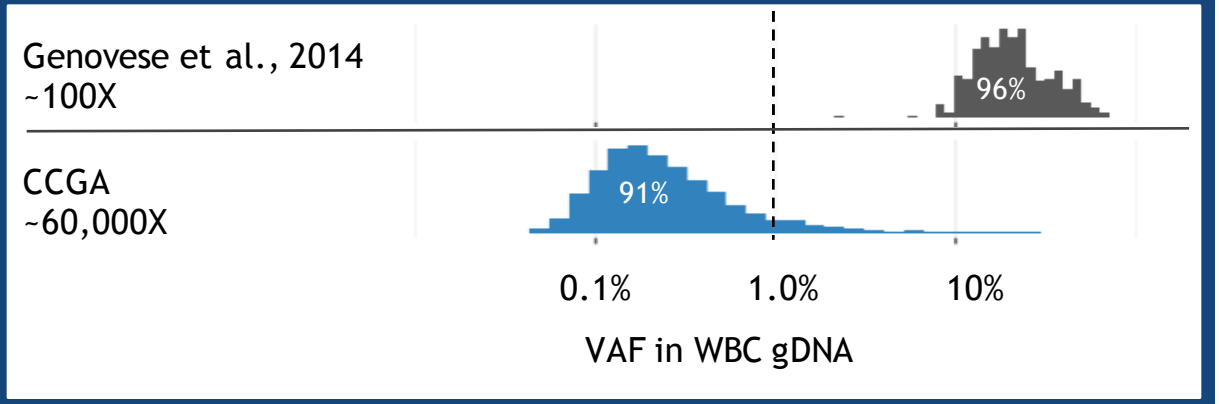
- Ultra-deep sequencing (this study): <10% of WBC-matched variants at >1% VAF



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Assessing CHIP Through Matched WBC and cfDNA Sequencing

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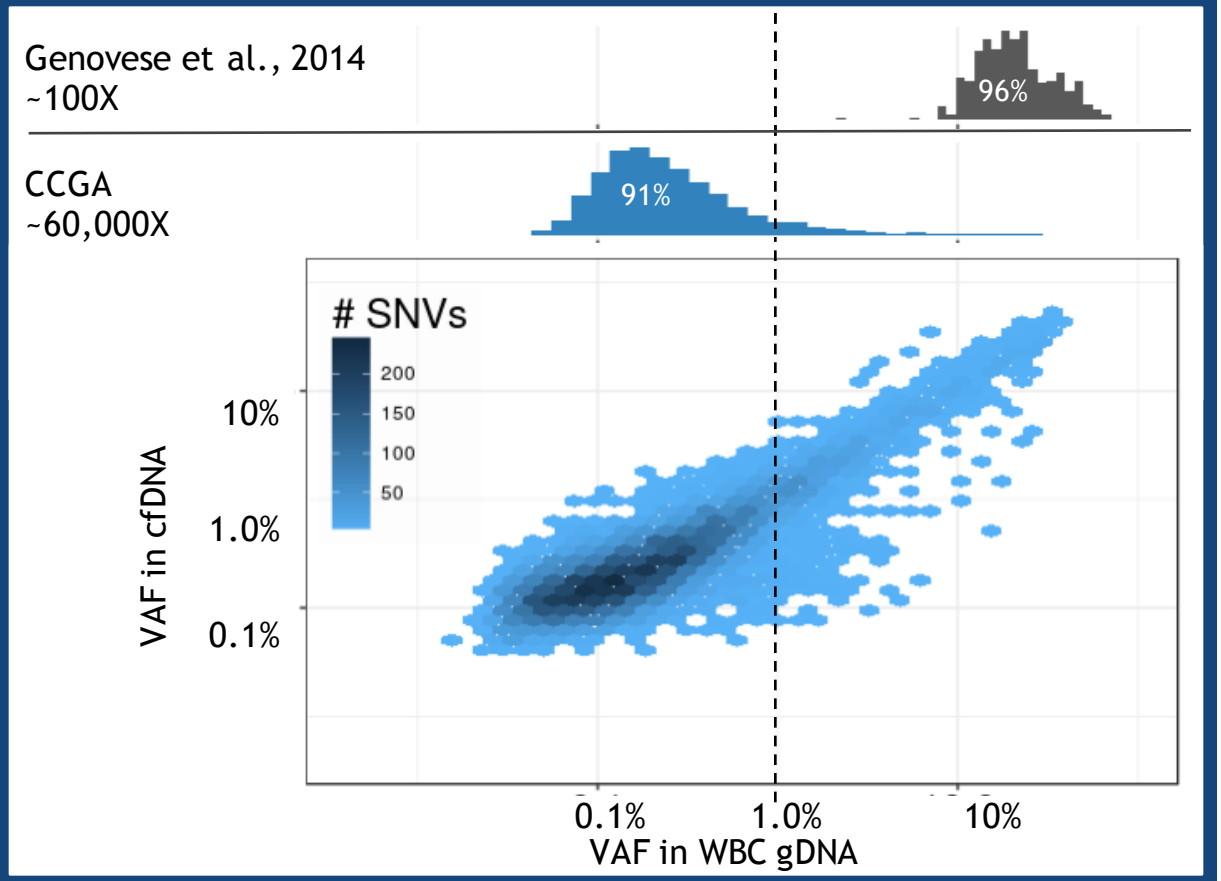
- Ultra-deep sequencing (this study): <10% of WBC-matched variants at >1% VAF
- Variants at lower VAFs will require high-depth sequencing of WBCs to effectively exclude this confounding signal in cfDNA-based assays



1. Genovese et al. *N Engl J Med* 2014;371:2477-2487.

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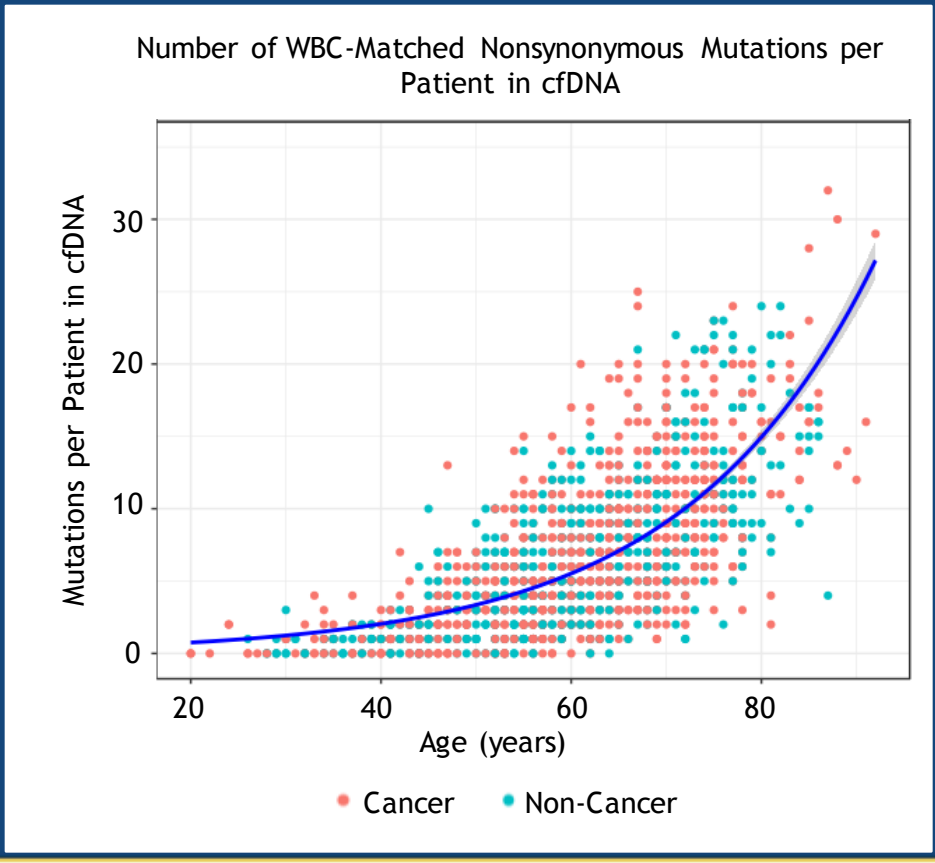
CHIP Increases with Age and has a Similar Prevalence in Cancer and Non-Cancer Participants

- 9,676 total nonsynonymous variants in 1,438 individuals were attributed to CHIP

	Avg. per patient per Mb	Total
Cancer	3.8	6024
Non-cancer	3.5	3652

- No statistically significant difference in age relationship in cancer vs non-cancer participants
- Estimated 160-170% increase in number of CHIP variants per decade ($p=2x10^{-16}$)
- At age 65, the predicted CHIP burden is 6.5 variants per individual in the panel



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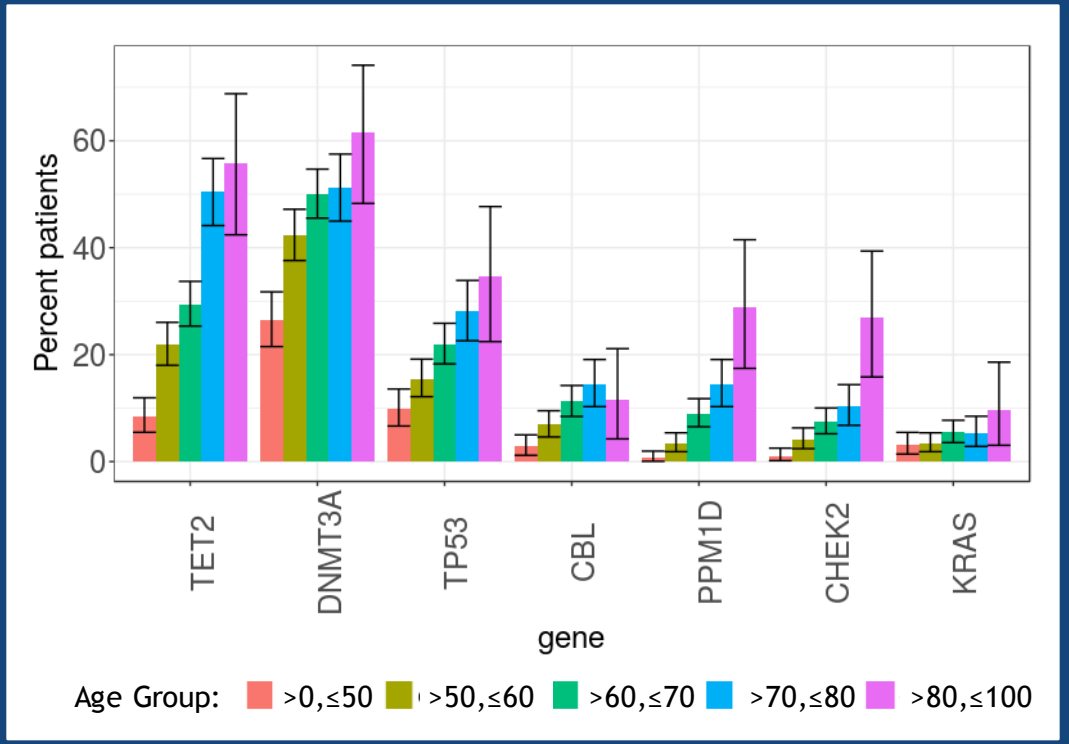
CHIP is Prevalent in Genes Implicated in Various Malignancies And Shows Age-Dependence

- Genes implicated in hematologic and solid malignancies have non-synonymous CHIP mutations that could confound cfDNA-based assays

Gene	60 - 70 yo with mutation (%, 95% CI)
KRAS	6% (4, 8)
TP53	22% (18, 26)
TET2	30% (26, 34)
DNMT3A	51% (46, 55)

- Suggests a screening population may have confounding CHIP variants in their cfDNA that must be accounted for



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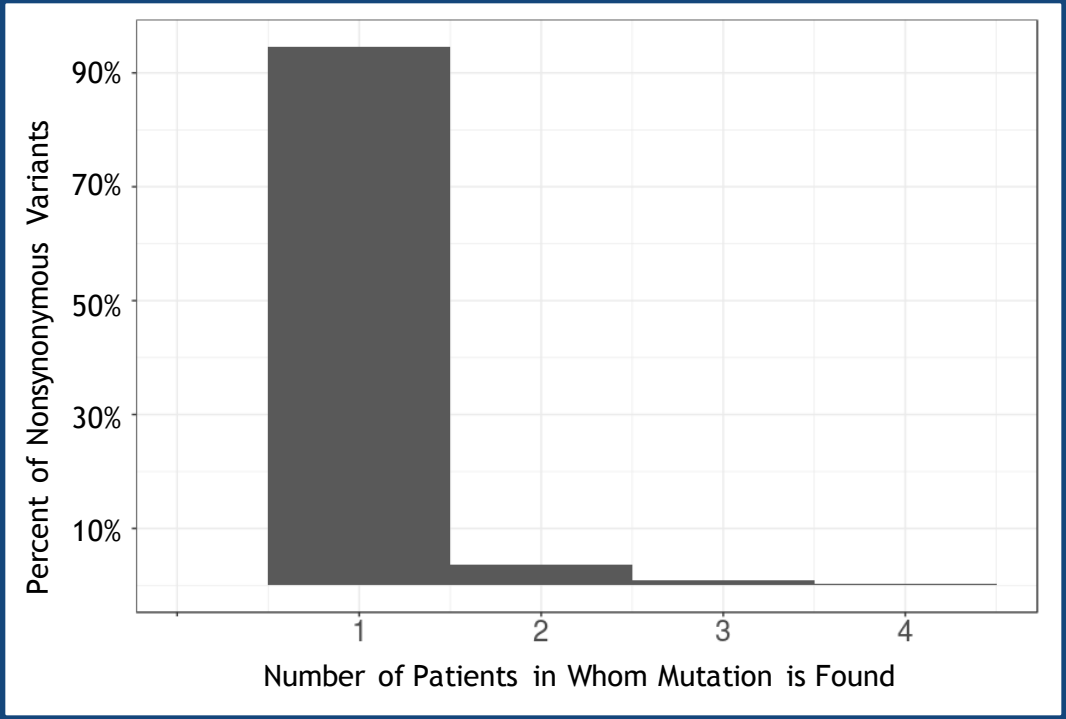
Nearly All WBC-Matched Variants in cfDNA are Specific to Individual Patients in Cohort

- 94% of individual WBC-matched variants only observed in a single participant
- These "private" mutations suggest that targeted approaches will require patient-specific WBC-matched correction to minimize false positive results



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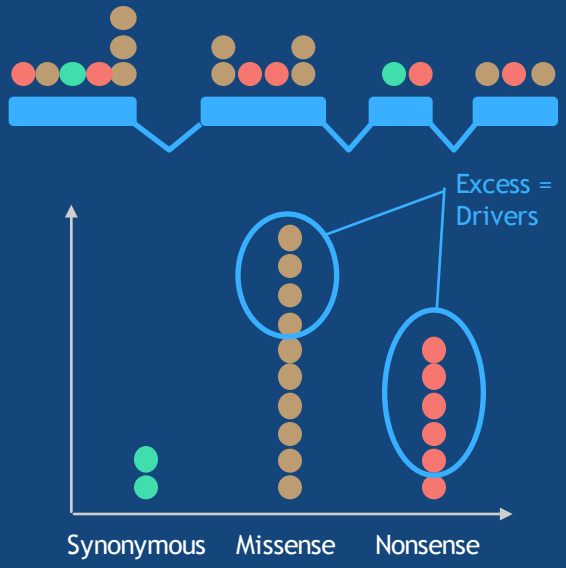
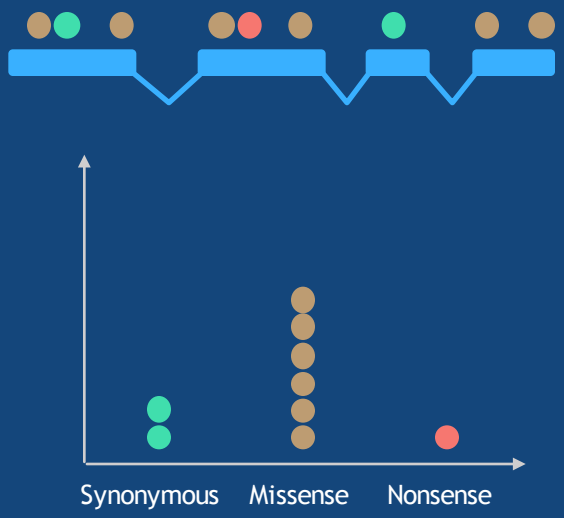
De novo Identification of Mutations that Drive WBC Clonal Expansions

Positively Selected Genes: >1



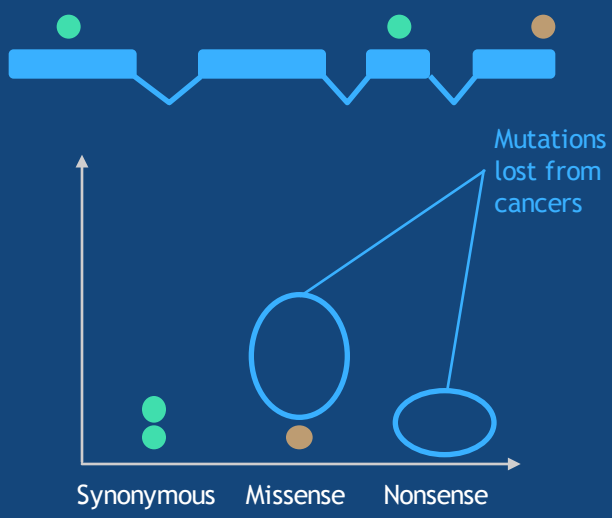
Positive selective pressure results in more driver mutations

Neutral Genes: "dNdS" score of 1



Codon-specific models of sequence evolution compares rate of non-synonymous versus synonymous mutations

Negatively Selected Genes: <1



Negative selective pressure results in fewer mutations

Adapted from Martincorena et al. *Cell* 2017;171:1029-1041.

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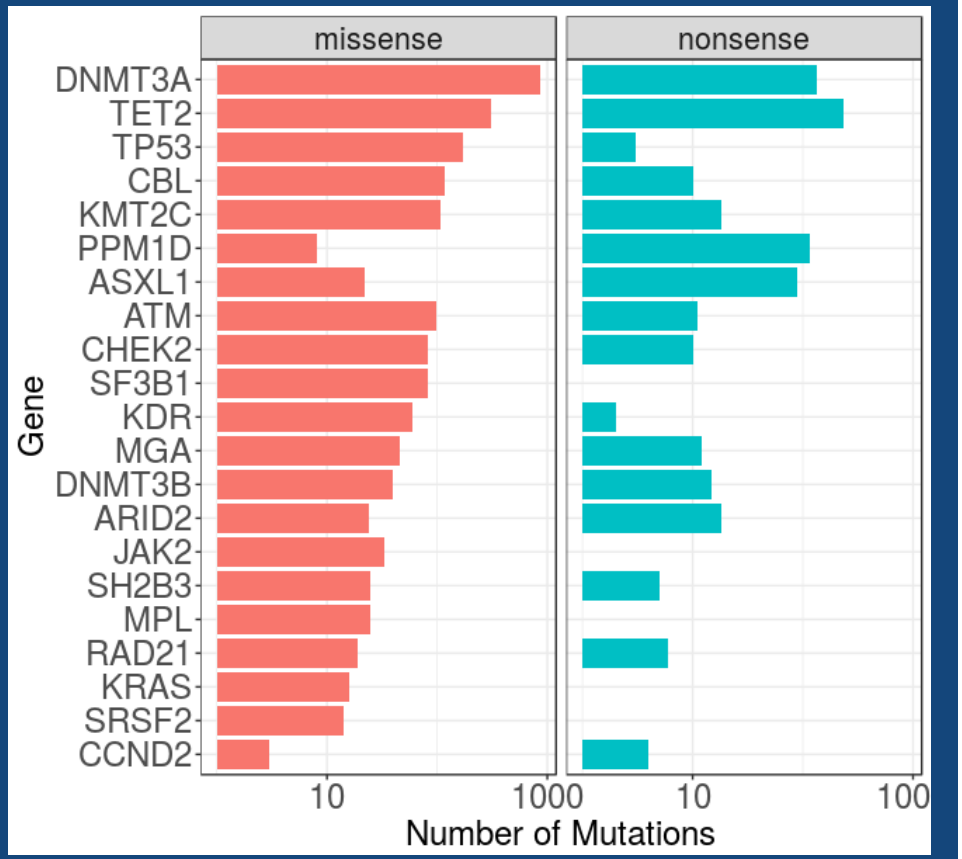
Genes Under Positive Selection

- Previous report showed more positive than negative selection on tumor mutations, largely in driver genes[1]
- This study also finds evidence for strong positive selection in CHIP
  - **21 driver genes with significant evidence for positive selection (cutoff q-score <0.05)**
  - Supports that these are real biological events
- Genes involved in hematologic malignancies identified (e.g., TET2, DNMT3A)[2]
  - Other identified genes (e.g., CHEK2) may also be implicated in hematologic or other malignancies



[1]Martincorena et al. *Cell* 2017;171:1029-1041.[2]Xie M et al. *Nat Med* 2014;20:1472-1478.

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Conclusions

- The WBC contribution to cfDNA somatic variation is diverse, common, and mostly private
  - Most variants were low frequency (<1% VAF) and private to a single individual in the cohort
- Unbiased analysis of selection identified strong positive selection of driver gene variants implicated in various malignancies in WBCs
- Additional studies into clinical and biological implications of CHIP are warranted
- For cfDNA-based assays, particularly in a screening population, accounting for CHIP may be critical to avoid false-positive results

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