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

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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


Charles Swanton, MBPhD FRCP FRS
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

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)

Tissue samples (cancer only)

Follow up for 5 years
All participants

Targeted sequencing cfDNA, WBCs
Whole-genome sequencing cfDNA, WBCs
Targeted & whole-genome bisulfite sequencing cfDNA
Whole transcriptome sequencing cfRNA
Whole genome sequencing of tumor tissue

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
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.

<table>
<thead>
<tr>
<th></th>
<th>Cancer</th>
<th>Non-Cancer</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Breast</td>
<td>Lung</td>
</tr>
<tr>
<td>Total</td>
<td>410</td>
<td>127</td>
</tr>
<tr>
<td>Age, Mean ± SD</td>
<td>58 ± 13</td>
<td>67 ± 9</td>
</tr>
<tr>
<td>Sex (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>100%</td>
<td>54%</td>
</tr>
<tr>
<td>Race/Ethnicity (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White, Non-Hispanic</td>
<td>86%</td>
<td>88%</td>
</tr>
<tr>
<td>African American</td>
<td>8%</td>
<td>5%</td>
</tr>
<tr>
<td>Hispanic, Asian, Other</td>
<td>6%</td>
<td>7%</td>
</tr>
<tr>
<td>Smoking Status (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never-smoker</td>
<td>60%</td>
<td>15%</td>
</tr>
</tbody>
</table>

*Other includes renal, uterine, pancreas, esophageal, lymphoma, head & neck, ovarian, hepatobiliary, melanoma, cervical, multiple myeloma, leukemia, thyroid, bladder, gastric, anorectal, unknown primary/other.
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
Sequencing to exhaustive depth allowed comprehensive quantification of CHIP prevalence in CCGA participants.
Assessing CHIP Through Matched WBC and cfDNA Sequencing

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

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- Variants at lower VAFs will require high-depth sequencing of WBCs to effectively exclude this confounding signal in cfDNA-based assays

\[\text{Genovese et al., 2014} \]
\[\sim 100X\]

\[\text{CCGA} \]
\[\sim 60,000X\]

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

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<thead>
<tr>
<th></th>
<th>Avg. per patient per Mb</th>
<th>Total</th>
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<tbody>
<tr>
<td>Cancer</td>
<td>3.8</td>
<td>6024</td>
</tr>
<tr>
<td>Non-cancer</td>
<td>3.5</td>
<td>3652</td>
</tr>
</tbody>
</table>

- No statistically significant difference in age relationship in cancer vs non-cancer participants
- Estimated 160-170% increase in number of CHIP variants per decade \((p=2 \times 10^{-16})\)
- At age 65, the predicted CHIP burden is 6.5 variants per individual in the panel
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

<table>
<thead>
<tr>
<th>Gene</th>
<th>60 - 70 yo with mutation (%)</th>
<th>95% CI</th>
</tr>
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<tbody>
<tr>
<td>KRAS</td>
<td>6% (4, 8)</td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>22% (18, 26)</td>
<td></td>
</tr>
<tr>
<td>TET2</td>
<td>30% (26, 34)</td>
<td></td>
</tr>
<tr>
<td>DNMT3A</td>
<td>51% (46, 55)</td>
<td></td>
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- Suggests a screening population may have confounding CHIP variants in their cfDNA that must be accounted for
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

![Graph showing the number of patients in whom a mutation is found and the percent of nonsynonymous variants.](image-url)
De novo Identification of Mutations that Drive WBC Clonal Expansions

Positively Selected Genes: >1

Neutral Genes: “dNdS” score of 1

Negatively Selected Genes: <1

Positive selective pressure results in more driver mutations

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

Negative selective pressure results in fewer mutations

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

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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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