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Interleukin-6 receptor polymorphism attenuates clonal hematopoiesis-mediated coronary artery disease risk among 451,180 individuals in the UK Biobank

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Clonal hematopoiesis of indeterminate potential (CHIP) is a prevalent age-related condition wherein hematopoietic stem cells acquire a pathogenic mutation in a blood cancer driver gene (most commonly *DNMT3A* or *TET2*) resulting in a clonal expansion. CHIP is associated with incident coronary artery disease (CAD) in large observational cohorts.[1] Mouse models suggest that IL-1β/IL-6 signaling mediates atherosclerosis in CHIP.[1] A retrospective analysis of CANTOS found that treatment with anti-IL1β antibody canakinumab was associated with lower risk of secondary cardiovascular events in individuals with *TET2* CHIP but not *DNMT3A* CHIP.[2] Additionally, we previously reported that individuals with CHIP who were carriers of a common inherited genetic variant associated with dampened IL-6 signaling (*IL6R* p.Asp358Ala, rs2228145-C) – a genetic proxy of IL-6 receptor blockade – had a lower risk of incident cardiovascular events in the initial 50,000-person exome sequencing release of the UK Biobank available in 2019.[3] More recently, the complete 450,000-person UK Biobank exome dataset has been made available. A preliminary analysis of this dataset by Kessler *et al.* showed a small effect size for CHIP and CAD risk, and failed to show a protective effect for rs2228145-C[4], however the authors employed a relaxed genetic variant filtering criteria for CHIP, increasing the likelihood of false positive CHIP calls such as sequencing artifacts and germline variants. We independently examined the dataset for CHIP applying a stringent filtering strategy to

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minimize false positives. Using this high-confidence dataset, we reproduced the association of CHIP with CAD and the interaction between CHIP and *IL6R* p.Asp358Ala.

We built on previously established methods to identify CHIP in 451,180 individuals in the UK Biobank with available exome data and without known hematologic cancers at time of blood draw. Access to UK Biobank was provided under application 43397, and local approval for secondary analyses of the data was obtained from the Vanderbilt University Medical Center institutional review board. The methods are described in detail in a separate manuscript.[5] Briefly, putative somatic CHIP mutations were called with Mutect2 in 74 canonical CHIP genes. Those present in a pre-established list of driver variants were included in the candidate variant list.[1] This candidate list was filtered for sequencing quality; for example, variants with low total sequencing depth (DP < 20) were removed. We then integrated novel population-based filtering parameters, such as removing hotspot variants (present ≥ 20 times in the dataset) that were not associated with at least one well-established marker of CHIP: age and an inherited *TERT* promoter variant (*rs7705526*). We then sought to define the optimal lower threshold for alternate allele read count depth (minAD). Previous studies ascertaining CHIP from exome data have used minAD ranging between 3 and 6. Lower minAD increases sensitivity but introduces false positives. We tested the association of CHIP calls filtered using these cutoffs with age and *rs7705526*. While Kessler *et al.* used minAD of ≥ 3, we identified that a minAD of ≥ 5 was optimally associated with age and TERT (Figure 1A). The CHIP calls and phenotype data are available by application to UK Biobank.

Using our high-confidence CHIP dataset, we assessed the risk of incident coronary artery disease using the same phenotype definition as Kessler *et al.*, *i.e.*, a composite of incident coronary artery or ischemic heart disease, myocardial infarction, revascularization (percutaneous coronary intervention or coronary artery bypass grafting) or death attributed to these causes. Individuals with a history of CAD at enrollment were excluded (N = 14,305). There were 30,967 events. CHIP was associated with an increased cumulative incidence of CAD (Figure 1B) as well as increased risk in a Cox proportional hazards model adjusted for age, age^2, sex, baseline diabetes, smoking history, baseline systolic blood pressure, baseline body-mass index, baseline HDL-C and LDL-C levels, and 10 principal components of genetic ancestry. We identified significant associations between CHIP and CAD as well as an interaction between CHIP and rs2228145-C and CAD (Figure 1C). Whereas DNMT3A CHIP was not independently associated with an increased risk of CAD, the effect sizes for CHIP and IL6R protection were greater for genes other than DNMT3A. Notably, these outcomes were not significant when using a more relaxed minAD threshold of 3 (Figure 1D) as was done by Kessler *et al.*

In conclusion, we show that CHIP – and especially non-DNMT3A CHIP – was associated with incident CAD and that genetic attenuation of IL-6 signaling was protective against this CHIP-associated risk in the full ~450,000 person UK Biobank exome dataset, consistent with our prior report on the 50,000 person subset of this data.[3] Our observation distinguishing DNMT3A CHIP vs non-DNMT3A CHIP is highly concordant with the CANTOS observation that *TET2* CHIP patients and not *DNMT3A* CHIP patients had increased benefit from canakinumab. This work highlights the challenges in distinguishing

true CHIP from artifact and presents strategies that can be used to derive high-confidence CHIP calls in population biobanks to robustly identify CHIP cardiovascular disease consequences.

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Conflict of Interest Disclosures:

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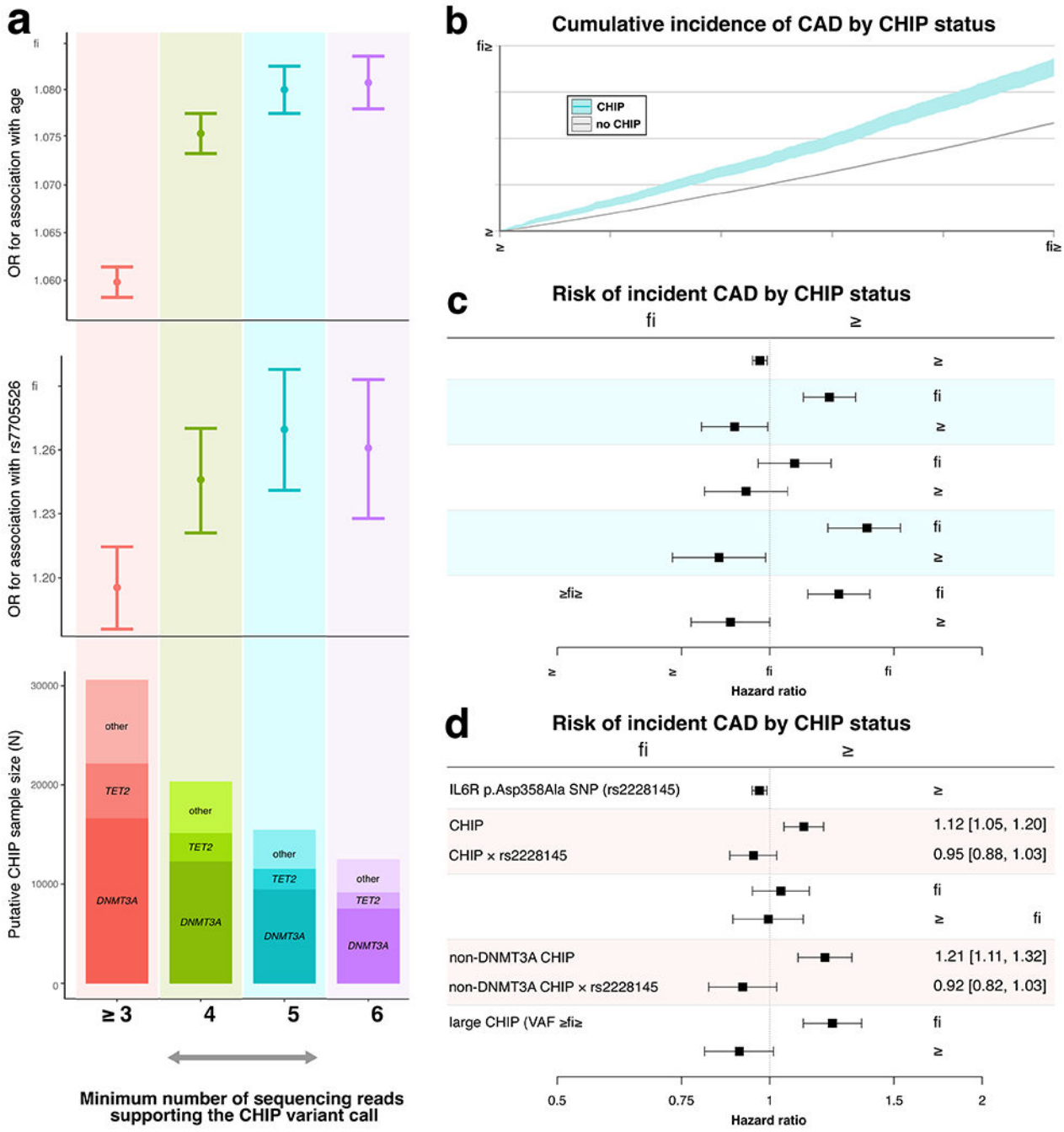

Figure 1. Genetic attenuation of IL6R signaling is protective against CHIP-associated CAD risk in the UK Biobank 450k dataset when high-confidence CHIP calls are used.

a) Associations between CHIP with increasing numbers of next generation sequencing reads that support the CHIP variant and age (top panel) and TERT genetic variant rs7705526 (middle panel), assessed using a logistic regression with binary CHIP status as the outcome. The rs7705526 genotype was coded using an additive model. The odds ratios represent per unit SD increase in the predictor variable. The total number of individuals putatively identified with CHIP at each of these thresholds is shown in the bottom panel. 5 supporting

reads was the optimal balance of sensitivity and specificity based on associations with age and TERT genetic variant.

b) Cumulative incidence of CAD by CHIP status, defined with at least 5 supporting reads.

c) When CHIP is defined based on at least 5 supporting reads, CHIP is associated with incident CAD and *IL6R* p.Asp358Ala SNP is protective against this risk. This association is observed for non-DNMT3A CHIP and CHIP with a large VAF (≥10%), but not DNMT3A-CHIP.

d) When CHIP is defined using a relaxed criteria of only 3 supporting reads, the association of CHIP with incident CAD is greatly attenuated and *IL6R* p.Asp358Ala SNP is not protective.