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JAK2-mutant Clonal Hematopoiesis is Associated with Venous Thromboembolism

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Abstract:
Venous thromboembolism (VTE) is common among older individuals, but provoking factors are not identified in many cases. Patients with myeloid malignancies, especially myeloproliferative neoplasms, are at increased risk for venous thrombosis. Clonal hematopoiesis of indeterminate potential (CHIP), a precursor state to myeloid malignancies, is common among the elderly and may similarly predispose to venous thrombosis. We evaluated overall and genotype-specific associations between CHIP and prevalent and incident VTE in >400,000 samples from the UK Biobank. CHIP was modestly associated with incident VTE with a hazard ratio of 1.17 (95% confidence interval (CI) 1.09-1.3; p= 0.002) but was not significantly associated with prevalent VTE with an odds ratio of 1.02 (95% CI 0.81-1.23; p= 0.81). TET2-mutant CHIP was associated with incident VTE with a hazard ratio of 1.33 (95% CI 1.05-1.69; p= 0.02). JAK2 mutations were highly associated with both prevalent and incident VTE risk with odds ratio of 6.58 (95% CI 2.65-16.29; p= 4.7 x 10-5) and hazard ratio of 4.2 (95% CI 2.18-8.08; p= 1.7 x 10-5), respectively, consistent with the thrombophilia associated with JAK2-mutant myeloproliferative neoplasms. The association between JAK2-mutant CHIP and VTE remained significant after excluding potential undiagnosed myeloproliferative neoplasms based on laboratory parameters. Compared to heterozygous factor V Leiden and heterozygous prothrombin gene mutation, JAK2-mutant CHIP was more strongly associated with VTE but was less common. These results indicate that most individuals with CHIP do not have an altered risk of thrombosis, but that individuals with JAK2-mutant CHIP have a significantly elevated risk of VTE.

Conflict of interest: COI declared - see note

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Clinical trial registration information (if any):

Figure 1

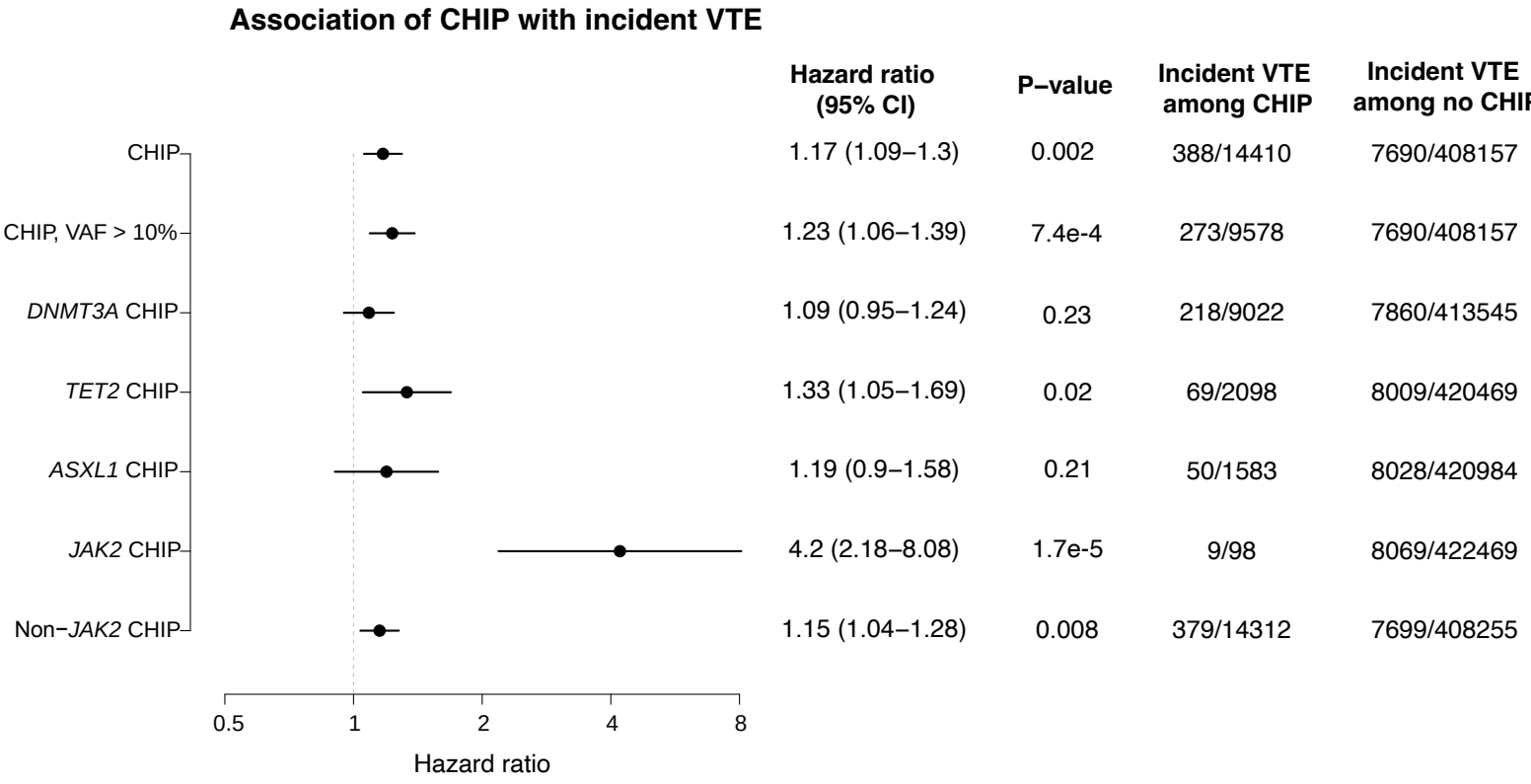


Figure 2

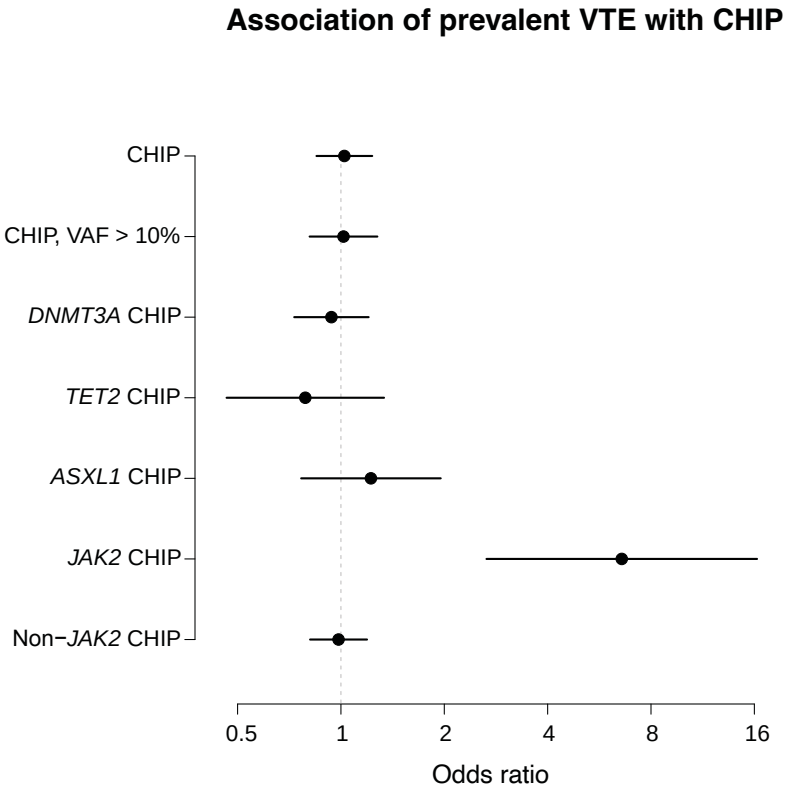
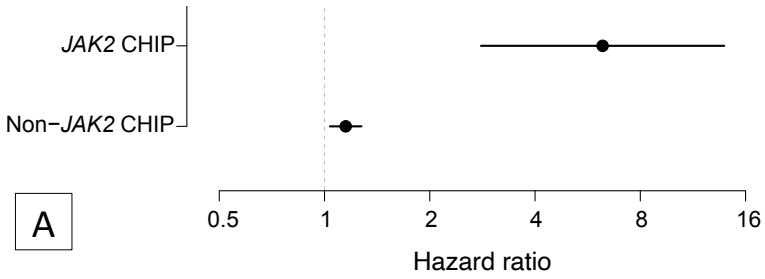
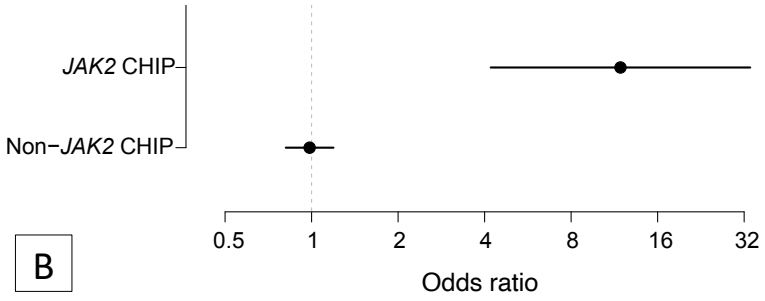


Figure 3



A

	Hazard ratio (95% CI)	P−value	Incident VTE among CHIP	Incident VTE among no CHIP
JAK2 CHIP	6.24 (2.8–13.9)	7.4e-6	6/43	8069/422469
Non−*JAK2* CHIP	1.15 (1.04–1.28)	0.008	379/14312	7696/408200



B

	Odds ratio (95% CI)	P−value	CHIP with prevalent VTE	CHIP without prevalent VTE
JAK2 CHIP	11.88 (4.2–33.59)	3.1e-6	4/2831	43/422512
Non−*JAK2* CHIP	0.98 (0.81–1.19)	0.87	112/2831	14312/422512

JAK2-mutant Clonal Hematopoiesis is Associated with Venous Thromboembolism

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The patient cohort used for this study is the UK Biobank. The source data are available to the approved researchers through the UK Biobank. Individual-level UK Biobank data are available for approved researchers from https://www.ukbiobank.ac.uk. This cohort has been used in multiple other studies, including: Niroula A, Sekar A, Murakami MA, Trinder M, Agrawal M, Wong WJ, Bick AG, Uddin MM, Gibson CJ, Griffin GK, Honigberg MC, Zekavat SM, Paruchuri K, Natarajan P, Ebert BL. Distinction of lymphoid and myeloid clonal hematopoiesis. Nat Med. 2021 Nov;27(11):1921-1927. doi: 10.1038/s41591-021-01521-4. Epub 2021 Oct 18. PMID: 34663986; PMCID: PMC8621497. Kishtagari A, Khan MAW, Li Y, Vlasschaert C, Marneni N, Silver AJ, von Beck K, Spaulding T, Stockton S, Snider C, Sochacki A, Dorand D, Mack TM, Ferrell PB Jr, Xu Y, Bejan CA, Savona MR, Bick AG. Driver mutation zygosity is a critical factor in predicting clonal hematopoiesis transformation risk. Blood Cancer J. 2024 Jan 15;14(1):6. doi: 10.1038/s41408-023-00974-9. PMID: 38225345; PMCID: PMC10789770.

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Key Points:

- *JAK2*-mutant clonal hematopoiesis is associated with a strong risk of venous thromboembolism (VTE).
- *JAK2*-mutant CHIP confers greater risk of VTE than heterozygous thrombophilias but is present in lower frequency in the general population.

Abstract

Venous thromboembolism (VTE) is common among older individuals, but provoking factors are not identified in many cases. Patients with myeloid malignancies, especially myeloproliferative neoplasms, are at increased risk for venous thrombosis. Clonal hematopoiesis of indeterminate potential (CHIP), a precursor state to myeloid malignancies, is common among the elderly and may similarly predispose to venous thrombosis. We evaluated overall and genotype-specific associations between CHIP and prevalent and incident VTE in >400,000 samples from the UK Biobank. CHIP was modestly associated with incident VTE with a hazard ratio of 1.17 (95% confidence interval (CI) 1.09-1.3; $p = 0.002$) but was not significantly associated with prevalent VTE with an odds ratio of 1.02 (95% CI 0.81-1.23; $p = 0.81$). *TET2*-mutant CHIP was associated with incident VTE with a hazard ratio of 1.33 (95% CI 1.05-1.69; $p = 0.02$). *JAK2* mutations were highly associated with both prevalent and incident VTE risk with odds ratio of 6.58 (95% CI 2.65-16.29; $p = 4.7 \times 10^{-5}$) and hazard ratio of 4.2 (95% CI 2.18-8.08; $p = 1.7 \times 10^{-5}$), respectively, consistent with the thrombophilia associated with *JAK2*-mutant myeloproliferative neoplasms. The association between *JAK2*-mutant CHIP and VTE remained significant after excluding potential undiagnosed myeloproliferative neoplasms based on laboratory parameters. Compared to heterozygous factor V Leiden and heterozygous prothrombin gene mutation, *JAK2*-mutant CHIP was more strongly associated with VTE but was less common. These results indicate that most individuals with CHIP do not have an altered risk of thrombosis, but that individuals with *JAK2*-mutant CHIP have a significantly elevated risk of VTE.

Introduction

Venous thromboembolism (VTE) affects nearly 10 million people worldwide every year[1]. Risk factors for VTE include recent major surgery, malignancy, and inherited thrombophilia. However, 25-50% of venous thromboembolic events do not have a clear, identifiable cause[2]. Myeloid malignancies increase the risk of venous thrombosis, especially among those with myeloproliferative neoplasms (MPN)[3,4]. Studies have shown a 10-fold increased risk of MPN-associated thrombotic events compared to healthy individuals[5-7].

Clonal hematopoiesis of indeterminate potential (CHIP) is a premalignant state characterized by myeloid malignancy driver mutations with a variant allele fraction ≥0.02 in individuals without blood count abnormalities[8,9]. CHIP increases in prevalence with age, occurring in approximately 10% of individuals over 70 years old[10,11]. CHIP is associated with an increased risk of hematologic malignancies and has also been associated with non-hematologic pathologies. These conditions, many of which are etiologically linked to inflammation, include cardiovascular disease[12,13], stroke[14], kidney disease[15,16], rheumatologic conditions[17], and liver disease[18].

Studies evaluating the relationship between CHIP and VTE have arrived at conflicting results. Retrospective studies have found prevalent CHIP in 19% of patients with unprovoked pulmonary embolism[19], 37.8% and 46% in patients with splanchnic vein thrombosis[20,21], and 3.7% in patients with unprovoked proximal VTE[22]. In a larger cohort study, in which 45% of individuals had schizophrenia, incident venous thrombosis rates in patients with CHIP were 5% compared to 2.1% in patients without CHIP[23]. Examination of genotype-specific associations between CHIP and VTE requires substantially larger cohorts. We, therefore, examined CHIP and VTE in the UK Biobank, a study with >400,000 individuals linked to exome sequencing of peripheral blood DNA and detailed clinical phenotypes.

Methods

UK Biobank and Analyses:

502,490 individuals from the UK Biobank were evaluated for inclusion in this study. Data were obtained for these participants through UK Biobank application 50834. Individuals with whole-exome sequencing data and non-missing data across all covariates were included. Individuals were excluded if genomic analyses did not pass quality control, individuals were outliers for heterozygosity or missing rate, the individual had a prevalent diagnosis of a hematologic malignancy (list of diagnosis codes as previously described[24]) prior to DNA sampling or within 6 months after enrollment in the study, or consent was withdrawn.

VTE events were identified based on ICD10 codes (Supplementary Table 1). Prevalent and incident VTE cases were ascertained with respect to the time of DNA sampling. Prevalent VTE was defined as having a VTE at or prior to the time of DNA sampling. An incident VTE was defined as an event that happened after DNA sampling. Median follow-up time for incident VTE was 11.8 years. Associations of CHIP and VTE were assessed using logistic regression for prevalent analyses and Cox proportional hazards for incident analyses. For the incident analysis, we excluded those with prevalent VTE at the time of DNA sampling. The association analyses were adjusted for age at the time of DNA sampling, age^2, sex, European ancestry, first five genetic principal components, smoking status (ever vs. never), and body mass index (BMI) as covariates. Analyses were conducted in R Studio.

CHIP detection:
CHIP mutations were identified as described previously[24] using whole-exome sequencing data from blood DNA from UK Biobank participants. Somatic variants in genes associated with clonal hematopoiesis and/or myeloid malignancies were detected using Mutect2[10,25-27]. The list of genes in which pathogenic mutations were identified for CHIP calling and gene-level sequencing coverage statistics for this cohort are described elsewhere[28]. The variant allele fraction (VAF) had to be at least 0.02 to be considered a CHIP mutation. A minimum total read depth of 20 and a minimum read depth of 5 supporting the alternative allele were required. To minimize likelihood of detecting germline variants and artifacts, the Genome Aggregation Database (gnomAD) was used as a germline reference, and a panel-of-normal derived from the youngest participants in the UK Biobank cohort was utilized.

Definition of potential undiagnosed cases of MPN:
With the inclusion of a *JAK2* mutation, laboratory findings suggestive of myeloproliferative neoplasm were defined as polycythemia vera with hemoglobin >16.5 g/dL for males or hemoglobin >16 g/dL for females or essential thrombocytosis with platelets >450 x 10^9/L[29] based on the International Consensus Classification[29]. *JAK2* mutations in this analysis were all V617F.

Association of VTE with heterozygous inherited thrombophilias:
Association of VTE with factor V Leiden (heterozygous) and prothrombin gene mutation (heterozygous) was analyzed in the UK Biobank based on single nucleotide polymorphism genotypes for rs6025 and rs1799963, respectively. The risk associated with heterozygous factor V Leiden or prothrombin gene mutation was compared to individuals who were homozygous wildtype (individuals with homozygous factor V Leiden mutation or homozygous prothrombin mutation were not included in the analysis given their low frequency). Association was analyzed in a logistic regression model with VTE as the binary outcome variable and SNP genotype as the predictor variable with covariates of age, age^2, sex, BMI, European ancestry, smoking status (ever vs. never), and the first five genetic principal components as covariates.

Results

Analysis of CHIP with incident VTE:
Given the established association between myeloid neoplasms and VTE, we sought to determine whether a similar relationship existed between CHIP and VTE. Of 502,490 individuals in the UK Biobank, 425,399 individuals had high-quality exome sequencing, did not have prevalent hematologic malignancies by ICD10 codes, and had documented covariates. Among these individuals, the mean age of participants was 56.6 years, 54% were female, 84% were of European ancestry, and 60% had ever smoked.

CHIP was modestly associated with incident VTE with a hazard ratio of 1.17 (95% CI=1.09-1.3; p= 0.002), and CHIP with VAF greater than 10% had a hazard ratio of 1.23 (95% CI=1.06-1.39; p= 7.4 x 10^{-4}) (Figure 1). We evaluated the three most commonly mutated genes in CHIP and found that *TET2*-mutant CHIP was modestly associated with incident VTE with a hazard ratio of

1.33 (95% CI=1.05-1.69; p= 0.02), but *DNMT3A*-mutant and *ASXL1*-mutant CHIP were not significantly associated with VTE.

As *JAK2*-mutant myeloproliferative neoplasms are a potent risk factor for VTE, we hypothesized that *JAK2*-mutant CHIP may be particularly associated with an increased risk of VTE. Indeed, we found that *JAK2*-mutant CHIP was also strongly associated with incident VTE with a hazard ratio (HR) of 4.2 (95% CI=2.18-8.08; $p = 1.7 \times 10^{-5}$). Among 98 individuals with *JAK2*-mutant CHIP, 9 had a documented incident VTE during a median of 6.98 years of follow-up (Figure 1). Given the strong association between *JAK2*-mutant CHIP and VTE and the fact that patients with *JAK2*-mutant myeloproliferative neoplasms have been found to have venous thrombotic events at unusual sites, such as splanchnic vein thromboses[30,31], we evaluated the types of venous thrombotic events seen in our cohort. In the nine individuals with *JAK2*-mutant and incident VTE, four had deep vein thrombosis, four had pulmonary embolism, and one had portal vein thrombosis. In these nine individuals, six developed an MPN after DNA sampling and before or at the time of incident VTE.

<u>*JAK2*-mutant CHIP is strongly associated with prevalent VTE:</u>
Since CHIP may exist for many years, we analyzed the frequency of CHIP in individuals with and without prevalent VTE (defined as a VTE event at or prior to DNA sampling). We again found that *JAK2*-mutant CHIP was strongly associated with prevalent VTE, with an odds ratio (OR) of 6.58 (95% CI=2.65-16.29; $p = 4.7 \times 10^{-5}$). Among 2,832 individuals with a prevalent VTE, 5 individuals had *JAK2* mutations. Of these five individuals, two had deep vein thrombosis, one had pulmonary embolism, one had portal vein thrombosis, and one had Budd Chiari based on ICD10 codes. In contrast, there was no association found between prevalent VTE and CHIP overall (OR= 1.02; 95% CI=0.81-1.23; p= 0.81) or CHIP overall with VAF greater than 10% (OR 1.02; 95% CI=0.72-1.28; p= 0.88) (Figure 2).

<u>*JAK2*-mutant CHIP is strongly associated with VTE after excluding potential undiagnosed MPNs:</u>
We next considered the possibility that the VTE association with *JAK2*-mutant CHIP could be driven by undiagnosed cases of myeloproliferative neoplasms that are not diagnosed or captured by ICD10 codes. To identify potential *JAK2*-mutated MPNs in the UK Biobank that were not identified based on ICD10 codes, we analyzed elevations in laboratory values of hemoglobin and platelet counts using criteria established by the International Consensus Classification (Supplementary Table 2).[9] With MPN diagnoses excluded by both ICD10 codes and in those with cytoses, *JAK2*-mutant CHIP remained significantly associated with incident VTE with a hazard ratio of 6.24 and prevalent VTE with an odds ratio of 11.88 (Figure 3A and 3B, respectively). While those with cytopenias may represent advanced disease and would therefore be more likely to have a formal diagnosis, we further analyzed the association of *JAK2*-mutant CHIP with both incident and prevalent VTE when MPN diagnoses were excluded based on ICD10 codes, cytoses, and cytopenias; the hazard ratio for incident VTE with *JAK2*-mutant CHIP was 7.49 and the prevalent odds for VTE ratio was 14.59 (Supplementary Figure 1).

<u>Risk of VTE with *JAK2*-mutant CHIP relative to inherited heterozygous thrombophilias:</u>
Although *JAK2*-mutant CHIP was associated with a significantly increased risk of incident VTE (HR= 6.24), it accounted for only a small fraction (5/2,832) of individuals with prevalent VTE

(Table 1). The median follow-up time to VTE for *JAK2*-mutant CHIP was 6.98 years, and for non-*JAK2*-mutant CHIP was 7.23 years. To contextualize these findings with well-established risk factors for VTE in the same cohort, we examined the risk of inherited thrombophilias. Using the exome sequencing data in the UK Biobank, we identified pathogenic mutations in factor V Leiden and the prothrombin gene. Heterozygous factor V Leiden and heterozygous prothrombin gene mutation G20210A had hazard ratios of 2.36 and 1.91, respectively. The median age of VTE diagnosis for both heterozygous thrombophilias was 64.88 years. These studies demonstrate that the impact of *JAK2*-mutant CHIP on risk of VTE may be greater than common heterozygous inherited thrombophilias, though the prevalence of *JAK2*-mutant CHIP is lower than these germline risk alleles.

Discussion

Our study demonstrates definitively that, in a large population-based cohort with whole exome sequencing, *JAK2*-mutant CHIP is most strongly associated with both incident and prevalent VTE compared to other CHIP genotypes. While other CHIP genotypes have been associated with inflammatory disorders, VTE is selectively associated with *JAK2* mutations[10,12,32,33]. Our findings validate reports from smaller cohorts, non-population-based cohorts, or cohorts that had more limited genotyping of somatic mutations[23,34-36]. The relationship between *JAK2*-mutant CHIP and VTE likely involves multiple mechanisms including increased NETosis and impacts on platelets and red blood cells[23,37]. *JAK2*-mutant CHIP was also recently found to increase risk of arterial thrombosis due to younger and more activated platelets[38].

The UK Biobank cohort enabled us to examine the strength of association between *JAK2*-mutant CHIP and VTE, as well as the impact of germline predisposition to VTE in the same population. Similarly, the relative prevalence of these germline and somatic risk factors was assessed in the same cohort. Somatic *JAK2* mutations carry a higher risk of thrombosis in the UK Biobank than inherited mutations in the Factor V or Prothrombin genes. On the other hand, somatic *JAK2* mutations were much rarer than the major inherited thrombophilias. The prevalence of *JAK2*-mutant CHIP in our study was 0.02%, which is lower than prior documented rates ranging from 0.14-3.1% depending on the depth of sequencing coverage[11,34-36]. The exome sequencing data in the UK Biobank had lower coverage of the *JAK2* locus than other CHIP genes, thereby decreasing the sensitivity of detection for smaller *JAK2*-mutant clones. On the other hand, it is likely that the VTE risk from smaller *JAK2* clones is lower than the risk associated with larger clones, as has been demonstrated for other CHIP-associated phenotypes[10,12,39]. A Danish Population Study of around 20,000 individuals showed an odds ratio for prevalent VTE of 2.8 in individuals with *JAK2* VAF $\geq$0.01 versus an odds ratio of 0.71 with *JAK2* VAF<0.01, further supporting the increased risk of VTE with larger *JAK2* clone sizes as found in our cohort[35]. Using an estimated prevalence of *JAK2* V617F of 2/1000 in the general population based on the middle of the range from our study and others[11,34-36], and approximately 77 million individuals over age 60 in the US[40], 154,000 individuals in the US would be estimated to have *JAK2*-mutant CHIP[39].

Our findings demonstrate that, among individuals with CHIP, those with *JAK2*-mutant CHIP have a striking risk of developing a VTE. While we do not recommend screening for *JAK2* mutations, particularly due to the low prevalence of *JAK2*-mutant CHIP, the index of suspicion may be increased by blood count parameters or VTE location. In the UK Biobank, individuals

with CHIP who developed a VTE event tended to have higher hemoglobin and/or platelet counts, even though values were within the normal range and would therefore not be consistent with a diagnosis of MPN based on these parameters. In addition, individuals with *JAK2*-mutant CHIP may have VTE events in unusual locations, such as splanchnic vein thrombosis, based on data from the UK Biobank and prior publications[41,42]. Even without screening, widespread use of next-generation sequencing panels has resulted in an increased number of individuals diagnosed with *JAK2*-mutant CHIP. Future studies will aid in determining approaches to VTE risk reduction for individuals with *JAK2*-mutant CHIP.

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Author Contributions
RLZ, AS, and BLE designed the research, performed the research, analyzed the data, and wrote the paper. KC performed the research, contributed data for the Factor V Leiden and Prothrombin gene mutation analyses, and contributed analytical tools. KC, OO, AN, AB, CJG, GG, MMU, PN contributed analytical tools. DN performed the research and analyzed the data.

Disclosure of Conflicts of Interest
BLE has received research funding from Celgene, Deerfield, Novartis, and Calico and consulting fees from Abbvie and GRAIL. He is a member of the scientific advisory board and shareholder for Neomorph Inc., TenSixteen Bio, Skyhawk Therapeutics, and Exo Therapeutics.
PN reports research grants from Allelica, Apple, Amgen, Boston Scientific, Genentech / Roche, and Novartis, personal fees from Allelica, Apple, AstraZeneca, Blackstone Life Sciences, Foresite Labs, Genentech / Roche, GV, HeartFlow, Magnet Biomedicine, and Novartis, scientific advisory board membership of Esperion Therapeutics, Preciseli, and TenSixteen Bio, scientific co-founder of TenSixteen Bio, equity in MyOme, Preciseli, and TenSixteen Bio, and spousal employment at Vertex Pharmaceuticals, all unrelated to the present work. AB is on the Scientific Advisory board membership at TenSixteen Bio. KC is on the Advisory board for United Therapeutics, and receives consulting fees from Amgen, Tectonic Therapeutics, and United Therapeutics. AS reports stock in Vertex. RLZ is a stockholder and receives consultancy fees in Triveni Bio. OO, AN, CJG, GG, and MMU report no conflicts of interest.

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Tables

Table 1. *JAK2*-mutant CHIP relative incident risk of venous thromboembolism and prevalence in the general population compared to heterozygous factor V Leiden and heterozygous prothrombin gene mutation G20210A in the UK Biobank.

Thrombophilia	**Hazard ratio of first episode of VTE compared with controls**	**Prevalence in the population**
Heterozygous Factor V Leiden	2.36 (95% CI: 2.21-2.52)	4.4%
Heterozygous prothrombin G20210A	1.91 (95% CI: 1.74-2.10)	2.3%
JAK2 CHIP	6.24 (95% CI: 2.8-13.9)	98/430,000 = 0.02% *0.14-3% in other studies[11,37-39]
Non-*JAK2* CHIP	1.15 (95% CI: 1.04-1.28)	14,417/430,000= 3.4%

Figure Legends

Figure 1. Incidence of venous thromboembolism in individuals with CHIP using logistic regression with analysis corrected for age, sex, European ancestry, genetic principal components, ever smoked status, and body mass index.
VTE: venous thromboembolism
VAF: variant allele fraction

Figure 2. Association of CHIP with prevalent venous thromboembolism using Cox proportional hazards with analysis corrected for age, sex, European ancestry, genetic principal components, ever smoked status, and body mass index.
VTE: venous thromboembolism
VAF: variant allele fraction

Figure 3. Association between *JAK2*-mutant CHIP and incident VTE (A) and *JAK2*-mutant CHIP and prevalent VTE (B) with myeloid neoplasms excluded by ICD10 codes and potential cases of undiagnosed myeloproliferative neoplasms excluded based on cytoses.

Association of Clonal Hematopoiesis with Venous Thromboembolism

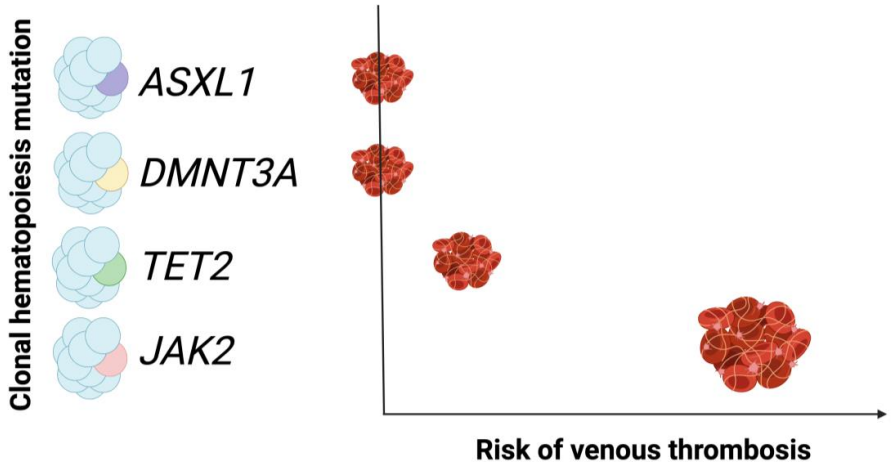
Context of Research

Myeloid malignancies are associated with increased risk of venous thrombosis but associations between clonal hematopoiesis driven by specific genotypes and venous thrombosis are less clear

Aim of This Study

In this work, we evaluated overall and genotype-specific associations between CHIP and prevalent and incident venous thromboembolism in >400,000 samples from the UK Biobank

Main Findings



Conclusions: ***JAK2*-mutant clonal hematopoiesis is strongly associated with venous thromboembolism in contrast to clonal hematopoiesis with other gene mutations.**

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blood Visual Abstract