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Platelets, Thrombosis and Haemostasis

Association of clonal haematopoiesis with recurrent venous thromboembolism: A case–control study

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Summary

Venous thromboembolism (VTE) is the third most common cardiovascular disease. Clonal haematopoiesis (CH) is linked to cardiovascular disease risk, but its potential association with VTE remains poorly understood. We assessed the prevalence of CH in patients with recurrent VTE ($n = 107$; median age [IQR] 57 [48–63] years, 44.9% female) and matched healthy controls ($n = 127$; median age [IQR] 53 [45–60] years, 51.2% female) to investigate a putative association of CH with VTE risk. We detected 12 CH-associated mutations in 11 (10.3%) VTE cases and six mutations in 5 (3.9%) controls. Thus, patients with recurrent VTE tended to have higher odds of presenting with CH compared to controls (OR: 2.74, 95% CI: 0.95–9.16). Moreover, the odds of detecting CH were significantly higher in VTE cases in the subgroup of individuals without thrombophilia (OR: 4.58, 95% CI: 1.48–15.99). VTE cases with CH showed elevated platelet counts compared to cases and controls without CH (median [IQR]: 292 [254–298], 223 [198–260] and 220 [185–259] $\times 10^9/L$; both $p < 0.01$). Fibrinogen, sP-selectin, D-dimer and hsCRP levels did not differ according to CH status. Overall, we identified a trend for an association between CH and recurrent VTE, particularly in individuals without underlying thrombophilia, warranting further research in this patient group.

KEYWORDS

clonal haematopoiesis, risk factors, thromboembolism, thrombophilia, venous thromboembolism

INTRODUCTION

Venous thromboembolism (VTE), a disease entity comprising deep vein thrombosis and pulmonary embolism (PE), has an estimated incidence of 100–200 per 100 000 person-years in Europe,¹ making it the third most common cardiovascular disease (CVD). The disease burden of VTE is high due to increased morbidity, functional limitations, working disability and health-care utilization.^{2–4}

VTE risk factors can be categorized as transient or chronic as well as major or minor.⁵ Genetic or acquired factors can cause abnormalities in the coagulation system, resulting

in an increased risk of thrombosis, collectively referred to as thrombophilia. In clinical practice, about 60% of VTE patients have identifiable risk factors (e.g. cancer, immobilization), but this is not the case in 40%, defining these as unprovoked VTE.¹ Unprovoked VTE has a high recurrence risk, significantly impacting quality of life and increasing bleeding risk due to the indefinite anticoagulant treatment recommended in guidelines to prevent recurrence.⁶

Clonal haematopoiesis (CH) involves the expansion of haematopoietic stem cells due to acquired mutations that provide a proliferative or survival advantage. CH is prevalent in the ageing population and has been linked to

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increased all-cause mortality and CVD such as coronary artery disease, peripheral artery disease and stroke.^{7–11} This is likely due to an altered inflammatory profile mediated by CH-associated mutations in immune cells, especially macrophages.^{12,13} Platelet and endothelial cell activation, leading to increased P-selectin expression, may also play a role in CH affecting CVD.¹² Similar to their role in arterial disease, these mechanisms in general are also involved in venous thrombus formation.¹⁴ Therefore, an implication of CH in VTE might exist, particularly as CH prevalence increases with advancing age, the latter representing a modifying factor for VTE risk.^{1,10} Moreover, previous studies have reported an increased prevalence of CH in patients with unprovoked VTE compared to the general population,^{15,16} with recent UK Biobank data supporting an association between CH and VTE.¹⁷ These studies were in part limited by either low sample size, absence of matched controls or lack of detailed clinical information of the included patients. Furthermore, reported CH frequencies are heterogeneous between studies and the use of different detection methods impedes direct comparisons.

Our aim was to assess CH prevalence in patients with recurrent VTE, with at least one unprovoked event, in comparison with age- and sex-matched controls and to explore potential associations between CH and inflammatory and haemostatic biomarkers as well as biomarkers of biological ageing.

METHODS

Patient cohort

This case–control study was conducted at the Medical University of Vienna (Vienna, Austria) and details have been described previously.^{18,19} In brief, consecutive unrelated patients with a history of recurrent VTE, including at least one unprovoked event, referred to the outpatient department for an assessment of thrombosis risk factors were recruited and provided written informed consent for study inclusion. Unprovoked VTE which was objectively verified was defined as VTE not occurring in the context of a triggering condition such as surgery, trauma causing immobilization, pregnancy, delivery, malignancy, hospital stay or bed-ridden period due to illness, hormone replacement therapy or oral contraception.²⁰ Established risk factors for VTE leading to thrombophilia (protein C-, protein S- or antithrombin deficiency; lupus anticoagulant; elevated homocysteine levels; factor V Leiden and prothrombin G20210A variation) were routinely evaluated in all study participants (cases and controls). Whole-group—not pairwise—age- and sex-matched healthy controls were selected from the same ethnic and geographic background. Healthy controls were either spouses or acquaintances of patients, hospital staff or friends or relatives of hospital staff but were unrelated to patients and had no history of VTE or arterial thromboembolic events, as described previously.²⁰ The study was approved by the local

ethics committee. We analysed 116 cases and 128 controls with available whole-blood samples, of which nine cases and one control had to be excluded from analyses due to insufficient read coverage in the sequencing analysis.

Sample preparation and biomarker measurements

DNA was isolated from patients' peripheral blood samples, which were collected ≥ 3 months after the most recent VTE event as described previously,²⁰ using the Maxwell® RSC Blood DNA Kit on a Maxwell® RSC Instrument (all Promega, Vienna, Austria). Furthermore, routine laboratory parameters were measured centrally at the Department of Laboratory Medicine of the Medical University of Vienna and biomarkers were measured as described previously.^{18–20}

Targeted resequencing

Library preparation for targeted resequencing of selected genomic regions including hotspots for CH-associated mutations was performed as previously described,²¹ expanded to include screening of selected DNA damage repair genes. In brief, genomic regions of interest involving the genes *DNMT3A*, *TET2*, *ASXL1*, *JAK2*, *PPM1D*, *CHEK2* and *TP53* were amplified using a Multiplex-PCR approach. Individual patient samples were subsequently indexed using the Nextera XT Index Kit v2 (Illumina, San Diego, CA) and purified using a solid-phase reversible immobilization (SPRI) approach using Agencourt AmPure XP magnetic beads (Beckman Coulter, Indianapolis, IN). Finally, library pools of up to 384 uniquely tagged samples were sequenced on an MiSeq instrument (MiSeq Reagent Kit v3 in 2×300 bp paired-end, all Illumina, San Diego, CA). Demultiplexing and subsequent somatic variant calling were performed using the Local Run Manager v2 software of the MiSeq instrument. Briefly, reads were aligned to the human reference genome version hg19 using the Burrows-Wheeler Aligner (BWA) implementation of the Local Run Manager. A custom manifest file containing the amplicon regions of interest was used. Somatic variants were called from a variant allele frequency of 0.01 at a minimum variant site depth of 10. Indel Realignment was switched on. All patient samples included in the study underwent targeted resequencing in a screening run. Samples carrying positively selected variants were subjected to a validation run, which involved a second round of sequencing subsequent to a separate library preparation under identical conditions.

Variant annotation and filtering

For variant annotation, RefSeq was selected. Variants were filtered as previously described.²¹ In brief, the following inclusion criteria were used: variant site depth ≥ 100 , variant reported

as somatic in COSMIC,²² population allele frequency <0.01% in gnomAD databases,²³ protein-coding, non-synonymous, variant allele frequency (VAF) between 2%–40% or 60%–90% (except for *JAK2*^{V617F} mutations, where all VAF ≥2% was accepted). For variants passing filters applied on the screening run, samples were re-run to confirm validity at adjusted filter settings (inclusion criteria for validation run: variant site depth ≥200, VAF ≥1%). While variants not passing the validation criteria were removed, variant call characteristics including VAFs for validated variants were exported from the screening run.

Statistical considerations

Statistical analyses were performed using SPSS (version 28.0, IBM SPSS Statistics, Chicago, IL, USA) and R Studio (version 4.2.3, Vienna, Austria). Standard statistical summary modes were used to depict patient characteristics (absolute and relative frequencies, median, interquartile range [IQR]). Between-group differences of clinical characteristics, laboratory parameters and biomarker levels were assessed with a chi-square test or a Mann–Whitney *U*-test as appropriate. When the expected frequencies in the contingency table cells were below 5, a Fisher exact test was conducted. Odds ratios (OR) were calculated based on a 2 × 2 contingency table with corresponding 95% confidence intervals (CI) to assess the association between CH and prevalent VTE and a *p*-value was calculated using a chi-square test. A logistic regression model

was used to investigate the interaction between CH and thrombophilia on recurrent VTE which included only the interaction term. An OR for the interaction term was estimated along with its 95% confidence interval (CI). The alpha level was set at 0.05.

RESULTS

Patient characteristics

In this study, 107 patients with recurrent VTE (median age [IQR] 57 [48–63] years, 44.9% female) and 127 healthy controls (median age [IQR] 53 [45–60] years, 51.2% female) were included. Detailed patient characteristics are summarized in Table 1. Details on the type and location of VTE events that have occurred in cases are depicted in Table S1.

Detection of CH-associated mutations

Overall, we detected 18 somatic mutations in 16 individuals (6.8%). In 107 VTE cases, 12 variants were detected in 11 patients (10.3%), whereas six variants were detected in five controls (3.9%). Overall, the most commonly mutated gene was *JAK2* (*n* = 7, 38.8%) followed by *DNMT3A* (*n* = 5, 27.8%), whereas no mutations were found in *TP53*, *PPM1D* or *CHEK2* (Figure 1A). In patients with recurrent VTE, five *JAK2* (41.6%), three *DNMT3A* (25.0%), two *TET2* (16.7%)

TABLE 1 Patient characteristics of patients with recurrent VTE (cases, *n* = 107) and matched healthy controls (*n* = 127).

	Cases		Controls		<i>p</i> -value
	<i>n</i> of available data	<i>n</i> (%) / median (IQR)	<i>n</i> of available data	<i>n</i> (%) / median (IQR)	
Female	107	48 (44.9)	127	65 (51.2)	0.34
Age	107	57 (48–63)	127	53 (45–60)	0.02
BMI	106	27.4 (24.8–29.1)	127	25.8 (23.6–29.4)	0.06
Comorbidities					
Hypertension	99	27 (27.3)	122	23 (18.1)	0.14
Thrombophilia	102	45 (44.1)	127	15 (11.8)	<0.001
High risk ^a		15 (14.7)		1 (0.8)	
Low risk ^b		30 (29.4)		14 (11.0)	
Metabolic syndrome ^c	102	35 (35.5)	127	26 (20.5)	0.005
Blood group					
A	106	58 (54.7)	127	59 (46.5)	0.06
AB		12 (11.3)		13 (10.2)	
B		21 (19.8)		18 (14.2)	
O		15 (14.2)		37 (29.1)	
Current or prior smoking exposure	106	27 (25.5)	127	28 (22.0)	0.54

^aIncluding individuals with heterozygous FV: R50Q60 or FII: G20210A mutation.

^bIncluding individuals with homozygous FV: R50Q60 or FII: G20210A mutation, Protein C or S deficiency, antithrombin deficiency, lupus anticoagulant or a combined heterozygous FV: R50Q60 and FII: G20210A mutation.

^cAny three of the following five criteria constituted a diagnosis of metabolic syndrome: elevated waist circumference, elevated triglycerides, reduced HDL-C, elevated blood pressure, elevated fasting glucose.¹⁹

and two *ASXL1* (16.7%) mutations were detected (Figure 1B). In healthy controls, two *JAK2* (33.3%), two *DNMT3A* (33.3%), one *TET2* (16.7%) and one *ASXL1* (16.7%) mutation(s) were found (Figure 1C). All mutations represented single nucleotide variants and the overall majority were predicted to be missense mutations (83.3%, Figure 1D). Mutations detected in *JAK2* were all missense mutations resulting in *JAK2*^{V617F}. The age distribution of CH frequencies differed between cases and controls (Figure 1E).

In our study cohort, neither cases nor controls with CH were significantly older than the respective counterparts

without CH (Figure 2A). Individuals with CH were not more likely to have current or prior smoking exposure or comorbidities such as hypertension or the metabolic syndrome¹⁹ (CH vs. no CH [p value]: 6.7% vs. 24.8% smoker [0.20], 20.0% vs. 4.1% hypertension [0.74], 0% vs. 4.1% metabolic syndrome [0.99]). No individual with *ASXL1* mutation had current or prior smoking exposure. Cases with CH were equally likely to have post-thrombotic syndrome, or PE as index event as those without CH mutation (CH vs. no CH [p value]: 27.3% vs. 46.9% post-thrombotic syndrome [0.72], 36.3% vs. 38.5% PE as index event [0.71]).

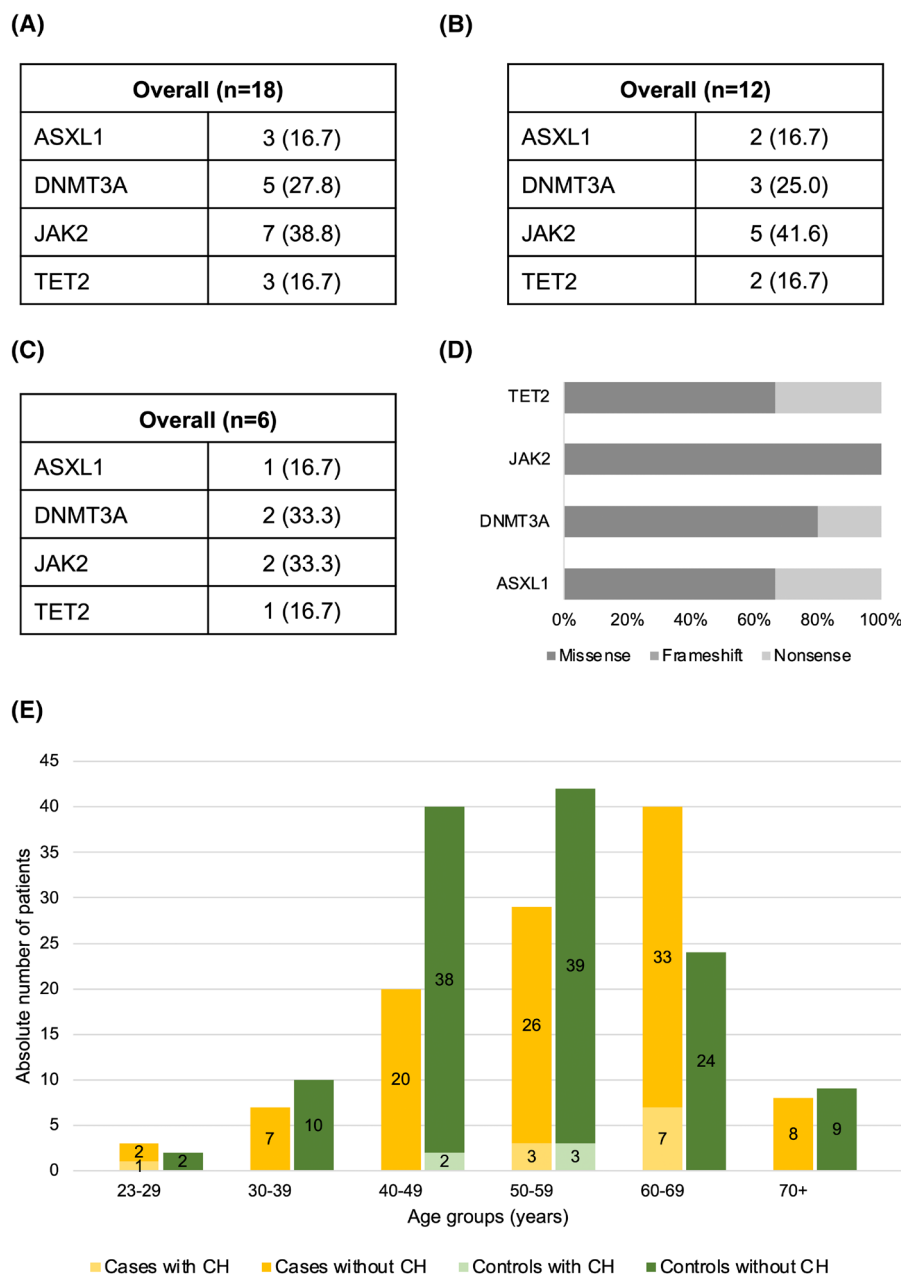


FIGURE 1 Characteristics of clonal haematopoiesis (CH) mutations detected in cases and controls. (A) Absolute frequency of genes affected by a CH mutation in all included individuals. (B) Absolute frequency of genes affected by a CH mutation in cases. (C) Absolute frequency of genes affected by a CH mutation in controls. (D) Types of mutations detected in individual genes. (E) Percent and absolute numbers of included individuals with and without CH carrier status, stratified according to age groups and case/control status.

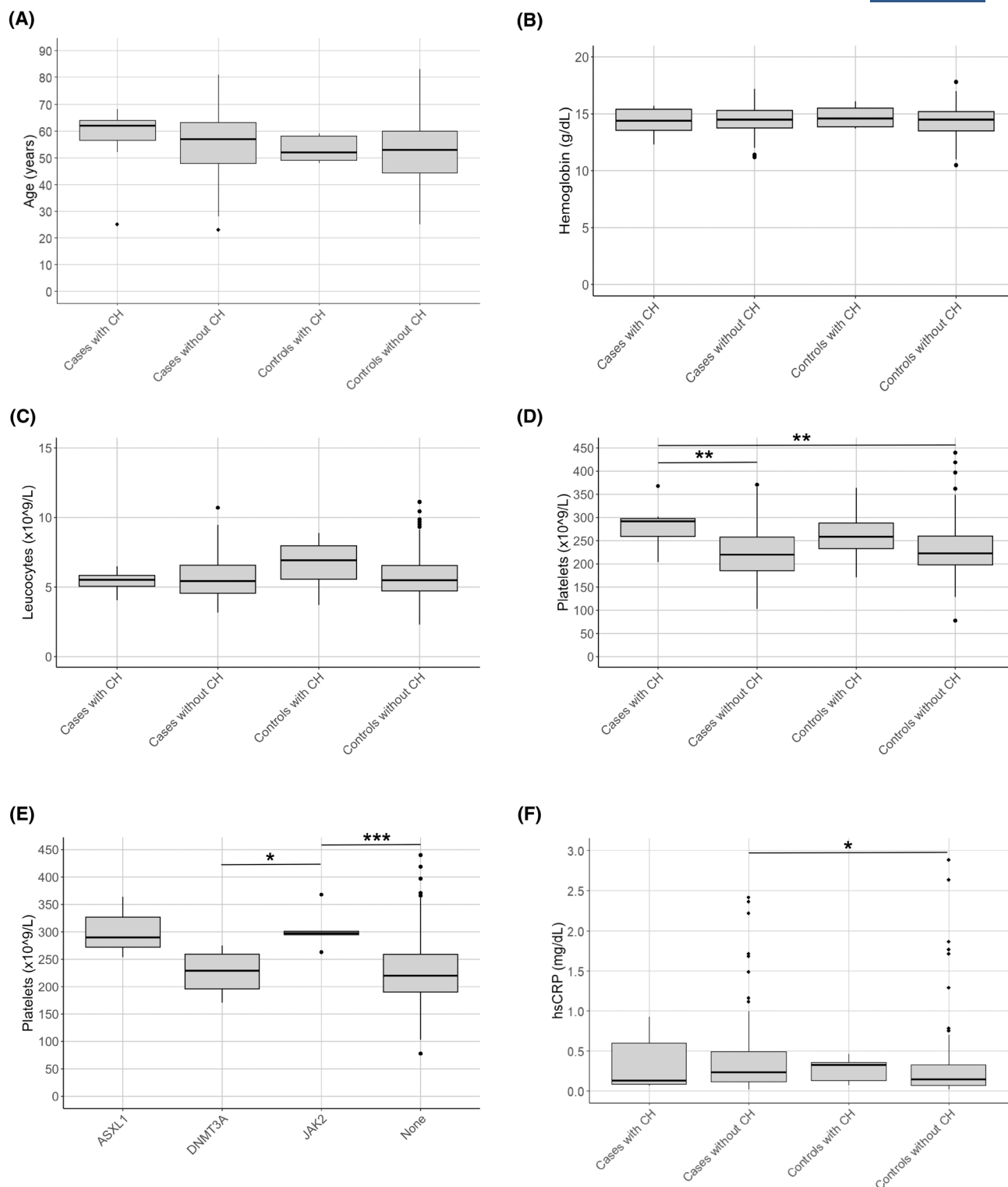


FIGURE 2 Age (A) and laboratory parameters (B–F) in cases and controls stratified according to CH carrier status. CH—clonal haematopoiesis, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Clonal haematopoiesis and its association with recurrent VTE

The frequency of CH was 10.3% in patients with recurrent VTE and 3.9% in the matched control individuals.

Therefore, the odds of having CH were higher in patients with VTE compared to healthy controls (OR: 2.74, 95% CI: 0.95–9.16). A total of 39 patients had two unprovoked VTE events, of which three exhibited CH (7.7%). Thus, the odds of having CH tended to be also higher in the subgroup of

patients with two unprovoked VTE events compared to controls (OR: 3.44, 95% CI: 0.60–20.78). When focusing on individuals without thrombophilia (cases and controls), CH was detected in 9 (15.8%) of 57 VTE cases without any thrombophilia, whereas 4 (3.6%) of 112 controls without thrombophilia exhibited CH. Thus, we observed a more pronounced association between VTE and CH in individuals without thrombophilia (OR: 4.58, 95% CI: 1.48–15.99). An additional analysis revealed no strong evidence of an interaction between CH and thrombophilia regarding the outcome recurrent VTE (OR: 2.42, 95% CI: 0.22–27.05). Details on the presence of specific types of thrombophilia and CH are summarized in Table S2 and details on the location of VTE events in cases with CH mutation is depicted in Table S3.

Clonal haematopoiesis and its association with laboratory parameters

While haemoglobin levels and leucocyte counts did not differ between groups (Figure 2B,C), cases with CH showed higher platelet counts compared to both cases and controls without CH (median [IQR]: 292 [254–298] vs. 223 [198–260] vs. 220 [185–259] $\times 10^9/L$; $p < 0.01$ for both direct comparisons, Figure 2D). Individuals (merged cases and controls) with *JAK2* mutation had higher platelet counts compared to individuals with *DNMT3A* or no mutation (median [IQR]: 298 [294–302] vs. 229 [188–265] vs. 221 [190–260] $\times 10^9/L$, respectively; $p = 0.03$; Figure 2E). Serum levels of high sensitivity (hs)CRP were higher in cases without CH compared to controls without CH, but did not differ within cases or controls according to CH status (Figure 2F).

Clonal haematopoiesis and its association with haemostatic biomarkers

As we have reported previously, VTE cases without CH exhibit the elevated levels of fibrinogen and soluble P-selectin (sP-selectin) as compared to controls without CH (Figure 3A,B).¹⁸ In our current study on the role of CH in

VTE, within both cases and control groups, there was no difference between CH carriers and non-carriers regarding fibrinogen or sP-selectin levels. D-dimer levels did not differ between cases and controls or depending on carrier status (i.e. with and without CH) (Figure 3C). Furthermore, fibrinogen, sP-selectin and D-dimer levels did not differ according to different gene mutations (data not shown).

Clonal haematopoiesis and its association with biomarkers of biological ageing

As we have recently reported, in our cohort patients with VTE exhibited lower mtDNA levels compared to healthy controls.²⁴ Our CH-centred analyses now revealed that the presence of CH had no significant influence on mtDNA levels (Figure 4A). Interestingly, on individual gene level, individuals with *JAK2* mutation showed decreased mtDNA levels compared to those with mutations in *DNMT3A* or *ASXL1* (Figure 4B). Telomere length did not differ between individuals with and without CH and was similar overall in cases and controls (Figure 4C).

DISCUSSION

In summary, we assessed the prevalence of selected CH mutations in a case–control study of patients with recurrent VTE and matched healthy controls. We found a higher frequency of CH mutations in recurrent VTE cases compared to healthy controls and the association between CH and recurrent VTE was most pronounced in individuals without established thrombophilia. Patient characteristics, laboratory parameters and haemostatic biomarkers did not differ according to CH carrier status. However, individuals carrying *JAK2*^{V617F} showed altered platelet counts and mtDNA levels.

As CH is highly prevalent in the ageing general population, it has emerged as an aspiring research topic.^{10,11} CH is associated with increased all-cause mortality and risk of transformation to haematological malignancies, but it is most strikingly linked to CVD-related morbidity and mortality

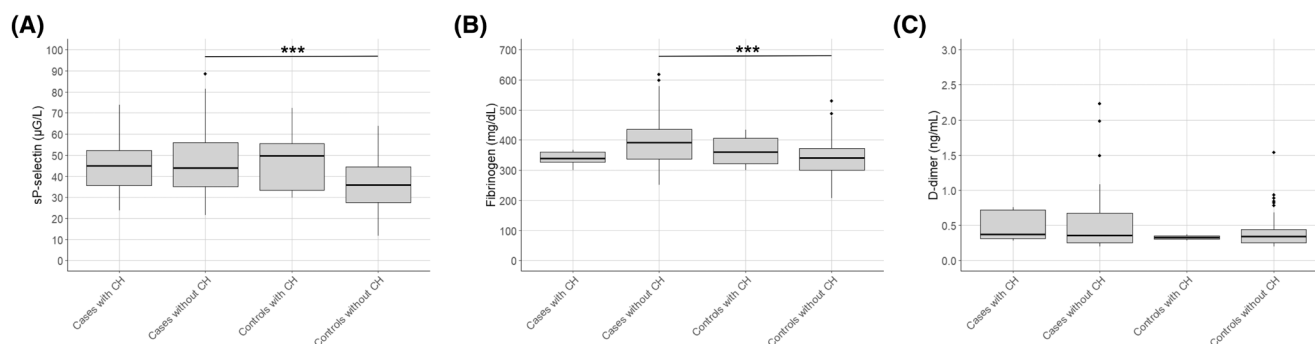


FIGURE 3 Levels of haemostatic biomarkers in cases and controls stratified according to CH carrier status. sP-selectin (A), fibrinogen (B) and D-dimer (C) according to case/control and CH carrier status. CRP—C-reactive protein, CH—clonal haematopoiesis, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

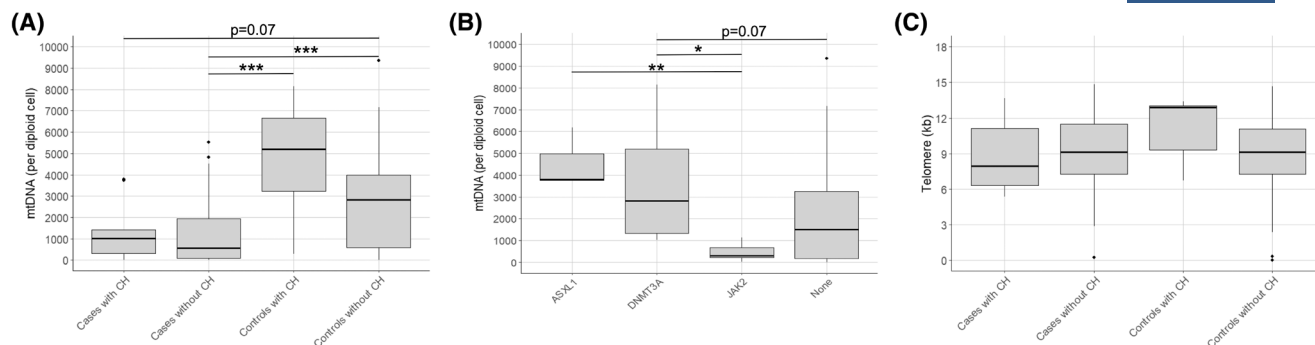


FIGURE 4 Levels of biomarkers of biological aging in cases and controls stratified according to CH carrier status and to mutated gene. (A) Levels of mtDNA according to case/control and CH carrier status, (B) mtDNA levels stratified by mutated gene, (C) telomere length according to case/control and CH carrier status. CH—clonal haematopoiesis, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

due to higher risk of, for example, coronary artery disease, stroke and peripheral artery disease.^{7–10} Most recently, a large biobank-based study described an association between CH and VTE as well.¹⁷ Previously, two smaller studies screened for CH-associated mutations in modest size patient cohorts with unprovoked VTE.^{15,16} In one study, the authors reported a CH prevalence of 20% using a VAF cut-off of 1% and of 6% with a 2% VAF cut-off.¹⁶ The other study, reported as a conference abstract, similarly described a CH frequency of 9% in patients with unprovoked VTE.¹⁵ However, both studies were lacking a control group and detailed patient characteristics. Our study involving patients with recurrent VTE and matched healthy controls revealed increased odds of having CH in recurrent VTE cases. Interestingly, as we had detailed clinical history and clinical characteristics available, we discovered that the association between CH and recurrent VTE was especially pronounced in individuals without thrombophilia. This may suggest that, in individuals with thrombophilia, the impact of CH on recurrent VTE risk is masked, as they already possess an elevated baseline risk, and thus, this impact is most clearly visible in patients without thrombophilia.

In our cohort, mutations in *JAK2* were most common in the overall cohort, and in cases with recurrent VTE, aligning with previous studies.¹⁷ In previous studies, an increased VTE risk in *JAK2*-mutated subjects without overt myeloproliferative neoplasm (MPN) was observed.^{25,26} Specifically, it was found that 25% of patients with *JAK2* mutation had a history of unprovoked VTE.²⁶ Another study reported an odds ratio of nearly 3 for prevalent VTE in individuals with mutated *JAK2*.²⁵ Moreover, the recently published large study, based on the UK biobank, reported an association of overall CH with incident VTE at a small effect size, whereas *JAK2*-driven CH resulted in a 6.5-fold higher risk for incident VTE.¹⁷ In agreement with that, our study now provides additional clinical insights based on detailed patient information which may help formulate hypotheses for mechanistically investigating the link between CH and VTE.

Although CH is associated with advanced age, our study did not find significant age differences among CH carriers, possibly due to low sample numbers as we observed only one CH carrier younger than 40 years and three CH

carriers younger than 50 years. As we have previously reported on an association between VTE and biological ageing in our study,²⁴ we now had the possibility to assess a potential influence of CH on this observation. The results of our analyses do not support a role of CH in this phenomenon. Interestingly, *JAK2*-mutated individuals showed reduced mtDNA levels compared to patients with mutations in *DNMT3A* or *ASXL1*. mtDNA is a primary source of reactive oxygen species (ROS), which can lead to mitochondrial dysfunction and a subsequent decrease in mtDNA levels. Previously, elevated ROS levels and oxidative stress in the plasma of haematopoietic stem cells and in samples of patients with *JAK2* mutation were found.^{27,28} Our observation of decreased mtDNA levels in individuals with *JAK2* mutation might be the consequence of an increased oxidative stress state and may warrant further exploration.

While haemoglobin levels and leucocyte counts did not differ according to CH status, individuals with CH and especially with *JAK2* mutation exhibited higher platelet counts. This might indicate that we have included some cases of undiagnosed MPN in our cohort. However, while ruling out MPN in population-based studies using ICD-10 codes is hampered, we had access to detailed clinical histories and blood counts at the time of study inclusion. At the time of blood collection, there were no blood count abnormalities or other clinical symptoms indicative of MPN present. Clinically, it is of course important to think of MPN, especially when venous thrombosis manifests at unusual sites, that is, in the splanchnic or cerebral venous system.

Elevated sP-selectin is linked to VTE risk, as P-selectin plays an important role in thrombus formation as it facilitates leucocyte adhesion to stimulated endothelial cells and platelets and triggers increased expression of tissue factor.^{18,29,30} The observation of increased P-selectin expression and elevated sP-selectin levels in *TET2* knockout mice supports a putative role of CH in VTE development.¹² In our study, however, we neither observed a difference in sP-selectin levels between individuals with and without overall CH, nor between individuals with or without mutated *TET2*. Generally, we did not observe any influence of CH on haemostatic biomarkers in our study. Thus, the proposed

enhanced inflammatory environment associated with CH may have no detectable effect on haemostatic parameters. While literature on potential correlations of CH status with CRP levels as a downstream marker of inflammation is ambiguous,^{21,31–33} our analyses did not reveal differences in hsCRP levels according to CH status. Elevated hsCRP levels in patients with VTE have been previously reported^{19,34} and our observed lack of correlation between hsCRP and recurrent VTE in CH carriers might be due to restricted statistical power in this smaller subgroup.

There are some limitations that need to be acknowledged. First, our analyses were limited by the overall number of included individuals, which precluded a more detailed investigation of individual CH-associated mutations on single-gene level. However, detailed information on patient characteristics, laboratory parameters and biomarkers were available for all included patients. Further studies with higher sample size and power are needed to confirm our findings. Second, as our analyses are based on a retrospective study of recurrent VTE, we do not know whether CH was present before or after the first VTE event. Third, our analyses were restricted to mutational hotspots within selected genes, potentially resulting in an underestimation of CH frequencies. Nevertheless, the seven genes included in our screening assay comprise the majority of mutated genes described in previous pivotal studies of CH.¹⁰

In conclusion, our study reveals an elevated CH frequency in patients with recurrent VTE compared to healthy controls reflected by an association between CH and recurrent VTE in the subgroup of individuals without underlying thrombophilia. As thrombophilia might mask the effect of CH-associated mutations, further studies in large VTE cohorts with detailed patient characteristics available may be suitable to expand on our findings.

AUTHOR CONTRIBUTIONS

CE, RJ and CA did the conceptualization of the study. CE, RV, TS and RJ performed laboratory analyses. CE performed the statistical analysis. CE, RV, TS, IP and CA gathered clinical data. CE, RJ and CA analysed and discussed data. CE, RJ, CJB and CA acquired funding for this project. CJB, IP, RJ and CA supervised the project. All authors reviewed and edited the drafts and agreed to the final version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

CE, RV, RJ and TS have no conflict of interest to report. CJB has received honoraria for scientific advisory board participation and consultation from Amgen, AOP Pharma, Boehringer Ingelheim, Daiichi-Sankyo, Novartis, Sobi and is board member of Technoclone GmbH. IP has received honoraria for lectures or participation in advisory boards from Bayer, Bristol-Meyers Squibb, Pfizer, Rovi and Sanofi. CA has received honoraria for lectures or participation in advisory boards from Bayer, Daiichi-Sankyo, Bristol-Meyers Squibb, Pfizer and Sanofi.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Heit JA. Epidemiology of venous thromboembolism. *Nat Rev Cardiol.* 2015;12(8):464–74.
2. Cohen AT, Agnelli G, Anderson FA, Arcelus JL, Bergqvist D, Brecht JG, et al. Venous thromboembolism (VTE) in Europe. The number of VTE events and associated morbidity and mortality. *Thromb Haemost.* 2007;98(4):756–64.
3. Lutsey PL, Windham BG, Misialek JR, Cushman M, Kucharska-Newton A, Basu S, et al. Long-term Association of Venous Thromboembolism with Frailty, physical functioning, and quality of life: the atherosclerosis risk in communities study. *J Am Heart Assoc.* 2020;9(12):e015656.
4. Braekkan SK, Grosse SD, Okoroh EM, Tsai J, Cannegieter SC, Naess IA, et al. Venous thromboembolism and subsequent permanent work-related disability. *J Thromb Haemost.* 2016;14(10):1978–87.
5. Ortel TL, Neumann I, Ageno W, Beyth R, Clark NP, Cuker A, et al. American Society of Hematology 2020 guidelines for management of venous thromboembolism: treatment of deep vein thrombosis and pulmonary embolism. *Blood Adv.* 2020;4(19):4693–738.
6. Ainle FN, Kevane B. Which patients are at high risk of recurrent venous thromboembolism (deep vein thrombosis and pulmonary embolism)? *Blood Adv.* 2020;4(21):5595–606.
7. Zekavat SM, Viana-Huete V, Matesanz N, Jorshery SD, Zuriaga MA, Uddin MM, et al. TP53-mediated clonal hematopoiesis confers increased risk for incident atherosclerotic disease. *Nat Cardiovasc Res.* 2023;2:144–58.
8. Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, et al. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med.* 2017;377(2):111–21.
9. Bhattacharya R, Zekavat SM, Haessler J, Fornage M, Raffield L, Uddin MM, et al. Clonal hematopoiesis is associated with higher risk of stroke. *Stroke.* 2022;53(3):788–97.
10. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med.* 2014;371(26):2488–98.

11. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477–87.
12. Fuster JJ, MacLauchlan S, Zuriaga MA, Polackal MN, Ostriker AC, Chakraborty R, et al. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*. 2017;355(6327):842–7.
13. Rauch PJ, Silver AJ, Gopakumar J, McConkey M, Sinha E, Fefer M, et al. Loss-of-function mutations in Dnmt3a and Tet2 lead to accelerated atherosclerosis and convergent macrophage phenotypes in mice. *Blood*. 2018;132(1):745.
14. von Bruhl ML, Stark K, Steinhart A, Chandraratne S, Konrad I, Lorenz M, et al. Monocytes, neutrophils, and platelets cooperate to initiate and propagate venous thrombosis in mice in vivo. *J Exp Med*. 2012;209(4):819–35.
15. Fidalgo-Fernández Á, Hernández-Sánchez M, Marín-Quílez A, Benito R, Santos-Mínguez S, Miguel-García C, et al. Identification of somatic mutations associated with clonal hematopoiesis in unprovoked venous thromboembolism. *Res Pract Thromb Haemost*. 2020;4(Suppl 1).
16. Soudet S, Jedraszak G, Evrard O, Marolleau JP, Garçon L, Pietri MAS. Is hematopoietic clonality of indeterminate potential a risk factor for pulmonary embolism? *TH Open*. 2021;5(3):e338–e342.
17. Zon RL, Sekar A, Clapham K, Oren O, Niroula A, Bick A, et al. JAK2-mutant clonal hematopoiesis is associated with venous thromboembolism. *Blood*. 2024. Epub ahead of print.
18. Ay C, Jungbauer LV, Sailer T, Tengler T, Koder S, Kaider A, et al. High concentrations of soluble P-selectin are associated with risk of venous thromboembolism and the P-selectin Thr715 variant. *Clin Chem*. 2007;53(7):1235–43.
19. Ay C, Tengler T, Vormittag R, Simanek R, Dorda W, Vukovich T, et al. Venous thromboembolism a manifestation of the metabolic syndrome. *Haematologica*. 2007;92(3):374–80.
20. Ay C, Bencur P, Vormittag R, Sailer T, Jungbauer C, Vukovich T, et al. The angiotensin-converting enzyme insertion/deletion polymorphism and serum levels of angiotensin-converting enzyme in venous thromboembolism. Data from a case control study. *Thromb Haemost*. 2007;98(4):777–82.
21. Jäger R, Hoke M, Mayer FJ, Boden S, Englisch C, Ay C, et al. Combined effects of clonal hematopoiesis and carotid stenosis on cardiovascular mortality. *J Am Coll Cardiol*. 2024;83(18):1717–27.
22. Tate JG, Bamford S, Jubb HC, Sondka Z, Beare DM, Bindal N, et al. COSMIC: the catalogue of somatic mutations in cancer. *Nucleic Acids Res*. 2019;47(D1):D941–D947.
23. Chen S, Francioli LC, Goodrich JK, Collins RL, Kanai M, Wang Q, et al. A genomic mutational constraint map using variation in 76,156 human genomes. *Nature*. 2024;625(7993):92–100.
24. Vostatek R, Hohensinner P, Nopp S, Haider P, Englisch C, Pointner J, et al. Association of telomere length and mitochondrial DNA copy number, two biomarkers of biological aging, with the risk of venous thromboembolism. *Thromb Res*. 2023;223:168–73.
25. Cordua S, Kjaer L, Skov V, Pallisgaard N, Hasselbalch HC, Ellervik C. Prevalence and phenotypes of JAK2 V617F and calreticulin mutations in a Danish general population. *Blood*. 2019;134(5):469–79.
26. Wolach O, Sellar RS, Martinod K, Cherpokova D, McConkey M, Chappell RJ, et al. Increased neutrophil extracellular trap formation promotes thrombosis in myeloproliferative neoplasms. *Sci Transl Med*. 2018;10(436).
27. Hurtado-Nedelec M, Csillag-Grange MJ, Boussetta T, Belambri SA, Fay M, Cassinat B, et al. Increased reactive oxygen species production and p47phox phosphorylation in neutrophils from myeloproliferative disorders patients with JAK2 (V617F) mutation. *Haematologica*. 2013;98(10):1517–24.
28. Tibullo D, Palumbo GA, Giallongo C, Longo A, Duminuco A, Di Raimondo F, et al. ROS-driven structural and functional fibrinogen modifications are reverted by JAK inhibition in myeloproliferative neoplasms. *Blood*. 2023;142(1):6334.
29. Larsen E, Celi A, Gilbert GE, Furie BC, Erban JK, Bonfanti R, et al. PADGEM protein: a receptor that mediates the interaction of activated platelets with neutrophils and monocytes. *Cell*. 1989;59(2):305–12.
30. André P, Hartwell D, Hrachovinová I, Saffaripour S, Wagner DD. Pro-coagulant state resulting from high levels of soluble P-selectin in blood. *Proc Natl Acad Sci U S A*. 2000;97(25):13835–40.
31. Busque L, Sun M, Buscarlet M, Ayachi S, Feroz Zada Y, Provost S, et al. High-sensitivity C-reactive protein is associated with clonal hematopoiesis of indeterminate potential. *Blood Adv*. 2020;4(11):2430–8.
32. Arends CM, Liman TG, Strzelecka PM, Kufner A, Löwe P, Huo S, et al. Associations of clonal hematopoiesis with recurrent vascular events and death in patients with incident ischemic stroke. *Blood*. 2023;141(7):787–99.
33. Gumuser ED, Schuermans A, Cho SMJ, Sporn ZA, Uddin MM, Paruchuri K, et al. Clonal hematopoiesis of indeterminate potential predicts adverse outcomes in patients with atherosclerotic cardiovascular disease. *J Am Coll Cardiol*. 2023;81(20):1996–2009.
34. Kamphuisen PW, Eikenboom JC, Vos HL, Pablo R, Sturk A, Bertina RM, et al. Increased levels of factor VIII and fibrinogen in patients with venous thrombosis are not caused by acute phase reactions. *Thromb Haemost*. 1999;81(5):680–3.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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