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CHIP & HIPs: Clonal Hematopoiesis is Common in Hip Arthroplasty Patients and Associates with Autoimmune Disease

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Abstract:
Clonal hematopoiesis (CH) is an age-related condition predisposing to blood cancer and cardiovascular disease (CVD). Murine models demonstrate CH-mediated altered immune function and proinflammation. Low-grade inflammation has been implicated in the pathogenesis of osteoarthritis (OA), the main indication for total hip arthroplasty (THA). THA-derived hip bones serve as a major source of 'healthy' hematopoietic cells in experimental hematology. We prospectively investigated frequency and clinical associations of CH in 200 patients without known hematologic disease undergoing THA. Prevalence of CH was 50%, including 77 patients with CH of indeterminate potential (CHIP, defined as somatic variants with allele frequencies [VAF] ≥2%), and 23 patients harboring CH with lower mutation burden (VAF 1-2%). Most commonly mutated genes were *DNMT3A* (29.5%), *TET2* (15.0%) and *ASXL1* (3.5%). CHIP significantly associated with lower hemoglobin, higher mean corpuscular volume, prior/present malignant disease, and CVD. Strikingly, we observed a previously unreported association of CHIP with autoimmune diseases (AID; multivariate adjusted odds ratio, 6.6; 95% confidence interval [1.7, 30]; p=0.0081). These findings underscore the association between CH and inflammatory diseases. Our results have considerable relevance for management of patients with OA and AID or mild anemia, and question use of hip bone-derived cells as 'healthy' experimental controls.

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Keywords: clonal hematopoiesis, CHIP, total hip replacement surgery, osteoarthritis, autoimmune disease

Key Points

- Association between clonal hematopoiesis and autoimmune disease in osteoarthritis patients underscores the link between CH and inflammation
- High rate of CH precludes use of hip bone-derived hematopoietic cells as healthy controls in experimental set-ups without prior sequencing

Abstract

Clonal hematopoiesis (CH) is an age-related condition predisposing to blood cancer and cardiovascular disease (CVD). Murine models demonstrate CH-mediated altered immune function and proinflammation. Low-grade inflammation has been implicated in the pathogenesis of osteoarthritis (OA), the main indication for total hip arthroplasty (THA). THA-derived hip bones serve as a major source of ‘healthy’ hematopoietic cells in experimental hematology. We prospectively investigated frequency and clinical associations of CH in 200 patients without known hematologic disease undergoing THA. Prevalence of CH was 50%, including 77 patients with CH of indeterminate potential (CHIP, defined as somatic variants with allele frequencies [VAF] ≥2%), and 23 patients harboring CH with lower mutation burden (VAF 1-2%). Most commonly mutated genes were *DNMT3A* (29.5%), *TET2* (15.0%) and *ASXL1* (3.5%). CHIP significantly associated with lower hemoglobin, higher mean corpuscular volume, prior/present malignant disease, and CVD. Strikingly, we observed a previously unreported association of CHIP with autoimmune diseases (AID; multivariate adjusted odds ratio, 6.6; 95% confidence interval [1.7, 30]; p=0.0081). These findings underscore the association between CH and inflammatory diseases. Our results have considerable relevance for management of patients with OA and AID or mild anemia, and question use of hip bone-derived cells as ‘healthy’ experimental controls.

Introduction

Clonal hematopoiesis of indeterminate potential (CHIP) is defined as presence of somatic mutations with a variant allele frequency (VAF) ≥2% in the peripheral blood (PB) of individuals without evidence of hematologic disease[1]. Genome-wide or targeted sequencing revealed an ageing-associated increase of clonal hematopoiesis (CH) in healthy individuals[2–6]. Clonal expansion of hematopoietic cells is often driven by somatic mutations in genes implicated in myeloid disease, most frequently *DNMT3A*, *TET2* and *ASXL1*. CH is a pre-malignant state predisposing to blood cancer, although the absolute progression risk is low[6–8]. CH carriers also have higher risks of atherosclerotic cardiovascular disease (CVD), death from coronary heart disease, and all-cause mortality[3,9]. Leukocytes harboring somatic mutations exhibit altered immune properties, suggesting CH-mediated immune-cell dysfunction, with inflammasome-mediated endothelial injury driving atherosclerosis[10–12]. Chronic low-grade inflammation also plays a critical role in the pathogenesis of osteoarthritis (OA)[13], the main indication for total hip arthroplasty (THA). We prospectively studied CH in 200 THA patients without hematologic disease to characterize the spectrum of CH in this population and understand associations between CHIP and clinical parameters.

Methods

Bone marrow (BM) from femoral heads (n=109) and PB (n=91) were collected from 200 patients without known hematologic disease undergoing THA for OA between 07/2017 and 08/2020 within the German Cancer Consortium (DKTK CHOICE), after written informed consent (Table 1). The study was approved by the respective ethics committees in accordance with the Declaration of Helsinki. Detailed information about variant detection and data analysis are provided in the supplement. In brief, targeted sequencing of 68 genes recurrently mutated in hematologic malignancies identified non-synonymous variants with a VAF threshold of ≥1%. For correlative analyses of clinical parameters, only variants fulfilling the current CHIP definition (VAF ≥2%) were included. We tested for associations between CHIP and other variables by univariate analyses (with adjustment for multiple testing), and by multivariate analysis considering covariables with univariate q<0.1, as well as sex (see supplement for details).

Results and Discussion

Our prospective study in a large, well-annotated patient cohort shows that somatic mutations typical of CH are very frequent in patients undergoing elective THA for OA. At least one variant was identified in 100 patients (50%, Figure 1A), with no imbalances regarding treatment center or gender (p=0.26 and p=0.78, respectively; chi-squared test; Supplemental Figure 1A, B). CH became progressively more frequent with age (Figure 1B). Most patients (n=56) had 1 variant, 32 harbored 2 variants, and 10 carried 3 mutations, while two patients harbored 4 or 5 mutations, respectively. Number of variants per individual correlated with age (p=0.031, Spearman's rank correlation coefficient), whereas VAF (median 2.7%, range 1.0-32.7%) did not (p=0.73) (Supplemental Figure 2A, B). The prevalence of CH in our cohort is higher than previously reported in healthy individuals or specific patient groups, such as those with ischemic heart failure or CVD[3,9,14–16].

Overall, we detected 158 variants with VAFs ≥1%, affecting 25 different genes, most frequently *DNMT3A* (29.5%), *TET2* (15.0%), and *ASXL1* (3.5%) (i.e., ‘DTA’ genes, Figure

1C, Supplemental Figure 3A, Supplemental Table 1), similar to previous reports. Co-mutation was most commonly observed between *DNMT3A* and *TET2* (n=8). Most patients (n=61) had ≥1 DTA mutation with a VAF ≥2% or with VAFs between 1-2% (n=25) (Supplemental Figure 3B), but 14 patients carried exclusively non-DTA mutations.

We used a VAF threshold of ≥2% to compare clinical features of patients with variants fulfilling commonly accepted CHIP criteria (n=77; 38.5%) to patients without such variants. CHIP carriers were older (median, 74 years *vs*. 69 years for patients without CHIP; p=5.4x10^{-6} {Wilcoxon-Mann-Whitney test}, q=9.1x10^{-6}), had lower hemoglobin levels (median, 12.7 g/dL *vs*. 13.7 g/dL; p=0.0020, q=0.017) and higher mean corpuscular volume (MCV, median, 91.9 fl *vs*. 89.0 fl; p=0.0076, q=0.034) (Figure 1D, E). Other blood cell counts showed no significant differences (Table 1). Hemoglobin levels were available for 125/200 patients, including 50 with CHIP. Although no patient in our study had a known hematologic disorder, 27/125 (21.6%) presented with subnormal hemoglobin values (Figure 1F). Of note, 18/27 patients with anemia (66.7%, Supplemental Figure 4) had detectable mutations with VAF ≥2% and can thus be classified as clonal cytopenia of uncertain significance (CCUS), a higher proportion than recently reported in a large study of patients >60 years with unexplained anemia[17]. Prevalence of CHIP was lower in patients with normal hemoglobin levels (32.7%, p=0.0014). Most anemic patients in our cohort either had significant comorbidities, prior/present non-hematologic cancer, or were receiving anticoagulants or immunosuppressive treatment for autoimmune disease (AID) - all conditions that could predispose to anemia (Supplemental Table 2). We observed an enrichment of *SF3B1* and *TP53* mutations in anemic CHIP patients compared to those with normal hemoglobin levels when, in concordance with a previous report,[17] we applied a lower VAF threshold of ≥1% for these two genes (Supplemental Table 2, 4/27 vs. 2/98 patients, respectively; p=0.020, Fisher's exact test.

CHIP carriers were more likely to have CVD as previously reported, and showed a trend towards higher frequency of prior/present malignant disease (Figure 1G, H; Supplemental Table 3). Unexpectedly, CHIP carriers more commonly had AID (Figure 1I), comprising diverse autoimmune disorders (Supplemental Table 4). Multivariate logistic regression confirmed significant associations between CHIP and older age (adjusted odds ratio [OR], 2.1; 95% confidence interval [CI], [1.1, 4.0]; p=0.025), lower hemoglobin levels (adjusted OR, 0.67; 95% CI [0.49, 0.89]; p=0.0078), and AID (adjusted OR, 6.6; 95% CI [1.7, 30]; p=0.0081) (Figure 1J), but not with CVD or malignancy.

The association between CHIP and diverse AID is a novel finding. CHIP has been studied in patient cohorts with specific autoimmune conditions such as rheumatoid arthritis (RA) or ANCA-associated vasculitis (AAV), with reported prevalences of 17%[18] and 30%[19], respectively. Arends et al. found a higher prevalence of CHIP in AAV patients compared to age and gender-matched controls[19], whereas a smaller retrospective study did not show enrichment of CHIP among RA patients compared to published control cohorts[18]. In contrast to these reports, our prospective analysis of THA patients was unbiased with respect to AID, CVD and malignancy. Whether our findings can be generalized to individuals not undergoing THA remains to be studied.

THA is a common surgical procedure in older individuals, usually performed for advanced OA. Chronic low-grade inflammation is frequent in OA but a connection between OA and CH has not been reported. Inflammation can contribute to clonal expansion in the BM. In turn, clonal *TET2*-mutated monocytes may have a pro-inflammatory phenotype characterized by production of cytokines including interleukin-1ß (IL-1ß)[20]. Indirect evidence from the CANTOS trial suggests IL-1ß inhibition may prevent worsening of joint destruction[21,22], indicating that chronic inflammation in progressive OA is partially IL-1ß-mediated. This observation together with data presented here suggest a potential role for CH in OA pathogenesis. Unambiguous evidence of a specific association between OA and CH will require confirmation in a matched control cohort. The high sensitivity of our targeted sequencing assay, compared to earlier studies using genome-wide approaches, may provide an additional explanation for the high prevalence of CH in our cohort.

Recognition of CHIP is clinically relevant in allogeneic stem cell donors[23,24] and autologous transplant recipients[25], and in the context of CVD. We cannot draw conclusions on a causal relationship between AID and CHIP. However, given the relationship between CHIP and inflammation, it is tempting to speculate whether CHIP contributes to clinical features of AID, and/or autoimmune-mediated inflammation reinforces outgrowth of CH clones. Further studies in this direction are needed to confirm this relationship and CH should be considered as a confounder in studies evaluating anti-inflammatory therapies for OA or AID.

In summary, our prospective analysis of a large cohort of elderly THA patients, using a sensitive targeted sequencing assay, revealed a high frequency of CH/CHIP. Mutations were particularly frequent in patients with (mostly mild) anemia, indicating that CCUS commonly contributes to pathogenesis of anemia in older persons. Our data point to a potential, previously unappreciated role for CHIP in the pathogenesis of inflammatory and autoimmune disorders, supporting the concept of interplay between CH and systemic inflammation which has possible implications for future therapies.

Finally, our results question the routine use of femoral heads as a source of ‘healthy’ hematopoietic cells for use in basic research. Given the high CH prevalence in THA specimens, such experiments must include screening for somatic variants.

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Authorship contributions

J.S.H. collected clinical patient characteristics, analyzed data and drafted the manuscript. L.H. performed sequencing analyses, analyzed data and drafted the manuscript. B.K. and M.R.-T. performed sequencing analyses. J.R., M.C.B. and M.v.d.G. performed sample processing and biobanking and assisted with data analysis. L.F., S.W., F.Z., K.S., M.S. and A.S.K. collected clinical patient data. M.R., E.T., M.N., D.H., A.C.P., J.L. and A.R. provided femoral heads. J.S.H and J.R. performed statistical tests with C.M. F.B, K.S., L.C.H. and U.P. provided samples, conceptual advice and critical comments. K.H.M. and K.S.G. designed the study, analyzed data and wrote the manuscript. All authors read and agreed with the final version of the manuscript.

Disclosure of conflicts of interest

The authors declare no competing financial interests.

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Table 1: Baseline characteristics of the total cohort.

Variable	All patients	VAF 1-2%	CHIP (VAF ≥2%)	Non-CHIP	*p*-value (CHIP vs. non-CHIP)	*q*-value (CHIP vs. non-CHIP)
n (%)	200	23 (11.5)	77 (38.5)	123 (61.5)		
Center					0.12	0.28
TUM, Munich, n (%)	109 (54.5)	13 (56.5)	47 (61.0)	62 (50.4)		
LMU, Munich, n (%)	31 (15.5)	5 (21.7)	7 (9.1)	24 (19.5)		
TUD/UHL, n (%)	60 (30.0)	5 (21.7)	23 (29.9)	37 (30.1)		
Indication for hip arthroplasty					0.17	0.33
primary (idiopathic) OA, n (%)	149 (74.9)	18 (78.3)	61 (80.3)	88 (71.5)		
secondary OA, n (%)	50 (25.1)	5 (21.7)	15 (19.7)	35 (28.5)		
Age (years), median (range)	71 (18-91)	75 (53-85)	74 (60-86)	69 (18-91)	5.4×10^{-6}***	9.1×10^{-5}***
Male sex, n (%)	66 (33.0)	9 (39.1)	24 (31.2)	42 (34.1)	0.66	0.81
BMI, median (range)	27.0 (16.5-51.0)	26.7 (21.3-36.0)	28.0 (19.3-37.0)	27.0 (16.5-51.0)	0.14	0.31
Obesity (BMI ≥30), n (%)	52 (26.1)	5 (21.7)	22 (28.9)	30 (24.4)		
Blood counts						
Leukocytes (10^9/L), median (range)	6.7 (3.6-13.8)	6.8 (5.4-13.6)	6.7 (5.1-13.8)	6.7 (3.6-13.6)	0.43	0.56
Hemoglobin (g/dL), median (range)	13.4 (8.0-18.3)	13.9 (10.9-15.5)	12.7 (8.0-18.3)	13.7 (9.4-16.8)	0.0020**	0.017*
MCV (fL), median (range)	90.0 (80.0-102.0)	91.3 (86.0-95.4)	91.9 (82.0-102.0)	89.0 (80.0-97.7)	0.0076**	0.034*
Platelets (10^9/L), median (range)	272.0 (112.0-563.0)	274.0 (175.0-426.0)	264.5 (112.0-563.0)	275.0 (120.0-490.0)	0.38	0.53
Comorbidities, n (%)	175 (87.9)	20 (87.0)	70 (92.1)	105 (85.4)		
Cardiovascular disease, n (%)	132 (66.3)	16 (69.6)	59 (77.6)	73 (59.3)	0.0080**	0.034*
Hypertension, n (%)	122 (61.3)	15 (65.2)	57 (75.0)	65 (52.8)		
Coronary heart disease, n (%)	30 (15.1)	4 (17.4)	15 (19.7)	15 (12.2)		
Myocardial infarction, n (%)	22 (11.1)	3 (13.0)	12 (15.7)	10 (8.1)		
Stroke, n (%)	13 (6.5)	1 (4.3)	6 (7.9)	7 (5.7)		
Cardiac arrhythmia, n (%)	29 (14.6)	2 (8.7)	15 (19.7)	14 (11.4)		
Hypercholesterolemia, n (%)	40 (20.1)	5 (21.7)	18 (23.7)	22 (17.9)	0.32	0.51
Type 2 Diabetes, n (%)	19 (9.5)	1 (4.3)	7 (9.2)	12 (9.8)	0.90	0.90
Hypothyroidism, n (%)	38 (19.1)	3 (13.0)	15 (19.7)	23 (18.7)	0.86	0.90
Autoimmune disease, n (%)	20 (10.1)	1 (4.3)	12 (15.8)	8 (6.5)	0.034*	0.097
Malignant disease, n (%)	26 (13.1)	2 (8.7)	15 (19.7)	11 (8.9)	0.028*	0.096
History of thrombosis/pulmonary embolism, n (%)	12 (6.0)	1 (4.3)	3 (3.9)	9 (7.3)	0.33	0.51
Anti-inflammatory drug use, n (%)	66 (34.3)	9 (39.1)	26 (34.7)	40 (32.5)	0.76	0.86
NSAID, n (%)	64 (32.2)	9 (39.1)	25 (33.3)	39 (31.7)		
Steroids, n (%)	4 (2.0)	0 (0.0)	0 (0.0)	4 (3.3)		
Other, n (%)	7 (3.5)	0 (0.0)	2 (2.7)	5 (4.1)		

We applied chi-squared test for categorical and Wilcoxon-Mann-Whitney test for continuous variables, respectively. *q*-values (i.e., FDR-adjusted *p*-values) were obtained using Benjamini-Hochberg procedure. *$p<0.05$, **$p<0.01$, ***$p<0.001$. BMI, body mass index; CHIP, clonal hematopoiesis of indeterminate potential; FDR, false discovery rate; LMU, University Hospital of Munich; MCV, mean corpuscular volume; NSAID, non-steroidal anti-inflammatory drug; OA, osteoarthritis; TUD, Technical University of Dresden; TUM, Technical University of Munich; UHL, University Hospital Leipzig; VAF, variant allele frequency.

Figure Legend

Figure 1. Frequency, mutational spectrum and clinical correlates of CH in patients undergoing THA. (A) Occurrence of CH with VAF ≥1% in 200 patients. (B) Frequency of CH among different age groups (not showing 1 patient with missing information about age and 1 patient >90 years). (C) Mutation frequency of recurrently mutated genes with VAF ≥1%. **Correlation of CHIP with clinical parameters:** Representation of hemoglobin levels (D), and MCV levels (E) according to absence or presence of CHIP. Red bars showing the prevalence of anemia (F, hemoglobin <13 g/dL in men and <12 g/dL in women), cardiovascular disease (G), prior/present malignant disease (H), and autoimmune disease (I) in patients with and without CHIP.

Non-adjusted *p*-values were obtained using Wilcoxon-Mann-Whitney test for continuous variables (D, E) and chi-squared test for categorical variables (F-I). (J) Multivariate logistic regression analysis. $ per 10 years increase. ¢ female versus male. † per 1g/dL increase. *$p<0.05$, **$p<0.01$. Adjusted OR, odds ratio adjusted for the effects of the other covariates; CH, clonal hematopoiesis; CHIP, clonal hematopoiesis of indeterminate potential; CI, confidence interval; MCV, mean corpuscular volume; VAF, variant allele frequency.

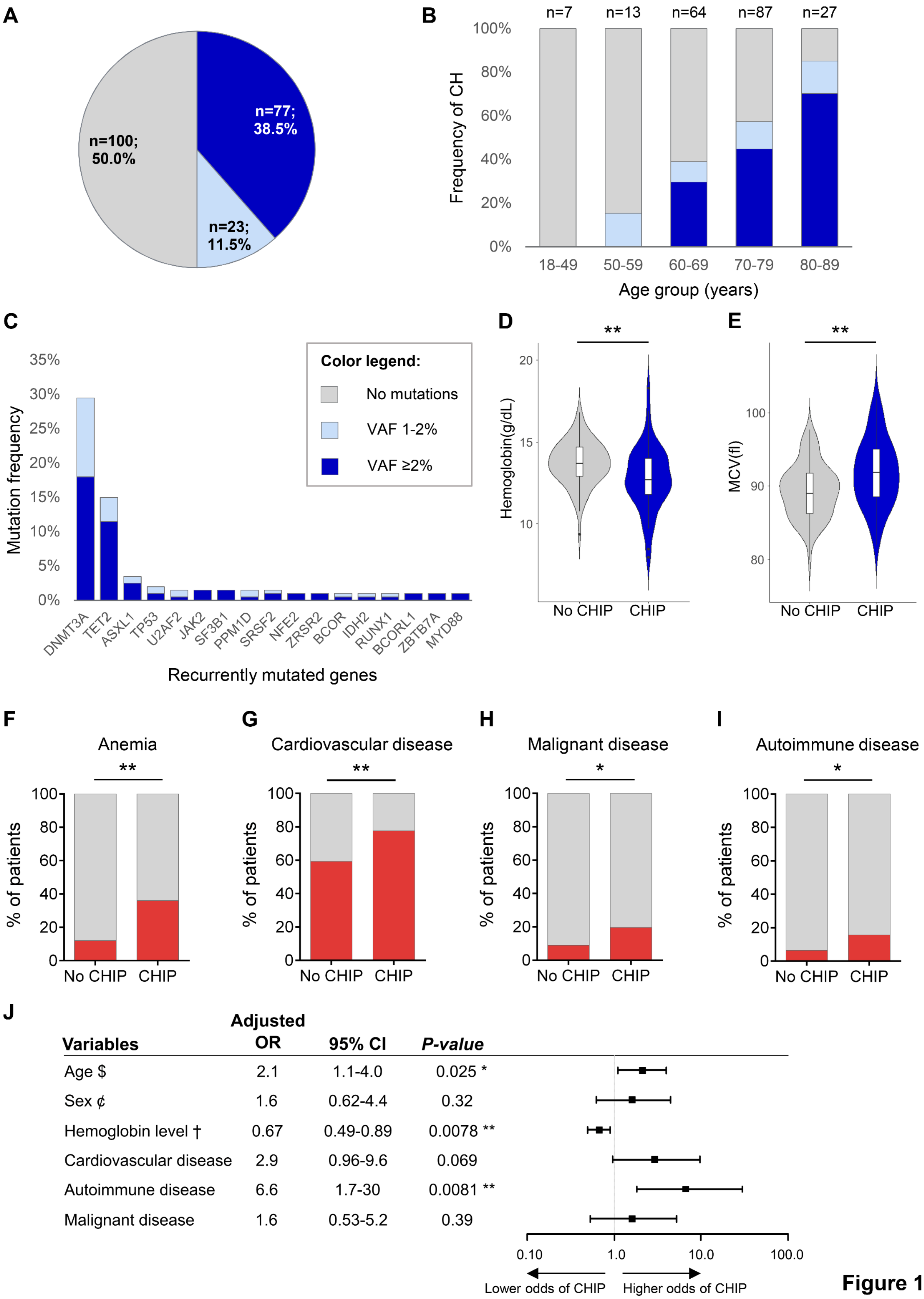
A
n=77; 38.5%
n=23; 11.5%
n=100; 50.0%
B
n=7
n=13
n=64
n=87
n=27
Frequency of CH
100%
80%
60%
40%
20%
0%
18-49
50-59
60-69
70-79
80-89
Age group (years)
C
Mutation frequency
35%
30%
25%
20%
15%
10%
5%
0%
Color legend:
No mutations
VAF 1-2%
VAF ≥2%
DNMT3A
TET2
ASXL1
TP53
U2AF2
JAK2
SF3B1
PPM1D
SRSF2
NFE2
ZRSR2
BCOR
IDH2
RUNX1
BCORL1
ZBTB7A
MYD88
Recurrently mutated genes
D
**
Hemoglobin(g/dL)
20
15
10
No CHIP
CHIP
E
**
MCV(fl)
100
90
80
No CHIP
CHIP
F
Anemia
**
G
Cardiovascular disease
**
H
Malignant disease
*
I
Autoimmune disease
*
% of patients
100
80
60
40
20
0
No CHIP
CHIP
J
Variables
Adjusted OR
95% CI
P-value
Age $ 2.1 1.1-4.0 0.025 *
Sex ¢ 1.6 0.62-4.4 0.32
Hemoglobin level † 0.67 0.49-0.89 0.0078 **
Cardiovascular disease 2.9 0.96-9.6 0.069
Autoimmune disease 6.6 1.7-30 0.0081 **
Malignant disease 1.6 0.53-5.2 0.39
0.10
1.0
10.0
100.0
Lower odds of CHIP
Higher odds of CHIP

Figure 1