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## Clonal haematopoiesis of indeterminate potential

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# Clonal haematopoiesis of indeterminate potential: associations with heart failure incidence, clinical parameters and biomarkers

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## Aim

We aimed to analyse the association of clonal haematopoiesis of indeterminate potential (CHIP) with incident heart failure (HF) in a European population cohort.

## Methods and results

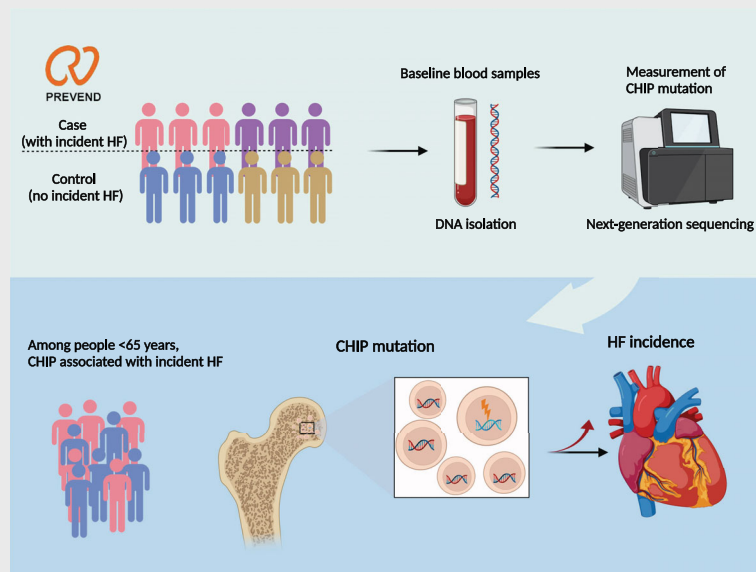
From the prospective Prevention of Renal and Vascular End-stage Disease (PREVEND) cohort, we included all 374 participants with incident HF and selected 1:1 age- and sex-matched control subjects. Peripheral blood samples of 705 individuals were successfully analysed by error-corrected next generation sequencing for acquired mutations at a variant allele frequency  $\geq 2\%$  in 27 CHIP driver genes. The median age of the study population was 65 years (interquartile range 58–70) and 35.6% were female. CHIP mutations positively correlated with age, smoking, hypertension and cardiovascular biomarkers including N-terminal pro-B-type natriuretic peptide and mid-regional pro-A-type natriuretic peptide, but the frequency of CHIP was comparable in individuals with incident HF and in control participants (18.4% vs. 17.3%;  $p = 0.69$ ). In multivariable Cox regression models, CHIP was not significantly associated with incident HF (hazard ratio [HR] 1.24, 95% confidence interval [CI] 0.93–1.65;  $p = 0.144$ ). This association, however, was modified by age ( $p$  for CHIP–age interaction = 0.002). Among people younger than 65 years, CHIP mutations were more frequently detected in the case cohort compared to the control cohort (14.2% vs. 5.8%;  $p = 0.009$ ), and were significantly associated with new-onset HF (HR 2.07, 95% CI 1.30–3.29;  $p = 0.002$ ).

## Conclusion

Clonal haematopoiesis of indeterminate potential correlates with HF risk factors and biomarkers, and is associated with incident HF in subjects <65 years of age.

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## Graphical Abstract



Association of clonal haematopoiesis of indeterminate potential with incident heart failure.

## Keywords

CHIP • Clonal haematopoiesis • Heart failure • Risk factors • Biomarkers

## Introduction

Somatic mutations occur and gradually accumulate among a variety of healthy tissues, including skin and blood, potentially contributing to aging and tumorigenesis.<sup>1,2</sup> Expansion of haematopoietic stem or progenitor cells carrying somatic mutations in certain genes implicated in haematologic malignancies results in detectable, mutated clones of cells in the peripheral blood and bone marrow. These mutations can also be detected in individuals without haematologic malignancy, which has been defined as clonal haematopoiesis of indeterminate potential (CHIP).<sup>3,4</sup> Among all mutations, DNMT3A, TET2 and ASXL1 are three predominant driver genes of CHIP, involved in the epigenetic regulation of inflammation.<sup>3,5</sup>

The prevalence of CHIP increases with age, and associates with incident cancer and mortality.<sup>6,7</sup> Interestingly, recent data demonstrated that CHIP also increases the risks of incident coronary heart disease, myocardial infarction (MI) and stroke.<sup>3,8,9</sup> In mechanistic studies, loss of TET2 potentiates atherosclerosis in low-density lipoprotein receptor (Ldlr) knockout mice, and worsens cardiac remodelling and function in mice with heart failure (HF) by upregulating CXC chemokines and cytokines such as interleukin (IL)-6 and IL-1 $\beta$ .<sup>10,11</sup>

As such, CHIP has been positioned as a link between cancer and cardiovascular disease (CVD), as a potential common genetic risk factor in the cardio-oncology field.<sup>12,13</sup> In the CVD spectrum, HF in particular has emerged as a potential precipitant for incident

cancer, and several pathophysiological factors are shared between HF and cancer.<sup>12–14</sup>

Recent clinical data have shown that in patients with chronic HF, CHIP is significantly associated with all-cause mortality and HF hospitalization, best characterized by DNMT3A and TET2 mutations.<sup>15,16</sup> Similar associations were reported among HF patients with reduced ejection fraction (HFrEF).<sup>17</sup> Moreover, a large American cohort study including over 50 000 participants found that CHIP correlated with a 25% increased risk of new-onset HF.<sup>18</sup>

However, the effects of CHIP mutations on HF risk factors and biomarkers, and HF incidence among the European population, and whether the effects vary between HF phenotypes remain unexplored. On this basis, we designed a case–control study to assess the associations of CHIP mutations with incident HF and HF subtypes (heart failure with preserved ejection fraction [HFpEF] and HFrEF) in a well-characterized general European population cohort (the Prevention of Renal and Vascular End-stage Disease [PREVEND] study).<sup>19,20</sup>

## Methods

### Study population

For the present study data and biomaterials were used from the PREVEND cohort, a prospective population-based cohort which has been fully described in detail before.<sup>19,21</sup> In brief, from 1997 to 1998, all residents ( $n = 85\,421$ ) in Groningen, the Netherlands, within an

age range of 25 to 75 years, were asked to send in a first-morning urine sample and complete a short questionnaire about demographics and CVD history. In total 47.8% participants ( $n = 40\,856$ ) responded. From those, 7786 subjects with urinary albumin excretion (UAE)  $>10$  mg/L and 3395 randomly selected control individuals with a UAE  $<10$  mg/L were invited to an outpatient clinic for a detailed assessment of cardiovascular and renal risk factors. After excluding pregnant women, subjects with insulin-dependent diabetes mellitus, or unable or unwilling to participate, a total of 8592 subjects completed the initial screening. The PREVEND study complied with the Declaration of Helsinki and was approved by institutional medical ethics committee. Written informed consent was provided by the participants enrolled in the study.

Subsequently, 374 individuals developed new-onset HF during a median 11.5 year follow-up, of whom 241 (66%) people were diagnosed with HFrEF and 125 (34%) subjects developed a HFpEF phenotype, based on the European Society of Cardiology (ESC) guidelines for diagnosis of new-onset HF (left ventricular ejection fraction [LVEF]  $\leq 40\%$  for HFrEF or  $\geq 50\%$  for HFpEF, respectively); the adjudication of HF events has been published in detail.<sup>20</sup> We selected all individuals with incident HF as a case cohort, and a 1:1 age and sex-matched control cohort. The median age for both control and case cohorts was 65 years (interquartile range [IQR] 57–69;  $p = 1.0$ ) and 64.4% were male ( $p = 1.0$ ). The sample matching is shown in online supplementary Figure S1.

## Sample preparation and error-corrected next generation sequencing

DNA samples at baseline were isolated from peripheral blood with Qiamp DNA extraction kits (Qiagen). A custom panel of single-molecule-tagged molecular inversion probes (smMIP) was designed, and a pooled smMIP-DNA library was prepared for error-corrected next generation sequencing with a standard protocol.<sup>22,23</sup> The panel targeted regions in 27 driver genes associated with haematological malignancies (online supplementary Table S1). Sequencing was performed for 300 cycles on a NovaSeq 6000 or NextSeq500 instrument (Illumina, San Diego, CA, USA), resulting in  $2 \times 150$  bp paired-end reads. Somatic variants were called and included in our analysis with a variant allele frequency (VAF)  $\geq 2\%$  and  $\geq 10$  mutant unique smMIP reads after being manually inspected and curated to exclude recurrent artifacts and polymorphisms. Variants with VAF  $>45\%$  were further excluded, as we could not determine whether these are from germline or somatic origin. The median number of error-corrected consensus reads for the entire cohort was 6274 and the median coverage was 3231, with a coverage  $>500\times$  for 97.8% of all targeted regions (online supplementary Figure S2). Details regarding the design of sequencing panel, library preparation and variant calling are described in online supplementary Appendix S1.

## Study endpoints

We considered HF incidence, incident HFrEF and incident HFpEF as the endpoints.

## Statistical analyses

Categorical variables are presented as numbers with percentages (%) and were compared using the Chi-squared test. Continuous variables are displayed as medians with IQR and were compared using Student's

independent t-test if variables were normally distributed, whereas skewed variables were compared using Mann–Whitney U test. The distribution of CHIP was observed according to age quartiles.

A stepwise logistic regression model was used to identify clinical correlates of CHIP, and the final model included all clinical covariates with a  $p$ -value  $<0.05$ . Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated to estimate the correlations of CHIP with clinical factors. Linear regression models were conducted to analyse the associations of CHIP occurrence with CVD biomarkers including N-terminal pro-B-type natriuretic peptide (NT-proBNP), mid-regional pro-A-type natriuretic peptide (MR-proANP), troponin T, C-reactive protein (CRP), mid-regional pro-adrenomedullin (MR-proADM), C-terminal pro-endothelin-1, procalcitonin, plasminogen activator inhibitor (PAI), galectin-3, cystatin C and UAE.<sup>24–26</sup> All biomarker measurements were natural log-transformed prior to analyses to obtain normal distribution. Univariable analyses with  $p < 0.05$  for all biomarkers associated with CHIP were adjusted for age and sex, which were further adjusted for smoking, diabetes, hypertension, body mass index (BMI), cholesterol, history of MI, atrial fibrillation (AF) and cerebrovascular accident. Since age is a strong risk factor for incident HF and significantly associated with CHIP, an interaction test of age to determine the effects of CHIP on new-onset HF was performed.

Univariable and multivariable Cox proportional hazards regression analyses were performed to evaluate associations between the presence of CHIP or specific single gene mutation(s) or clone size and outcomes. Age, sex, smoking, diabetes, hypertension, BMI, cholesterol, history of MI, AF and cerebrovascular accident were included as covariates in multivariable models. For each single gene analysis, the control group included individuals without CHIP mutations and people without variants in the specific single gene who may carry mutations in other CHIP driver genes. HRs are reported with 95% CIs. A 2-tailed  $p$ -value  $<0.05$  was considered to denote statistically significant differences. All data analyses were performed with Stata15.1 (StataCorp, 2017, College Station, TX, USA, StataCorp LLC).

## Results

### Baseline characteristics

In the PREVEND cohort, all 374 individuals who developed HF were included together with 374 age- and sex-matched control subjects. After excluding missing DNA of 28 people, four variant carriers with VAFs  $>45\%$  and 11 samples failing to be sequenced, the final sequencing results were obtained for 347 controls and 358 cases, as illustrated in online supplementary Figure S3. In the case cohort, 238 (66.5%) participants developed HFrEF and 120 people (33.5%) developed HFpEF. The baseline characteristics of the study population are presented in Table 1. The median age of all participants was 65 (IQR 58–70) and 35.6% were female. Compared to the control cohort, people with incident HF showed higher baseline levels of BMI, and CVD biomarkers including NT-proBNP, MR-proANP, troponin T, CRP, MR-proADM, PAI-1, cystatin C and UAE, accompanied by greater prevalence of MI, AF, diabetes and hypertension.

### Distribution of CHIP mutations

CHIP mutations were detected in 60 (17.3%) people in the control cohort and 66 (18.4%) individuals from the case cohort.

**Table 1** Baseline characteristics of the study population

Factor	Control (n = 347) (no HF)	Case (n = 358) (incident HF)	p-value
CHIP, n (%)	60 (17.3)	66 (18.4)	0.69
Clinical characteristics			
Age, years, median (IQR)	65 (58–70)	65 (58–70)	0.95
Female sex, n (%)	123 (35.4)	128 (35.8)	0.95
BMI, kg/m <sup>2</sup> , median (IQR)	26.6 (24.8–29.3)	27.9 (25.4–30.8)	<0.001
Medical history, n (%)			
Diabetes	19 (5.5)	46 (12.9)	<0.001
Hypertension	210 (60.5)	277 (77.6)	<0.001
Myocardial infarction	38 (11.0)	91 (25.4)	<0.001
Atrial fibrillation	7 (2.0)	22 (6.1)	0.006
Cerebrovascular accident	7 (2.0)	11 (3.1)	0.37
Malignancy	14 (4.0)	25 (7.0)	0.087
Smoking			0.12
Never	–88 (25.4)	–77 (21.6)	
Past	–153 (44.2)	–145 (40.7)	
Current	–105 (30.3)	–134 (37.6)	
Laboratory, median (IQR)			
Cholesterol, mmol/L	5.9 (5.2–6.5)	5.9 (5.3–6.7)	0.14
Telomere length, T/S	0.91 (0.77–1.11)	0.93 (0.77–1.13)	0.78
NT-proBNP, ng/L	50.8 (25.2–104.1)	104.8 (43.0–284.7)	<0.001
MR-proANP, pmol/L	57.8 (41.6–83.2)	73.4 (50.3–111.7)	<0.001
Troponin T, µg/L	4 (2.5–8)	7 (4–10)	<0.001
CRP, mg/L	1.9 (1.0–3.7)	2.5 (1.2–4.8)	0.006
MR-proADM, nmol/L	0.45 (0.34–0.54)	0.47 (0.37–0.59)	0.006
CT-proET-1, pmol/L	38.4 (27.4–47.3)	41.6 (29.3–52.2)	0.048
Procalcitonin, ng/L	0.017 (0.014–0.022)	0.018 (0.015–0.023)	0.017
PAI-1, mg/L	84.1 (52.5–124.7)	93.3 (57.9–160.2)	0.005
Galectin-3, mg/L	12.2 (10.2–14.4)	12.4 (10.4–14.7)	0.5
Cystatin C, mg/L	0.95 (0.87–1.07)	1.02 (0.90–1.14)	<0.001
UAE, mg/24 h	11.3 (6.9–26.2)	19.5 (9.3–53.9)	<0.001

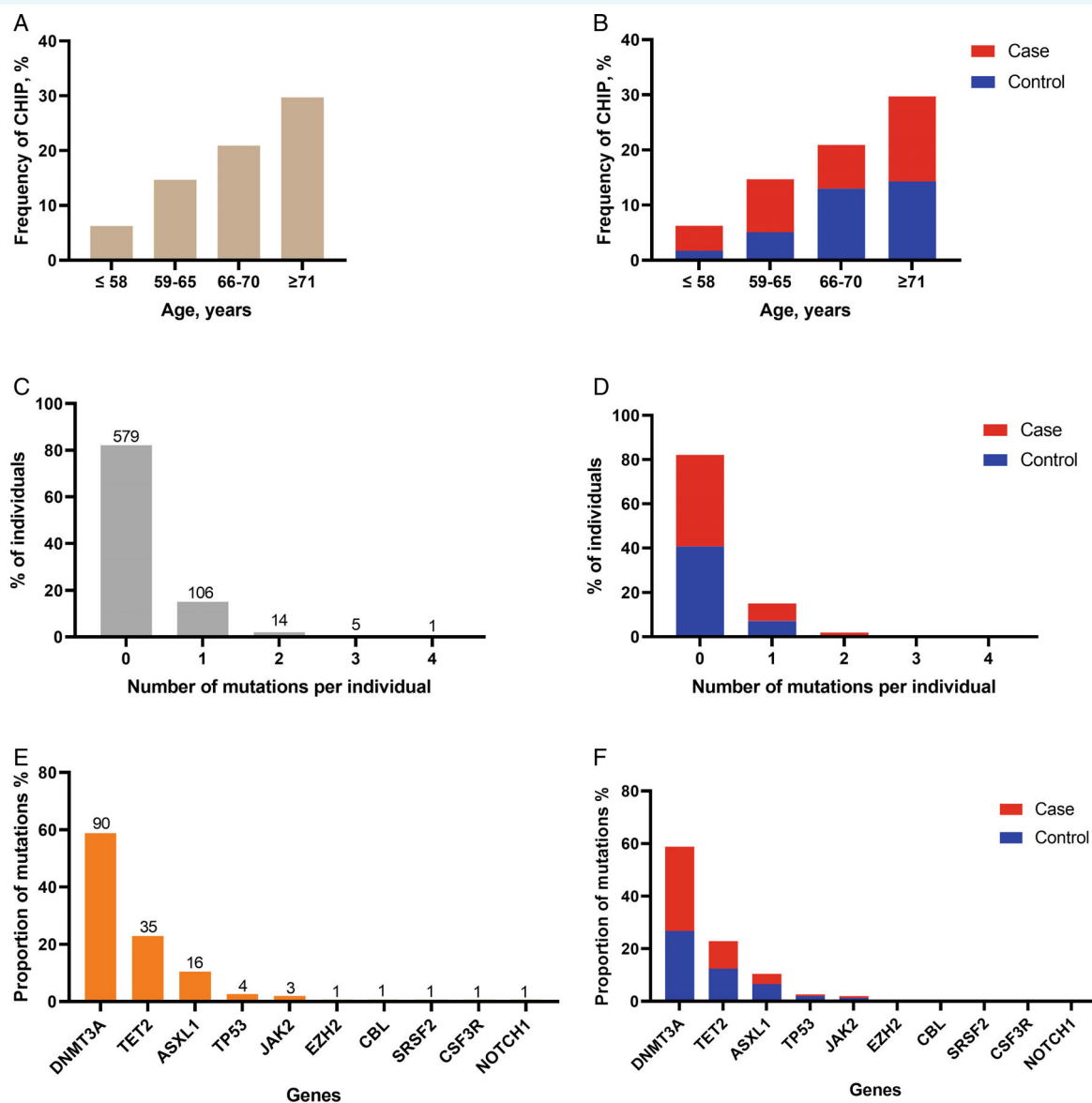
BMI, body mass index; CHIP, clonal haematopoiesis of indeterminate potential; CRP, C-reactive protein; CT-proET-1, C-terminal pro-endothelin-1; HF, heart failure; IQR, interquartile range; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro-A-type natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAI, plasminogen activator inhibitor; UAE, urine albumin excretion.

Details of the identified CHIP variants were listed in online supplementary *Table S2*. All participants were categorized according to age quartiles as depicted in *Figure 1A,B*. The prevalence of CHIP mutations increased from 6.3% among people with ages ≤58 (11 out of 176 persons) to 29.7% in elderly people above 71 years (52 out of 175 persons). In total, 153 mutations were identified among 126 individuals, of which 106 people (84%) had only one mutation, and 14 and 6 persons were found carrying 2 or >2 mutations, respectively (*Figure 1C*). The majority of the variants occurred in three genes: DNMT3A (58.8%), TET2 (22.9%) and ASXL1 (10.5%), as presented in *Figure 1E*. However, there were no significant differences observed between the case and control cohorts regarding the CHIP frequency, detected number of mutations, mutational spectrum and maximum VAF (*Figure 1B, D, F*; online supplementary *Figure S4*).

## CHIP is associated with cardiovascular risk factors and biomarkers

As displayed in online supplementary *Table S3*, 126 participants carrying CHIP mutations were older than the non-carriers, and had significantly higher baseline levels of NT-proBNP and MR-proANP, which are biomarkers for incident HF.

Linear regression analyses in *Table 2* showed that CHIP significantly correlated with 20.7% and 19.5% increases of log-standardized NT-proBNP ( $p = 0.019$ ) and MR-proANP ( $p = 0.034$ ) levels, respectively, after adjustment for age, sex, smoking, diabetes, hypertension, BMI, cholesterol, history of MI, AF and cerebrovascular accident. Logistic regression model (online supplementary *Table S4*) demonstrated that an increase of age by 10 years strongly correlated with the presence of CHIP (OR 2.30, 95% CI 1.66–3.18;  $p < 0.001$ ). Women had 66%



**Figure 1** Distribution of clonal haematopoiesis of indeterminate potential (CHIP) mutations. Frequency of CHIP mutations according to age quartiles in (A) the entire cohort, and (B) control and case cohort. Number of mutations per participant in (C) the entire cohort, and (D) control and case cohort. Proportion of mutations according to gene spectrum in (E) the entire cohort, and (F) control and case cohort.

higher chance to carry CHIP mutations than men. Additionally, CHIP was positively associated with past smoking (OR 2.11, 95% CI 1.16–3.83;  $p = 0.015$ ) and hypertension (OR 1.83, 95% CI 1.06–3.19;  $p = 0.031$ ), and negatively correlated with BMI (OR 0.72, 95% CI 0.54–0.95;  $p = 0.022$ ) and prevalent MI (OR 0.54, 95% CI 0.30–0.95;  $p = 0.033$ ).

### Association of CHIP with incident heart failure and heart failure subtypes

There were 43 people with incident HF<sub>rEF</sub> detected with CHIP variants, and 23 individuals with incident HF<sub>pEF</sub> carrying CHIP

mutations. Multivariable Cox regression models were performed to determine the associations of CHIP with incident HF and HF subtypes (online supplementary Table S5). However, the presence of CHIP was not significantly associated with overall HF incidence (HR 1.24, 95% CI 0.93–1.65;  $p = 0.144$ ), HF<sub>rEF</sub> (HR 1.20, 95% CI 0.84–1.71;  $p = 0.321$ ) or HF<sub>pEF</sub> (HR 1.09, 95% CI 0.67–1.77;  $p = 0.733$ ). Among individual genes, DNMT3A variants were significantly associated with new-onset HF with a HR of 1.52 (95% CI 1.10–2.10;  $p = 0.01$ ). This association between DNMT3A and incident HF remained significant after further adjustment for NT-proBNP (HR 1.40, 95% CI 1.01–1.94;  $p = 0.041$ ). No associations were observed between HF incidence and variants in

**Table 2** Correlation analyses of clonal haematopoiesis of indeterminate potential with cardiovascular biomarkers

	Univariable		Multivariable <sup>1</sup>		Multivariable <sup>2</sup>	
	Sβ (SE)	p-value	Sβ (SE)	p-value	Sβ (SE)	p-value
NT-proBNP	+0.411 (0.098)	0.000	+0.222 (0.096)	0.021	+0.207 (0.086)	0.019
MR-proANP	+0.443 (0.102)	0.000	+0.200 (0.096)	0.038	+0.195 (0.092)	0.034
Troponin T	+0.200 (0.099)	0.043	+0.015 (0.092)	0.867		
CRP	-0.027 (0.100)	0.785				
MR-proADM	+0.140 (0.103)	0.177				
CT-proET-1	+ 0.016 (0.104)	0.881				
Procalcitonin	-0.073 (0.104)	0.484				
PAI-1	-0.155 (0.099)	0.117				
Galectin-3	+0.127 (0.099)	0.2				
Cystatin C	+0.170 (0.102)	0.096				
UAE	-0.024 (0.098)	0.804				

CRP, C-reactive protein; CT-proET-1, C-terminal pro-endothelin-1; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro-A-type natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAI, plasminogen activator inhibitor; Sβ, standardized regression coefficient; SE, standard error; UAE, urine albumin excretion.

Multivariable model 1 was adjusted for age and sex.

Multivariable model 2 was further adjusted for smoking, diabetes, hypertension, body mass index, cholesterol, history of myocardial infarction, atrial fibrillation and cerebrovascular accident.

TET2 (HR 0.97, 95% CI 0.54–1.74;  $p = 0.919$ ) or ASXL1 (HR 0.74, 95% CI 0.30–1.80;  $p = 0.507$ ).

## CHIP frequency is higher in younger individuals with incident heart failure

There was a significant interaction between CHIP and age for incident HF ( $p$  for CHIP–age interaction = 0.002). Therefore, we stratified our analyses by age categories <65 years and ≥65 years (65 years being the median age).

In total 35 (10.1%) individuals were detected with CHIP variants from 348 people younger than 65 years, while 91 (25.5%) out of 357 participants ≥65 years carried CHIP mutations, as shown in Table 3. Interestingly, among individuals <65 years CHIP mutations were more frequently detected in the case cohort ( $n = 25$ , 14.2%) compared to the control subjects ( $n = 10$ , 5.8%;  $p = 0.009$ ), whereas in people ≥65 years the prevalence of CHIP was comparable between case and control cohorts ( $n = 41$ , 22.5% in cases vs.  $n = 50$ , 28.6% in controls;  $p = 0.19$ ).

## CHIP is associated with cardiovascular biomarkers and new-onset heart failure among people <65 years of age

Linear regression models in online supplementary Table S6 showed that previously observed associations between CHIP and natriuretic peptides including NT-proBNP and MR-proANP in the entire cohort were mainly driven by individuals younger than 65 years. In people ≥65 years, no correlations of cardiovascular biomarkers with CHIP were observed.

Multivariable Cox regression models in Table 4 demonstrated that in people younger than 65 years, CHIP was significantly associated with HF incidence (HR 2.07, 95% CI 1.30–3.29;  $p = 0.002$ ) and

incident HFpEF (HR 2.11, 95% CI 1.01–4.41;  $p = 0.048$ ) but not HFrEF (HR 1.45, 95% CI 0.79–2.66;  $p = 0.233$ ). Those associations were mainly driven by DNMT3A mutations. After further adjusting for NT-proBNP, the associations stayed significant between incident HF and CHIP (HR 1.72, 95% CI 1.08–2.76;  $p = 0.023$ ) or DNMT3A mutations (HR 1.73, 95% CI 1.01–2.97;  $p = 0.045$ ). There were no correlations between CHIP or DNMT3A variants with incident HF or HF subtypes among people older than 65 years.

## Associations of clone size with heart failure incidence and heart failure subtypes

To assess whether larger clone size could increase the risk of incident HF or HF subtypes, we categorized highest VAFs into two subgroups: VAF 2–5% and VAF >5%. As shown in Table 5, in multivariate analyses with adjustment for HF risk factors, we found only in people <65 years VAF 2–5% was significantly associated with incident HF (HR 1.98, 95% CI 1.15–3.41;  $p = 0.014$ ), larger clone size (VAF >5%) increased the risk of HF events (HR 2.30, 95% CI 1.05–5.02;  $p = 0.037$ ) and was associated with incident HFpEF (HR 4.74, 95% CI 1.76–12.74;  $p = 0.002$ ).

We additionally studied the potential association between the extent of clonal expansion and incident HF by lowering the cutoff of CHIP mutation calling to VAF ≥1%, which led to the identification of 124 additional mutations in 73 people. We categorized VAFs into three groups: 1–2%, 2–5%, and >5%. However, CHIP with lower threshold (VAF ≥1%) was not associated with HF incidence (HR 1.03, 95% CI 0.81–1.31,  $p = 0.831$ ), nor was larger clonal size (VAF >5%; HR 1.13, 95% CI 0.72–1.75,  $p = 0.591$ ) in the entire cohort after adjustment for HF risk factors (online supplementary Table S7). Among the younger individuals, the

**Table 3** Clonal haematopoiesis of indeterminate potential and baseline characteristics in individuals <65 and ≥65 years of age

Factor	Individuals <65 years (n = 348)			Individuals ≥65 years (n = 357)		
	Control (n = 172) (no HF)	Cases (n = 176) (incident HF)	p-value	Control (n = 175) (no HF)	Cases (n = 182) (incident HF)	p-value
CHIP, n (%)	10 (5.8)	25 (14.2)	0.009	50 (28.6)	41 (22.5)	0.19
Clinical characteristics						
Age, years, median (IQR)	57 (51–62)	58 (51–62)	0.91	69 (67–73)	70 (67–73)	0.99
Female sex, n (%)	63 (36.6)	64 (36.4)	0.96	60 (34.3)	64 (35.2)	0.86
BMI, kg/m <sup>2</sup> , median (IQR)	26.9 (24.9–29.5)	27.9 (25.6–31.1)	0.017	26.2 (24.6–29.1)	27.9 (25.2–30.3)	0.003
Medical history, n (%)						
Diabetes	8 (4.7)	21 (12)	0.015	11 (6.3)	25 (13.7)	0.019
Hypertension	75 (43.6)	118 (67.4)	<0.001	135 (77.1)	159 (87.4)	0.011
Myocardial infarction	14 (8.1)	38 (21.6)	<0.001	24 (13.7)	53 (29.1)	<0.001
Atrial fibrillation	1 (0.6)	8 (4.5)	0.02	6 (3.4)	14 (7.7)	0.08
Cerebrovascular accident	3 (1.7)	5 (2.8)	0.49	4 (2.3)	6 (3.3)	0.56
Malignancy	5 (2.9)	7 (4)	0.58	9 (5.1)	18 (9.9)	0.09
Smoking			0.25			0.68
Never	–40 (23.4)	–33 (18.9)		–48 (27.4)	–44 (24.3)	
Past	–69 (40.4)	–59 (33.7)		–84 (48)	–86 (47.5)	
Current	–62 (36.3)	–83 (47.4)		–43 (24.6)	–51 (28.2)	
Laboratory, median (IQR)						
Cholesterol, mmol/L	5.9 (5.2–6.4)	6.1 (5.4–6.8)	0.047	5.9 (5.2–6.5)	5.8 (5.2–6.6)	0.88
Telomere length, T/S	0.93 (0.78–1.16)	0.95 (0.80–1.18)	0.43	0.90 (0.77–1.10)	0.90 (0.76–1.08)	0.66
NT-proBNP, ng/L	32.2 (15.9–60.8)	69.4 (27.5–171.7)	<0.001	84.9(36.3–153.5)	163.5 (77.5–427.4)	<0.001
MR-proANP, pmol/L	48.1 (36.1–63.8)	55.6 (42.2–82.6)	0.002	74.0 (50.9–101.3)	91.3 (63.2–131.8)	<0.001
Troponin T, µg/L	3 (2.5–5)	5 (2.5–8)	<0.001	6 (3–10)	8 (6–12)	<0.001
CRP, mg/L	1.8 (0.9–3.3)	2.3 (1.1–4.7)	0.022	1.9 (1.1–3.9)	2.5 (1.2–4.9)	0.15
MR-proADM, nmol/L	0.39 (0.32–0.51)	0.42 (0.35–0.52)	0.12	0.50 (0.40–0.57)	0.54 (0.40–0.63)	0.012
CT-proET-1, pmol/L	36.9 (26.0–45.3)	37.7 (25.4–48.7)	0.33	40.3 (30.8–51.4)	44.7 (33.0–55.3)	0.1
Procalcitonin, ng/L	0.017 (0.014–0.022)	0.018 (0.015–0.023)	0.20	0.018 (0.015–0.022)	0.019 (0.016–0.023)	0.04
PAI-1, mg/L	92.6 (55.5–164.0)	100.4 (60.4–162.1)	0.25	75.1 (50.4–113.8)	89.3 (56.1–156.6)	0.005
Galectin-3, mg/L	11.4 (9.7–13.8)	11.8 (9.4–13.1)	0.77	13 (11–14.8)	13.3 (11.4–15.9)	0.17
Cystatin C, mg/L	0.9 (0.82–0.99)	0.94 (0.85–1.03)	0.016	1 (0.91–1.17)	1.1 (1.01–1.21)	<0.001
UAE, mg/24 h	9.8 (6.4–20.5)	18.0 (8.6–52.9)	<0.001	12.2 (7.5–29.9)	22.3 (10.7–53.9)	<0.001

BMI, body mass index; CHIP, clonal haematopoiesis of indeterminate potential; CRP, C-reactive protein; CT-proET-1, C-terminal pro-endothelin-1; HF, heart failure; IQR, interquartile range; MR-proADM, mid-regional pro-adrenomedullin; MR-proANP, mid-regional pro-A-type natriuretic peptide; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PAI, plasminogen activator inhibitor; UAE, urine albumin excretion.

**Table 4** Associations of clonal haematopoiesis of indeterminate potential with incident heart failure, heart failure subtypes, according to age categories

	Individuals <65 years				Individuals ≥65 years			
	CHIP HR (95% CI)	p-value	DNMT3A HR (95% CI)	p-value	CHIP HR (95% CI)	p-value	DNMT3A HR (95% CI)	p-value
Incident HF	2.07 (1.30–3.29)	0.002	2.13 (1.25–3.61)	0.005	0.95 (0.66–1.36)	0.773	1.29 (0.86–1.95)	0.220
Incident HF <sub>rEF</sub>	1.45 (0.79–2.66)	0.233	1.35 (0.66–2.76)	0.416	1.01 (0.65–1.58)	0.957	1.23 (0.74–2.06)	0.425
Incident HF <sub>pEF</sub>	2.11 (1.01–4.41)	0.048	2.26 (1.01–5.04)	0.047	0.78 (0.41–1.49)	0.447	1.10 (0.54–2.23)	0.789

CHIP, clonal haematopoiesis of indeterminate potential; CI, confidence interval; HF, heart failure; HF<sub>pEF</sub>, heart failure with preserved ejection fraction; HF<sub>rEF</sub>, heart failure with reduced ejection fraction; HR, hazard ratio.

Cox regression models were adjusted for age, sex, smoking, diabetes, hypertension, body mass index, cholesterol, history of myocardial infarction, atrial fibrillation and cerebrovascular accident.



**Table 5** Associations of highest variant allele frequencies with incident heart failure and its subtypes

	Entire cohort			Individuals <65 years			Individuals ≥65 years			
	VAF 2–5% HR (95% CI)	p-value	VAF >5% HR (95% CI)	p-value	VAF 2–5% HR (95% CI)	p-value	VAF >5% HR (95% CI)	p-value	VAF >5% HR (95% CI)	
Incident HF	1.29 (0.91–1.82)	0.147	1.16 (0.75–1.80)	0.506	1.98 (1.15–3.41)	0.014	2.30 (1.05–5.02)	0.037	1.04 (0.66–1.63)	0.864
Incident HFpEF	1.42 (0.94–2.14)	0.092	0.87 (0.48–1.57)	0.637	1.67 (0.87–3.22)	0.126	0.85 (0.20–3.49)	0.817	1.29 (0.75–2.19)	0.357
Incident HFrEF	0.83 (0.44–1.54)	0.549	1.67 (0.87–3.22)	0.123	1.32 (0.50–3.51)	0.573	4.74 (1.76–12.74)	0.002	0.62 (0.27–1.41)	0.254

CI, confidence interval; HF, heart failure; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; HR, hazard ratio; VAF, variant allele frequency. Cox regression models were adjusted for age, sex, smoking, diabetes, hypertension, body mass index, cholesterol, history of myocardial infarction, atrial fibrillation and cerebrovascular accident.

association between CHIP with new-onset HF was observed only in groups with VAF ≥2%.

## Discussion

In this study we investigated the prevalence and associations of CHIP mutations for incident HF by error-corrected targeted next generation sequencing. Firstly, our results confirm previous reports that CHIP mutations are more prevalent in older individuals, among which DNMT3A, TET2 and ASXL1 are three main driver mutations.<sup>3,15,18</sup> We found that the frequency of CHIP was comparable between subjects who developed incident HF and those who did not (control subjects). Next, we verified that CHIP correlated with cardiovascular risk factors, including age, smoking and hypertension, and was significantly associated with HF biomarkers including NT-proBNP and MR-proANP. Lastly, we demonstrated that the presence of CHIP did not associate with incident HF in the entire cohort but among people younger than 65 years. Larger clone size increased the risk of incident HF only in the younger individuals (*Graphical Abstract*).

Yu's study in five cohorts which collectively enrolled 56 597 participants showed that CHIP mutations detected by whole exome or genome sequencing (WES/WGS) were associated with a 25% increased risk for incident HF.<sup>18</sup> In our study, error-corrected targeted next generation sequencing was used to detect CHIP mutations, which provides better sensitivity to reliably capture CHIP variants with VAF 2–5% than WES or WGS.<sup>27</sup> Compared to Yu's finding, our study further suggests the association of CHIP with HF incidence varies among individuals with different ages. We only found a significant correlation between CHIP and new-onset HF in the relatively young subjects, while it was absent in people older than 65 years. This association was mainly driven by DNMT3A mutations instead of TET2 or ASXL1 variants observed in Yu's study,<sup>18</sup> which is in line with earlier basic research that inactivating mutations in gene DNMT3A promoted inflammation and cardiac hypertrophy,<sup>28</sup> and with clinical settings that DNMT3A variants significantly correlated with HF adverse outcomes.<sup>15,17</sup> Consistent with previous findings that large clone size associated with higher risk of incident HF, all-cause mortality and HF hospitalization,<sup>17,18</sup> we observed clonal expansion (VAF >5%) increased the risk of HF events but only in the younger population.

This phenomenon could be explained as age is a strong confounder, highly involved in modifying the effects of HF risk factors. The attributable risk conferred by a risk factor in younger population might be greater and more easily exposed than that in older people whose baseline risk for HF is higher, and where multiple risk factors weigh in. For example, known risk factors such as hypertension, diabetes, obesity and current smoking history have been reported to confer greater relative HF risk in younger compared with older population.<sup>29–31</sup> In line with this, CHIP mutations at a young age may afford a potential risk for incident HF, despite a lower incidence and absolute risk of HF among younger compared with older people.

Cross-sectional analyses showed that CHIP is associated with age, smoking, hypertension and CVD biomarkers. After

multivariable adjustment, NT-proBNP and MR-proBNP remained significantly associated with CHIP, which is driven by the younger individuals. There is no clear evidence on whether the mutation(s) can directly affect NT-proBNP production. Inflammation could be one of the possible mechanisms explaining the link between somatic mutations and natriuretic peptide release. For instance, experimental models for TET2, JAK2, and DNMT3A mutations demonstrated similar accelerating effects on cardiac dysfunction, hypertrophy and fibrosis.<sup>11,28,32</sup> Inactivating TET2 promoted the expression of IL-1 $\beta$ , IL-6, and chemokine C-C motif ligand 5 (Ccl5), whereas DNMT3A deactivation increased the expression of CXC ligand 1 (Cxcl1), Cxcl2, IL-6 and Ccl5.<sup>28</sup> In clinical settings, NT-proBNP levels positively correlate with the levels of inflammatory markers in HF patients.<sup>33,34</sup> A possible mechanism linking these two is that cytokines such as IL-6, IL-1 $\beta$ , IL-18 and tumour necrosis factor- $\alpha$  can stimulate the expression and secretion of natriuretic peptides.<sup>33,35,36</sup>

Classical risk factors including age, sex, smoking, hypertension, obesity, diabetes, smoking, lifestyle and pathophysiological pathways such as inflammation and oxidative stress, show strongly predictive value for incident CVD and cancer.<sup>12,37</sup> However, current risk models are only able to ascertain 53–75% of the individual's risk, and there is a substantial residual risk of genetics that remains unaccounted for.<sup>29</sup> Accumulated data support that CHIP has become a clinical entity with genetic susceptibility to incident HF, cancer and adverse outcomes, independent of environmental risk factors.<sup>3,8,15–18</sup> In our study, the greater attributable risk of CHIP at a young age for HF highlights the importance of early detection for CHIP and preventive intervention. Recently, the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) verified that the IL-1 $\beta$  neutralizing antibody canakinumab can reduce recurrent cardiovascular events, HF hospitalization, and elevated CRP levels in patients with stable coronary artery disease.<sup>38,39</sup> A CANTOS exploratory analysis further suggestively showed that patients with TET2 mutations have an improved response to canakinumab.<sup>40</sup> Whether CVD patients carrying CHIP mutations especially with young ages, are more sensitive to anti-inflammatory therapy, which might be specific for mutated genes, should be further explored in large clinical trials.

## Limitations

Our study suggesting that the presence of CHIP is associated with incident HF mainly in a relatively young population should be further validated in additional larger cohort studies. Although our cohort is clinically well annotated, due to the relative low number of cases, our study lacks sufficient statistical power to analyse the associations of specific gene mutations or number of mutations with incident HF or HF phenotypes. Similarly, correlations between CHIP and additional inflammatory markers such as IL-6 or IL-1 $\beta$  cannot be studied due to data unavailability of the PREVENT cohort. Subjects of <75 years were enrolled in the PREVENT cohort. Therefore, our results cannot be extrapolated to subjects of older than 75 years. Furthermore, we used the classic definition of CHIP with a VAF  $\geq 2\%$  to make this study comparable with previous data.<sup>15,17,18</sup> In Assmus's study,<sup>41</sup> lower VAF thresholds

for TET2 and DNMT3A have shown significant associations with HF outcomes. Whether certain cutoffs of VAF (either <2% or  $\geq 2\%$ ) for specific genes might confer a significant association with new-onset HF remain to be explored in large cohort studies.

## Conclusions

The current results support the notion that the somatic mutations in clonal haematopoiesis are associated with HF incidence mainly in individuals younger than 65 years.

## Supplementary Information

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

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