ESC HEART FAILURE
ESC Heart Failure 2022; **9**: 3954–3960
Published online 18 August 2022 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.14115

ORIGINAL ARTICLE

Clonal haematopoiesis of indeterminate potential-related mutations and outcome in dilated and ischaemic cardiomyopathy

Jasmine M.F. Wu[1], Tarek Bekfani[1,2], Anna Hinze[3], Julian Georg Westphal[1], Berit Steinacker[1], Max Zeller[1], Charlotte Hartmann[1], Sven Möbius-Winkler[1], Andreas Hochhaus[3], P. Christian Schulze[1]* and Thomas Ernst[3]

[1]*Department of Internal Medicine I, Division of Cardiology, University Hospital Jena, FSU Jena, Am Klinikum 1, 07747, Jena, Germany;* [2]*Clinic for Cardiology, Angiology and Pneumology, University Hospital Magdeburg A.ö.R, Magdeburg, Germany; and* [3]*Department of Internal Medicine II, Division of Hematology and Oncology, University Hospital Jena, FSU Jena, Jena, Germany*

Abstract

Aims Clonal haematopoiesis of indeterminate potential (CHIP)-associated mutation is a risk factor for the development of ischaemic cardiomyopathy (ICM), but its association with non-ischaemic dilated cardiomyopathy (DCM) remains unclear. We aimed to determine the prevalence of CHIP in patients with DCM and define its risk for disease progression.

Methods and results Next-generation sequencing targeting 54 common CHIP-associated genes was performed in 48 ICM and 52 DCM patients. The patients were monitored for a median of 3.1 years, and a COX proportional hazards model was used to examine the association between CHIP and adverse clinical outcome with regard to all-cause death or all-cause hospitalization. Overall, the prevalence of CHIP mutations was 19% and 13% in DCM and ICM, respectively. Seventeen per cent of ICM patients over 75 years were CHIP carriers. In DCM cohort, mutation event had already been observed in the patients who were under the age of 45 (13%). Among 54 genes analysed, *DNMT3A* had the highest mutation frequency, followed by *TET2* and *CUX1*. Kaplan–Meier curve over a median of 3.1 year tracking period showed a trend towards poor clinical outcome in the DCM patients who carried *DNMT3A* or *TET2* mutation; however, such association was not statistically significant.

Conclusions The prevalence of CHIP is detected at a young age in DCM, and accumulation of mutational frequency in DCM patients is independent of age. However, a larger patient cohort is required to validate the association between CHIP and clinical progression in the DCM patients.

Keywords Clonal haematopoiesis of indeterminate potential (CHIP); Dilated cardiomyopathy; *DNMT3A*; *TET2*; Clinical outcome

Received: 2 March 2022; Revised: 19 July 2022; Accepted: 4 August 2022
**Correspondence to: P. Christian Schulze, MD, Professor of Medicine, Chair, Department of Internal Medicine I, Division of Cardiology, University Hospital Jena, FSU Jena, Am Klinikum 1, 07747 Jena, Germany. Tel: 03641-9324101; Fax: 03641-9324102. Email: christian.schulze@med.uni-jena.de*
P. Christian Schulze and Thomas Ernst are joint senior authors.

Introduction

Clonal haematopoiesis of indeterminate potential (CHIP) describes a phenomenon where the mutant clone expands without causing evident haematopoietic malignancy. Accumulation of CHIP-associated somatic mutations is age dependent, and they are associated with increased risk of coronary heart disease.[1,2] In support of this finding, a meta-analysis reveals that CHIP carriers have 1.9 times greater risk of having coronary heart disease and 4 times greater risk of developing myocardial infarction.[3] Further analysis in chronic ischaemic heart failure cohort confirms high CHIP frequency in the patients, and its prevalence rises in an age-dependent manner.[4] It is also shown that CHIP increases the heart failure risk by 25%, suggesting that it is a risk factor of heart failure.[5] Furthermore, CHIP carriers are two times more likely to develop worsen clinical outcome than the non-carriers.[6] Collectively, these findings show that CHIP-associated mutations are adversely associated with cardiomyopathy progression.

$JAK2^{VF}$ is a gain-of-function mutation known to contribute to clonal haematopoiesis. In $Ldlr^{-/-}$ mice that are susceptible to atherosclerosis, larger atherosclerotic lesions are observed

in animals transplanted with JAK2^{VF} bone marrow cells.[7] In addition to JAK2^{VF}, *DNMT3A* and *TET2*, the two most commonly mutated CHIP-associated genes, have been implicated in poor prognosis of ischaemic heart failure.[3,4,8] It is reported that *DNMT3A* and *TET2* mutations are associated with an increased inflammatory response, which may underlie atherosclerosis development and contribute to ischaemic heart failure.[3,9,10] In agreement with these findings, abrogation of inflammatory signalling improves atherosclerotic plaque stability and reduces cardiovascular risk.[7,10,11]

Although prior data have suggested a causal relationship between CHIP and ischaemic cardiomyopathy (ICM), the association between CHIP and non-ischaemic dilated cardiomyopathy (DCM) in the absence of atherosclerotic vascular disease remains unclear. The study aimed to determine and compare the prevalence of CHIP-associated mutations in non-ischaemic DCM and ICM. Furthermore, the association between CHIP and the risk of poor clinical progression with regard to all-cause death or all-cause hospitalization was also examined.

Methods

A total number of 100 patients were recruited prospectively between March 2017 and March 2019 as part of the Heart Failure Registry at the University Hospital Jena for the study. All patients were recruited consecutively from the outpatient clinic and singed an informed consent. The trial and the trial protocol were approved by the local ethics committee. We included patients with symptomatic chronic heart failure New York Heart Association (NYHA) class I–IV regardless of aetiology or left ventricular ejection fraction (LVEF). Patients with an active malignant disease were excluded. All patients were on stable guideline-directed heart failure medication at the time of inclusion. The blood was taken by venipuncture and immediately processed for centrifugation and blood separation. The fractionated blood was aliquoted and stored at −80°C.

The ICM patients were characterized by the presence of coronary artery disease with the need for revascularization, and 90% of patients who had received intervention were successfully treated. Severity of ischaemic heart disease was characterized by the number of affected coronary arteries, in which 44%, 27%, and 29% of patients had one-, two-, or three-vessel disease, respectively. Furthermore, 19% of patients were previously treated with CABG. Non-ischaemic cardiomyopathy was diagnosed in patients with impaired ventricular systolic or diastolic function without any evidence of significant coronary disease. If these patients improved their left ventricular function under guideline-directed medical therapy at the time of inclusion, record of severely reduced LVEF with a dilated ventricle must have been present.

Genomic DNA was extracted from buffy coat using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Additionally, constitutional DNA, if available, was isolated from oral mucosa cells using buccal swabs to verify the somatic origin of mutations.

Next-generation sequencing was performed on the MiniSeq platform using the TruSight Myeloid Sequencing panel (Illumina, San Diego, CA, USA) covering 54 genes either full or only the hotspot regions that are commonly mutated in myeloid malignancies. Paired-end sequencing was performed with 150 bp read length. Data analysis was performed using the VariantStudio Software (Version 3.0, Illumina) with a sensitivity of 5% variant allele frequency and a read depth of at least be 500 to minimize potential artefacts as recommended by the manufacturer. The software's own path filter was used as recommended by the developer. The polymorphisms, intronic mutations with more than three bases from a splice region, silent mutations, and missense mutations with an allele frequency of ≥1% in the general population were also excluded. *In silico* analysis of the mutation outcome was performed using four predictive software, the SIFT, PolyPhen-2, FATHMM, and Mutation Taster. The mutation was predicted to be harmful to protein function when the variant was identified as deleterious or damaging in three out of four bioinformatic tools.[12]

For baseline characteristics of patients, numerical data are presented as mean ± SEM whereas categorical data are presented as percentage. Statistical analysis of numerical data and categorical data was performed using unpaired *t*-test and Fisher's exact test, respectively. For survival analysis for a median 3.1 years, Kaplan–Meier analysis was used. To evaluate the correlation between adverse clinical progression with regard to all-cause death and all-cause hospitalization and CHIP mutations, a univariant COX proportional hazards model was used.

Results

The recruited 48 ICM and 52 DCM patients had a median age of 69 and 62 years old, respectively, with an age range 26–94 years. The ICM cohort was composed of 71% of males, whereas 63% of DCM patients were male. The majority of patients were with NYHA class II, which accounted for 48% and 62% of ICM and DCM cohorts, respectively, and had no significant difference in LVEF and BMI values (*Table 1*).

The amplicon read coverage ranged from 2347 to 17 607 reads per amplicon. The sequencing analysis revealed that a single gene mutation was identified in 16 patients (Supporting Information, *Table S1*), and the overall CHIP prevalence in DCM and ICM was 19% and 13%, respectively. The highest CHIP mutation frequency was observed in the ICM patients who were older than 75 years, in which 17% of

Table 1 Baseline characteristics of the patients recruited in the study

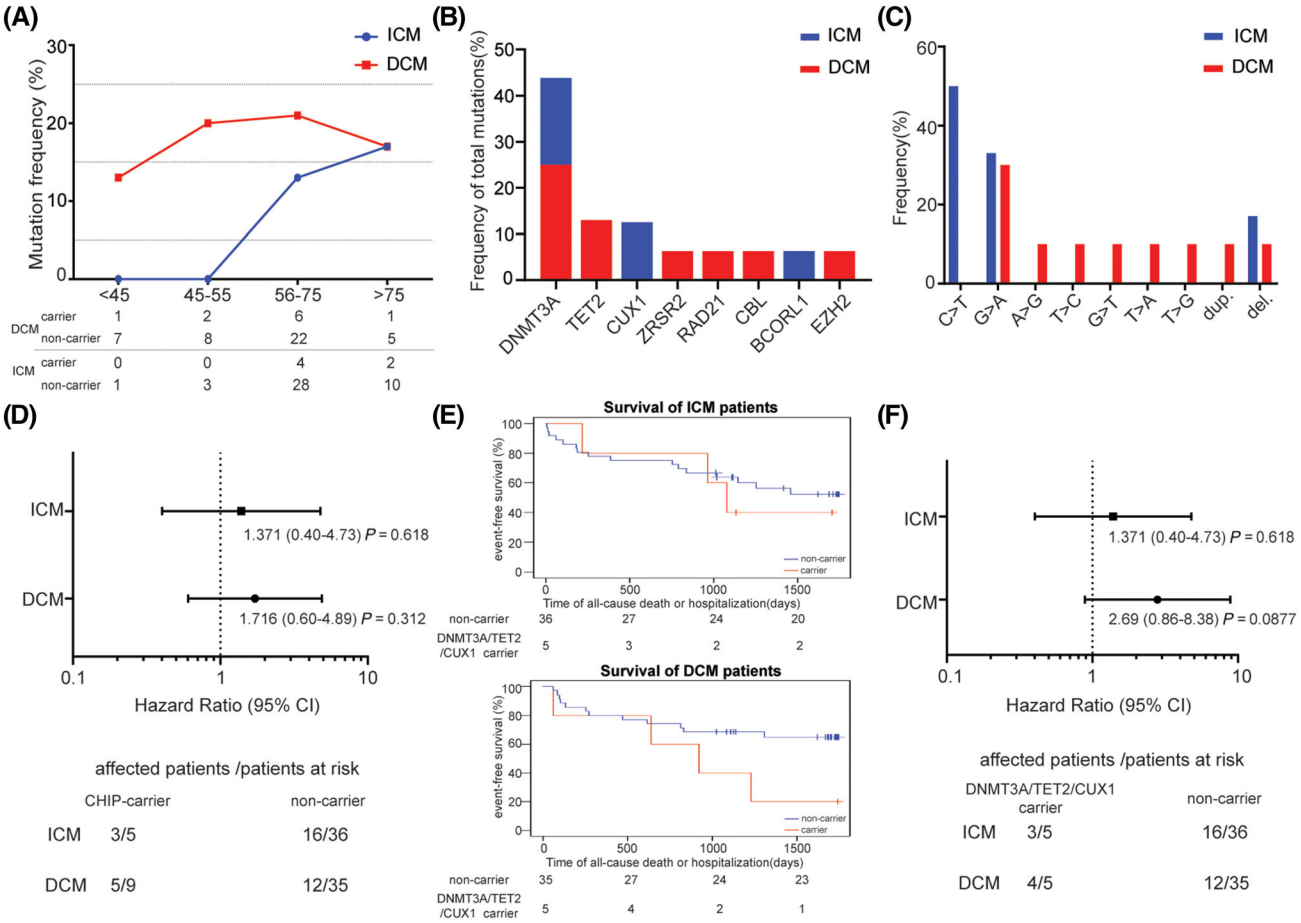
		ICM, n = 48	DCM, n = 52	P value
Basic characteristics				
Median age (years)		68.5	61.5	
Gender, male%		70.83%	63.46%	0.5245
BMI		27.7 ± 3.849, n = 46	28.5 ± 5.207, n = 50	0.3874
Smoking (%)		43.75%	34.6%	0.2193
Diabetes mellitus (%)		39.13%, n = 46	28.00%, n = 50	0.2834
Hypertension (%)		76.09%, n = 46	50.98%, n = 51	0.0124
Atrial fibrillation (%)		31.25%	32.7%	>0.999
	I	10%	13%	
NYHA class	II	48%	62%	
	III	38%	19%	
	IV	4%	6%	
	HFrEF	60.4%	57.7%	
Heart failure category	HFmrEF	18.8%	28.8%	
	HFpEF	20.8%	13.5%	
Echocardiographic parameters				
LVEF (%)		40.45 ± 1.975	36.81 ± 1.712	0.1651
LVEDd (mm)		56.08 ± 1.36	59.31 ± 1.246	0.0829
LVEDs (mm)		41.62 ± 2.18, n = 29	49.08 ± 1.631, n = 40	0.0067
LVEDV (mL)		166.4 ± 9.11, n = 46	174.9 ± 8.985	0.5090
LVEDVi (mL/m^2)		85.18 ± 4.714, n = 46	96.47 ± 10.1	0.3337
LAVol (mL)		85.48 ± 3.852, n = 44	103.4 ± 8.614, n = 47	0.0673
LAVoli (mL/m^2)		43.81 ± 1.976, n = 44	50.95 ± 3.652, n = 47	0.0953
E/A		1.233 ± 0.125, n = 37	1.416 ± 0.168, n = 33	0.3782
E/e′		15.25 ± 0.996, n = 22	15 ± 1.04, n = 27	0.8681
Medication use				
Anti-coagulant		51.11%, n = 45	15.69%, n = 51	0.0004
Beta-blocker		88.89%, n = 45	94.12%, n = 51	0.4678
Diuretics		75.56%, n = 45	86.27%, n = 51	0.2005
ACEI/Sartan/ARNI		93.33%, n = 45	98.04%, n = 51	0.3379
Statin		86.67%, n = 45	43.14%, n = 51	<0.0001
MRA		66.67%, n = 45	84.31%, n = 51	0.0562
Laboratory values				
Leukocytes (Gpt/L)		8.64 ± 2.837, n = 45	7.694 ± 2.111, n = 49	0.0684
Thrombocytes (Gpt/L)		232.7 ± 78.37, n = 45	232.9 ± 60.45, n = 50	0.9917
Haemoglobin (mmol/L)		8.396 ± 0.8076, n = 45	8.273 ± 1.219, n = 49	0.5719
MCV (fL)		89.33 ± 4.221, n = 45	88.47 ± 6.959, n = 49	0.4734
MCHC (mmol/L)		20.45 ± 0.6222, n = 45	20.51 ± 0.812, n = 45	0.7058
Uric acid (μmol/L)		421.5 ± 117.7, n = 42	465.6 ± 142.4, n = 46	0.1192
BNP (pg/mL)		606.1 ± 1005, n = 26	666.4 ± 1231, n = 28	0.8450
Creatinine (μmol/L)		145.8 ± 181.4, n = 45	106.9 ± 43.92, n = 50	0.1451
HbA1c (%)		6.368 ± 1.447, n = 44	5.971 ± 0.7267, n = 48	0.0954
GFR (mL/min)		62.16 ± 25.33, n = 45	69.49 ± 27.49, n = 50	0.1811

ACEI, angiotensin-converting enzyme inhibitor; ARNI, angiotensin receptor-neprilysin inhibitor; BNP, B-type natriuretic peptide; DCM, dilated cardiomyopathy; GFR, glomerular filtration rate; HbA1c, haemoglobin A1c; HFmrEF, heart failure with mid-range ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; ICM, ischaemic cardiomyopathy; LAVol, left atrial volume; LAVoli, left atrial volume index; LVEDd, left ventricular end-diastolic dimension; LVEDs, left ventricular end-systolic dimension; LVEDV, left ventricular end-diastolic volume; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; MRA, mineralocorticoid receptor antagonist; NYHA, New York Heart Association.
Numerical data are presented as mean ± SEM and categorical data are presented as percentage. Results are calculated from 48 and 52 patients in ICM and DCM groups, respectively, unless otherwise specified. Statistical analyses were performed using Fisher's exact test or unpaired t-test.

patients had mutation. Of note, one mutational event was observed in the DCM patient who was under 45 years old, and this accounted for 13% of mutation frequency in this age group. Nevertheless, no mutational event in this age group was observed in the ICM cohort (*Figure 1A*).

The predominant gene that was mutated in both DCM and ICM was *DNMT3A* (DCM, four variants; ICM, three variants), followed by *TET2* (DCM, two variants), *CUX1* (ICM, two variants), *ZRSR2*, *RAD21*, *CBL*, *BCORL1*, and *EZH2* (one variant each) (*Figure 1B*). Analyses of the mutation variants in both cohorts showed distinct base-pair change patterns. The G > A nucleotide change was commonly observed in the DCM and contributed to 30% of total mutation variants identified. In the ICM cohort, 50% of the base-pair change was found to be C > T nucleotide transition, which was not found in the DCM group (*Figure 1C*). Interestingly, the somatic C > T nucleotide change was reported to be enriched in age-associated mutational signature.[13] The majority of

Figure 1 Prevalence of clonal haematopoiesis of indeterminate potential (CHIP)-associated mutations and clinical outcomes in patients with ischaemic cardiomyopathy (ICM) or non-ischaemic dilated cardiomyopathy (DCM). (A) Graph represents the frequency of CHIP mutation observed in different age groups in DCM and ICM cohorts. (B) The frequency of mutated genes identified in both ICM and DCM cohorts. (C) The percentages of different types of nucleotide changes observed in the mutated genes. (D) A COX proportional hazards model was performed to examine the association between CHIP mutation and all-cause death and all-cause hospitalization in the DCM and ICM cohorts over a median follow-up time of 3.1 years. Patients who became lost to follow-up were excluded for the analysis. (E) Kaplan–Meier survival curves showing the event-free survival in the patients who carried *DNMT3A*, *TET2*, or *CUX1* mutations in ICM and DCM cohorts. Patients who were lost to follow-up and/or carried other CHIP mutation were excluded from analysis. (F) The prognostic significance of *DNMT3A*, *TET2*, and *CUX1* mutations with regard to all-cause death and all-cause hospitalization during the follow-up period was analysed using a hazard-risk model. del, deletion; dup, duplication.



identified mutations were classified as missense mutations. Using *in silico* predictive tools, these mutations were predicted to be deleterious with damaged protein function (Supporting Information, *Table S1*).

The patients in the study were monitored for a median 3.1 years (interquartile range: 1.9 to 4.7 years) for the events of all-cause death or all-cause hospitalization. During this period of time, 36 patients reached the endpoint. Association between CHIP mutations and adverse clinical outcomes in ICM patients was not significant [hazard ratio (HR) = 1.371, 95% confidence interval (CI) = 0.40–4.73, P = 0.618]. In DCM cohort, 56% of patients who reached the endpoint during the follow-up period were CHIP carriers, whereas 34% of non-carriers had poor disease progression (HR = 1.716, 95% CI = 0.60–4.89, P = 0.312) (*Figure 1D*).

DNMT3A and *TET2* mutations are known to increase the risk of ischaemic heart disease and are adversely associated with heart failure progression.[3,6] In our study, *DNMT3A*, *TET2*, and *CUX1* were the most frequently mutated genes observed. Therefore, the association between these mutations and the adverse disease progression with regard to all-cause death and all-cause hospitalization during the follow-up period was examined. Kaplan–Meier survival curve showed a trend towards poor clinical outcome in the DCM patients who carried *DNMT3A* or *TET2* mutation (*Figure 1E*). However, the association was not statistically significant (HR = 2.69, 95% CI = 0.86–8.38, P = 0.0877) (*Figure 1F*). Acquisition of *DNMT3A* or *CUX1* mutation had no significant negative effect on disease progression in ICM cohort (HR = 1.371, 95% CI = 0.40–4.73, P = 0.618) (*Figure 1E* and *1F*).

Discussion

Using a targeted gene sequencing panel, eight CHIP-associated genes were found to be mutated in our ICM and DCM cohorts. In consistent with literatures, *DNMT3A* remained the most commonly mutated CHIP gene in heart diseases.[4,6] In normal aging, 1.3% and 5% of CHIP mutation frequencies are observed among people under 50 years of age and those who are between 50 and 79 years of age, respectively. The mutation rate increases to an average of 13% in persons who are older than 80 years of age.[1] In DCM cohort, 19% of CHIP mutation frequency is observed. Such frequency is higher than the mutational event reported in normal aging[1] and compared with 13% mutation prevalence in our ICM cohort. Furthermore, 13% of DCM patients under the age of 45 years already carry CHIP mutation. Such mutational event is more frequent than that in our ICM cohort (0/2) and the previously reported ischaemic disease cohort (0/15).[4] In DCM cohort, a trend towards worse clinical outcome is observed in the carriers of *DNMT3A* or *TET2* mutation. Owing to limited sample size, the association is not statistically significant.

It is known that CHIP mutations in ischaemic heart diseases and normal aging accumulate in an age-dependent manner.[1,4] CHIP-associated changes in macrophage activity and inflammatory response have been shown to contribute to larger atherosclerotic lesion size and worse cardiac function, suggesting a causal role of CHIP in ICM.[3,9,10,14] On the contrary, our results demonstrate that CHIP mutation onset is observed at a relatively young age in DCM patients. Aetiology of DCM is heterogeneous, and approximately 30–50% of DCM cases are familial, potentially affecting young patients.[15] In animals with DCM due to genetic mutations, increased levels of inflammation and oxidative stress have been observed.[16–18] In support of the role of oxidative stress in DCM pathogenesis, antioxidants have been shown to ameliorate DCM-associated phenotypes in animals and improved cardiac function in young DCM patients.[19,20] Furthermore, it is known that failing myocardium exhibits a metabolic switch from fatty acid oxidation to glycosylation.[21] Metabolic regulation is a key to maintain haematopoietic stem cell quiescence, which is not only critical to sustain its function but also as a mechanism to protect itself against replication-associated stress.[22,23] In light of a high percentage of familial DCM incidence and the fact that CHIP-associated mutations could be observed in relatively young DCM patients, it is possible that DCM may predispose a pathophysiologic environment with elevated oxidative stress and disturbed metabolism, which may not only attenuate haematopoietic stem cell quiescence but also facilitate expansion of mutant clone, hence acquisition of CHIP. Alternatively, prior inflammatory changes associated with, for example, myocarditis might predispose to development and expansion of mutant clones.

The main limitation of the study is its sample size, which could underlie the failure to observe a connection between CHIP and ischaemic heart disease as previously reported.[3,4] Furthermore, the statistical power would also be reduced. A larger cohort will be necessary to validate the contribution of CHIP to adverse clinical outcome. However, our result has uncovered that DCM patients harbour CHIP mutation at relatively young age, and the overall mutation rate appears to be higher than that in ICM and normal aging. Many DCM cases are familial; it will also be important to extend this study with a pre-screened DCM cohort, which carries specific genetic background, to examine if it relates to CHIP incidence in more detail.

Conclusion

The study shows that CHIP mutation is more prevalent in DCM than in ICM. In contrast to ICM, CHIP mutation onset is independent of age and appears at relatively young age in DCM patients. It is possible that DCM may have predisposed a pathological condition to foster acquisition of CHIP-associated mutations. However, further studies are prerequisite to examine such possibility. In addition, a larger cohort is necessary to validate the impact of CHIP on clinical outcome in DCM.

Acknowledgements

Open Access funding enabled and organized by Projekt DEAL.

Conflict of interest

None declared.

Supporting information

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

Table S1. VAF of CHIP variants identified in blood and buccal swab.

References

1. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, Lindsley RC, Mermel CH, Burtt N, Chavez A, Higgins JM, Moltchanov V, Kuo FC, Kluk MJ, Henderson B, Kinnunen L, Koistinen HA, Ladenvall C, Getz G, Correa A, Banahan BF, Gabriel S, Kathiresan S, Stringham HM, McCarthy MI, Boehnke M, Tuomilehto J, Haiman C, Groop L, Atzmon G, Wilson JG, Neuberg D, Altshuler D, Ebert BL. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014; **371**: 2488–2498.
2. Bick AG, Weinstock JS, Nandakumar SK, Fulco CP, Bao EL, Zekavat SM, Szeto MD, Liao X, Leventhal MJ, Nasser J, Chang K, Laurie C, Burugula BB, Gibson CJ, Niroula A, Lin AE, Taub MA, Aguet F, Ardlie K, Mitchell BD, Barnes KC, Moscati A, Fornage M, Redline S, Psaty BM, Silverman EK, Weiss ST, Palmer ND, Vasan RS, Burchard EG, Kardia SLR, He J, Kaplan RC, Smith NL, Arnett DK, Schwartz DA, Correa A, de Andrade M, Guo X, Konkle BA, Custer B, Peralta JM, Gui H, Meyers DA, McGarvey ST, Chen IYD, Shoemaker MB, Peyser PA, Broome JG, Gogarten SM, Wang FF, Wong Q, Montasser ME, Daya M, Kenny EE, North KE, Launer LJ, Cade BE, Bis JC, Cho MH, Lasky-Su J, Bowden DW, Cupples LA, Mak ACY, Becker LC, Smith JA, Kelly TN, Aslibekyan S, Heckbert SR, Tiwari HK, Yang IV, Heit JA, Lubitz SA, Johnsen JM, Curran JE, Wenzel SE, Weeks DE, Rao DC, Darbar D, Moon JY, Tracy RP, Buth EJ, Rafaels N, Loos RJF, Durda P, Liu Y, Hou L, Lee J, Kachroo P, Freedman BI, Levy D, Bielak LF, Hixson JE, Floyd JS, Whitsel EA, Ellinor PT, Irvin MR, Fingerlin TE, Raffield LM, Armasu SM, Wheeler MM, Sabino EC, Blangero J, Williams LK, Levy BD, Sheu WHH, Roden DM, Boerwinkle E, Manson JAE, Mathias RA, Desai P, Taylor KD, Johnson AD, NHLBI Trans-Omics for Precision Medicine Consortium, Abe N, Albert C, Almasy L, Alonso A, Ament S, Anderson P, Anugu P, Applebaum-Bowden D, Arking D, Ashley-Koch A, Aslibekyan S, Assimes T, Avramopoulos D, Barnard J, Barr RG, Barron-Casella E, Barwick L, Beaty T, Beck G, Becker D, Beer R, Beitelshees A, Benjamin E, Benos P, Bezerra M, Bielak L, Bowler R, Brody J, Broeckel U, Bunting K, Bustamante C, Cardwell J, Carey V, Carty C, Casaburi R, Casella J, Castaldi P, Chaffin M, Chang C, Chang YC, Chasman D, Chavan S, Chen BJ, Chen WM, Choi SH, Chuang LM, Chung M, Chung RH, Clish C, Comhair S, Cornell E, Crandall C, Crapo J, Curtis J, Damcott C, das S, David S, Davis C, DeBaun M, Deka R, DeMeo D, Devine S, Duan Q, Duggirala R, Dutcher S, Eaton C, Ekunwe L, Boueiz AE, Emery L, Erzurum S, Farber C, Flickinger M, Franceschini N, Frazar C, Fu M, Fullerton SM, Fulton L, Gabriel S, Gan W, Gao S, Gao Y, Gass M, Gelb B, Geng X, Geraci M, Germer S, Gerszten R, Ghosh A, Gibbs R, Gignoux C, Gladwin M, Glahn D, Gong DW, Goring H, Graw S, Grine D, Gu CC, Guan Y, Gupta N, Haessler J, Hall M, Harris D, Hawley NL, Heavner B, Hernandez R, Herrington D, Hersh C, Hidalgo B, Hobbs B, Hokanson J, Hong E, Hoth K, Hsiung C, Hung YJ, Huston H, Hwu CM, Jackson R, Jain D, Jaquish C, Jhun MA, Johnson C, Johnston R, Jones K, Kang HM, Kelly S, Kessler M, Khan A, Kim W, Kinney G, Kramer H, Lange C, LeBoff M, Lee SS, Lee WJ, LeFaive J, Levine D, Lewis J, Li X, Li Y, Lin H, Lin H, Lin KH, Lin X, Liu S, Liu Y, Lunetta K, Luo J, Mahaney M, Make B, Manichaikul A, Margolin L, Martin L, Mathai S, May S, McArdle P, McDonald ML, McFarland S, McGoldrick D, McHugh C, Mei H, Mestroni L, Mikulla J, Min N, Minear M, Minster RL, Moll M, Montgomery C, Musani S, Mwasongwe S, Mychaleckyj JC, Nadkarni G, Naik R, Naseri T, Nekhai S, Nelson SC, Neltner B, Nickerson D, O'Connell J, O'Connor T, Ochs-Balcom H, Paik D, Pankow J, Papanicolaou G, Parsa A, Perez M, Perry J, Peters U, Peyser P, Phillips LS, Pollin T, Post W, Becker JP, Boorgula MP, Preuss M, Qasba P, Qiao D, Qin Z, Rasmussen-Torvik L, Ratan A, Reed R, Regan E, Sefuiva Reupena M', Rice K, Roselli C, Ruczinski I, Russell P, Ruuska S, Ryan K, Saleheen D, Salimi S, Salzberg S, Sandow K, Scheller C, Schmidt E, Schwander K, Sciurba F, Seidman C, Seidman J, Sheehan V, Sherman SL, Shetty A, Shetty A, Silver B, Smith J, Smith T, Smoller S, Snively B, Snyder M, Sofer T, Sotoodehnia N, Stilp AM, Storm G, Streeten E, Su JL, Sung YJ, Sylvia J, Szpiro A, Sztalryd C, Taliun D, Tang H, Taylor M, Taylor S, Telen M, Thornton TA, Threlkeld M, Tinker L, Tirschwell D, Tishkoff S, Tiwari H, Tong C, Tsai M, Vaidya D, Berg DVD, VandeHaar P, Vrieze S, Walker T, Wallace R, Walts A, Wang H, Watson K, Weir B, Weng LC, Wessel J, Willer C, Williams K, Wilson C, Wu J, Xu H, Yanek L, Yang R, Zaghloul N, Zhang Y, Zhao SX, Zhao W, Zhi D, Zhou X, Zhu X, Zody M, Zoellner S, Auer PL, Kooperberg C, Laurie CC, Blackwell TW, Smith AV, Zhao H, Lange E, Lange L, Rich SS, Rotter JI, Wilson JG, Scheet P, Kitzman JO, Lander ES, Engreitz JM, Ebert BL, Reiner AP, Jaiswal S, Abecasis G, Sankaran VG, Kathiresan S, Natarajan P. Inherited causes of clonal haematopoiesis in 97,691 whole genomes. *Nature*. 2020; **586**: 763–768.
3. Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, McConkey M, Gupta N, Gabriel S, Ardissino D, Baber U, Mehran R, Fuster V, Danesh J, Frossard P, Saleheen D, Melander O, Sukhova GK, Neuberg D, Libby P, Kathiresan S, Ebert BL. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med*. 2017; **377**: 111–121.
4. Dorsheimer L, Assmus B, Rasper T, Ortmann CA, Ecke A, Abou-el-Ardat K, Schmid T, Brüne B, Wagner S, Serve H, Hoffmann J, Seeger F, Dimmeler S, Zeiher AM, Rieger MA. Association of mutations contributing to clonal hematopoiesis with prognosis in chronic ischemic heart failure. *JAMA Cardiol*. 2019; **4**: 25–33.
5. Yu B, Roberts MB, Raffield LM, Zekavat SM, Nguyen NQH, Biggs ML, Brown MR, Griffin G, Desai P, Correa A, Morrison AC, Shah AM, Niroula A, Uddin MM, Honigberg MC, Ebert BL, Psaty BM, Whitsel EA, Manson JE, Kooperberg C, Bick AG, Ballantyne CM, Reiner AP, Natarajan P, Eaton CB, National Heart, Lung, and Blood Institute TOPMed Consortium. Supplemental association of clonal hematopoiesis with incident heart failure. *J Am Coll Cardiol*. 2021; **78**: 42–52.
6. Pascual-Figal DA, Bayes-Genis A, Díez-Díez M, Hernández-Vicente Á, Vázquez-Andrés D, de la Barrera J, Vazquez E, Quintas A, Zuriaga MA, Asensio-López MC, Dopazo A, Sánchez-Cabo F, Fuster JJ. Clonal hematopoiesis and risk of progression of heart failure with reduced left ventricular ejection fraction. *J Am Coll Cardiol*. 2021; **77**: 1747–1759.
7. Fidler TP, Xue C, Yalcinkaya M, Hardaway B, Abramowicz S, Xiao T, Liu W, Thomas DG, Hajebrahimi MA, Pircher J, Silvestre-Roig C, Kotini AG, Luchsinger LL, Wei Y, Westerterp M, Snoeck HW, Papapetrou EP, Schulz C, Massberg S, Soehnlein O, Ebert B, Levine RL, Reilly MP, Libby P, Wang N, Tall AR. The AIM2 inflammasome exacerbates atherosclerosis in clonal haematopoiesis. *Nature*. 2021; **592**: 296–301.
8. Assmus B, Cremer S, Kirschbaum K, Culmann D, Kiefer K, Dorsheimer L, Rasper T, Abou-el-Ardat K, Herrmann E, Berkowitsch A, Hoffmann J, Seeger F, Mas-Peiro S, Rieger MA, Dimmeler S, Zeiher AM. Clonal haematopoiesis in chronic ischaemic heart failure: prognostic role of clone size for DNMT3A- and TET2-driver gene mutations. *Eur Heart J*. 2021; **42**: 257–265.
9. Abplanalp WT, Cremer S, John D, Hoffmann J, Schuhmacher B, Merten M, Rieger MA, Vasa-Nicotera M, Zeiher AM, Dimmeler S. Clonal

hematopoiesis-driver DNMT3A mutations alter immune cells in heart failure. *Circ Res*. 2021; **128**: 216–228.
10. Fuster JJ, MacLauchlan S, Zuriaga MA, Polackal MN, Ostriker AC, Chakraborty R, Wu CL, Sano S, Muralidharan S, Rius C, Vuong J, Jacob S, Muralidhar V, Robertson AAB, Cooper MA, Andrés V, Hirschi KK, Martin KA, Walsh K. Clonal hematopoiesis associated with TET2 deficiency accelerates atherosclerosis development in mice. *Science*. 2017; **355**: 842–847.
11. Bick AG, Pirruccello JP, Griffin GK, Gupta N, Gabriel S, Saleheen D, Libby P, Kathiresan S, Natarajan P. Genetic interleukin 6 signaling deficiency attenuates cardiovascular risk in clonal hematopoiesis. *Circulation*. 2020; **141**: 124–131.
12. International CML Foundation Genomics Alliance, Branford S, Kim DDH, Apperley JF, Eide CA, Mustjoki S, Ong ST, Nteliopoulos G, Ernst T, Chuah C, Gambacorti-Passerini C, Mauro MJ, Druker BJ, Kim DW, Mahon FX, Cortes J, Radich JP, Hochhaus A, Hughes TP. Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. *Leukemia*. 2019; **33**: 1835–1850.
13. Alexandrov LB, Jones PH, Wedge DC, Sale JE, Campbell PJ, Nik-Zainal S, Stratton MR. Clock-like mutational processes in human somatic cells. *Nat Genet*. 2015; **47**: 1402–1407.
14. Sano S, Oshima K, Wang Y, MacLauchlan S, Katanasaka Y, Sano M, Zuriaga MA, Yoshiyama M, Goukassian D, Cooper MA, Fuster JJ, Walsh K. Tet2-mediated clonal hematopoiesis accelerates heart failure through a mechanism involving the IL-1β/NLRP3 inflammasome. *J Am Coll Cardiol*. 2018; **71**: 875–886.
15. McNally EM, Mestroni L. Dilated cardiomyopathy: genetic determinants and mechanisms. *Circ Res*. 2017; **121**: 731–748.
16. Rodriguez BM, Khouzami L, Decostre V, Varnous S, Pekovic-Vaughan V, Hutchison CJ, Pecker F, Bonne G, Muchir A. N-acetyl cysteine alleviates oxidative stress and protects mice from dilated cardiomyopathy caused by mutations in nuclear A-type lamins gene. *Hum Mol Genet*. 2018; **27**: 3353–3360.
17. Lynch TL 4th, Sivaguru M, Velayutham M, Cardounel AJ, Michels M, Barefield D, Govindan S, dos Remedios C, van der Velden J, Sadayappan S. Oxidative stress in dilated cardiomyopathy caused by MYBPC3 mutation. *Oxid Med Cell Longev*. 2015; **2015**: 424751.
18. Lynch TL 4th, Ismahil MA, Jegga AG, Zilliox MJ, Troidl C, Prabhu SD, Sadayappan S. Cardiac inflammation in genetic dilated cardiomyopathy caused by MYBPC3 mutation. *J Mol Cell Cardiol*. 2017; **102**: 83–93.
19. Kratsios P, Huth M, Temmerman L, Salimova E, al Banchaabouchi M, Sgoifo A, Manghi M, Suzuki K, Rosenthal N, Mourkioti F. Antioxidant amelioration of dilated cardiomyopathy caused by conditional deletion of NEMO/IKKγ in cardiomyocytes. *Circ Res*. 2010; **106**: 133–144.
20. Bajcetic M, Nikolic Kokic A, Djukic M, Kosutic J, Mitrovic J, Mijalkovic D, Jovanovic I, Simeunovic S, Spasic MB, Samardzic R. Effects of carvedilol on left ventricular function and oxidative stress in infants and children with idiopathic dilated cardiomyopathy: a 12-month, two-center, open-label study. *Clin Ther*. 2008; **30**: 702–714.
21. Schulze PC, Drosatos K, Goldberg IJ. Lipid use and misuse by the heart. *Circ Res*. 2016; **118**: 1736–1751.
22. Flach J, Bakker ST, Mohrin M, Conroy PC, Pietras EM, Reynaud D, Alvarez S, Diolaiti ME, Ugarte F, Forsberg EC, le Beau MM, Stohr BA, Méndez J, Morrison CG, Passegué E. Replication stress is a potent driver of functional decline in ageing haematopoietic stem cells. *Nature*. 2014; **512**: 198–202.
23. Ito K, Suda T. Metabolic requirements for the maintenance of self-renewing stem cells. *Nat Rev Mol Cell Biol*. 2014; **15**: 243–256.