

## AUTHORS' CONTRIBUTIONS

M. Anguita Sánchez, J.L. Bonilla Palomas, and F.J. Elola Somoza were responsible for the idea and conception of the study and for writing the article. M. García Márquez and J.L. Bernal Sobrino performed the design and statistical analysis and prepared the tables. F. Marín Ortuño was project coordinator and reviewed the document.

## CONFLICTS OF INTEREST

The authors state that they have no conflicts of interest with regard to this project.

## Acknowledgments

We would like to thank the Ministry of Health for providing the MDS database and the Institute of Health Information of the National Health System for assistance provided to the Spanish Cardiology Society to carry out the RECALCAR project.

Manuel Anguita Sánchez,<sup>a,b,\*</sup> Juan Luis Bonilla Palomas,<sup>c</sup> María García Márquez,<sup>d</sup> José Luis Bernal Sobrino,<sup>d,e</sup> Francisco Javier Elola Somoza,<sup>e</sup> and Francisco Marín Ortuño<sup>f</sup>

<sup>a</sup>Servicio de Cardiología, Hospital Universitario Reina Sofía, Córdoba, Spain

<sup>b</sup>Instituto Maimónides de Investigación Biomédica, Universidad de Córdoba, Córdoba, Spain

<sup>c</sup>Servicio de Cardiología, Hospital San Juan de la Cruz, Úbeda, Jaén, Spain

<sup>d</sup>Servicio de Control de Gestión, Hospital Universitario 12 de Octubre, Madrid, Spain

<sup>e</sup>Fundación Instituto para la Mejora de la Asistencia Sanitaria, Madrid, Spain

<sup>f</sup>Servicio de Cardiología, Hospital Virgen de la Arrixaca, El Palmar, Murcia, Spain

\* Corresponding author:

E-mail address: [manuelanguita@secardiologia.es](mailto:manuelanguita@secardiologia.es)

(M. Anguita Sánchez).

Available online 11 June 2021

## REFERENCES

1. Anguita M, Crespo-Leiro MG, de Teresa E, et al. Prevalencia de la insuficiencia cardíaca en la población general española mayor de 345 años Estudio PRICE. *Rev Esp Cardiol.* 2008;61:1041–1049.
2. Brotons C, Moral I, Ribera A, et al. Tendencias de la morbimortalidad por insuficiencia cardíaca en Cataluña. *Rev Esp Cardiol.* 1998;51:972–976.
3. López-Mesa JB, Andrés de Llano JM, López-Fernández L, et al. Evolución de las tasas de hospitalización y mortalidad hospitalaria por enfermedades cardiovasculares agudas en Castilla y León, 2001–2015. *Rev Esp Cardiol.* 2018;71:95–104.
4. Anguita Sánchez M, Bonilla Palomas JL, García Márquez M, Bernal Sobrino JL, Fernández Pérez C, Elola Somoza FJ. Tendencias temporales en ingresos y mortalidad hospitalaria por insuficiencia cardíaca en España, 2003–2015: diferencias por comunidades autónomas. *Rev Esp Cardiol.* 2020;73:1075–1077.
5. Martínez-Santos P, Bover R, Esteban A, et al. Mortalidad hospitalaria y reingresos por insuficiencia cardíaca en España Un estudio de los episodios índice y los reingresos por causas cardíacas a los 30 días y al año. *Rev Esp Cardiol.* 2019;72:988–1004.

<https://doi.org/10.1016/j.rec.2021.04.017>

1885-5857/ © 2021 Published by Elsevier España, S.L.U. on behalf of Sociedad Española de Cardiología.

## Prevalence and characteristics of clonal hematopoiesis in heart failure



## Prevalencia y características de la hematopoyesis clonal en insuficiencia cardíaca

### To the Editor,

Somatic mutations causing clonal expansion of hematopoietic cells (clonal hematopoiesis of indeterminate potential [CHIP]) increase with age and are associated with a higher risk of developing a hematological malignancy.<sup>1</sup> In the cardiovascular field, they have been found to be associated with atherosclerosis and inflammation.<sup>2</sup> Limited information is available in chronic heart failure (HF).

We studied a consecutive cohort of 60 patients with chronic HF without a previous history of cancer. Baseline characteristics are detailed in [table 1](#). The study was performed in accordance with the ethics committee of Germans Trias and Pujol Hospital and all patients signed an informed consent form. Targeted deep sequencing was performed on a custom panel including 43 myeloid- and CHIP-related genes, using deoxyribonucleic acid extracted from peripheral blood samples. Libraries were prepared using the SureSelect QXT capture chemistry (Agilent Technologies, United States) and sequenced on a HiSeq2500 following a 2 x 75 bp paired-end reads standard protocol (Illumina, United States) at a mean depth of coverage of 2905-fold. Reads were aligned using BWA 0.7.12. Packages SAMtools 1.2 and VarScan 2.4.0 were used for variant calling without variant allele frequency (VAF) threshold. Sequencing and mapping errors were removed by discarding variants with a low mapping quality (< 20), variants located at highly variable regions,

and variants occurring in  $\geq 5\%$  of the cohort. Synonymous variants and variants with a minor allele frequency  $> .01$ , according to available population databases, were also excluded. Statistical analysis was performed using the statistical package SPSS, version 23.0 (SPSS Inc, United States). We reviewed the largest reported CHIP cohorts to determine the prevalence of CHIP.

CHIP was found in 28% of patients with a total of 30 variants detected. Overall, 9 (15%) patients carried 1 mutation, 4 (7%) had 2 concurrent mutations, 3 (5%) had 3 mutations, and 1 (2%) patient had 4. Consistent with previous studies, the prevalence of CHIP increased with age: 67% in patients  $\geq 80$  years ( $n = 8$ ), 36% between 70 and 79 years ( $n = 15$ ), 25% between 60 and 69 years ( $n = 16$ ) and 17% between 50 and 59 years ( $n = 12$ ) ( $P = .01$ ; [figure 1A](#)). Of note, no CHIP carriers were detected in patients  $< 50$  years ( $n = 9$ ). The mean number of mutations detected per patient also increased with age: 2.3 ( $\geq 80$  years), 1.7 (70–79 years), 1.4 (60–69 years), and 1 ( $< 60$  years). The frequency of CHIP in HF was considerably higher in all age groups than in previously published cohorts, including unselected populations as well as patients with coronary heart disease.<sup>2</sup> However, this observation needs to be interpreted cautiously given that the prevalence of CHIP in the reported cohorts may have been influenced by the variety of sequencing technologies used in these studies, some of which did not reach the sensitivity reported for some of the variants detected in the current study. According to previous studies, the most frequently affected gene was *DNMT3A* (17%), followed by *TET2* (8%). Experimental studies have reported that mutations in these 2 genes promote cardiac dysfunction in murine models of HF.<sup>3,4</sup> Recurrent mutations were also detected in 2 patients (3%) in *JAK2* and *PPM1D*,

**Table 1**

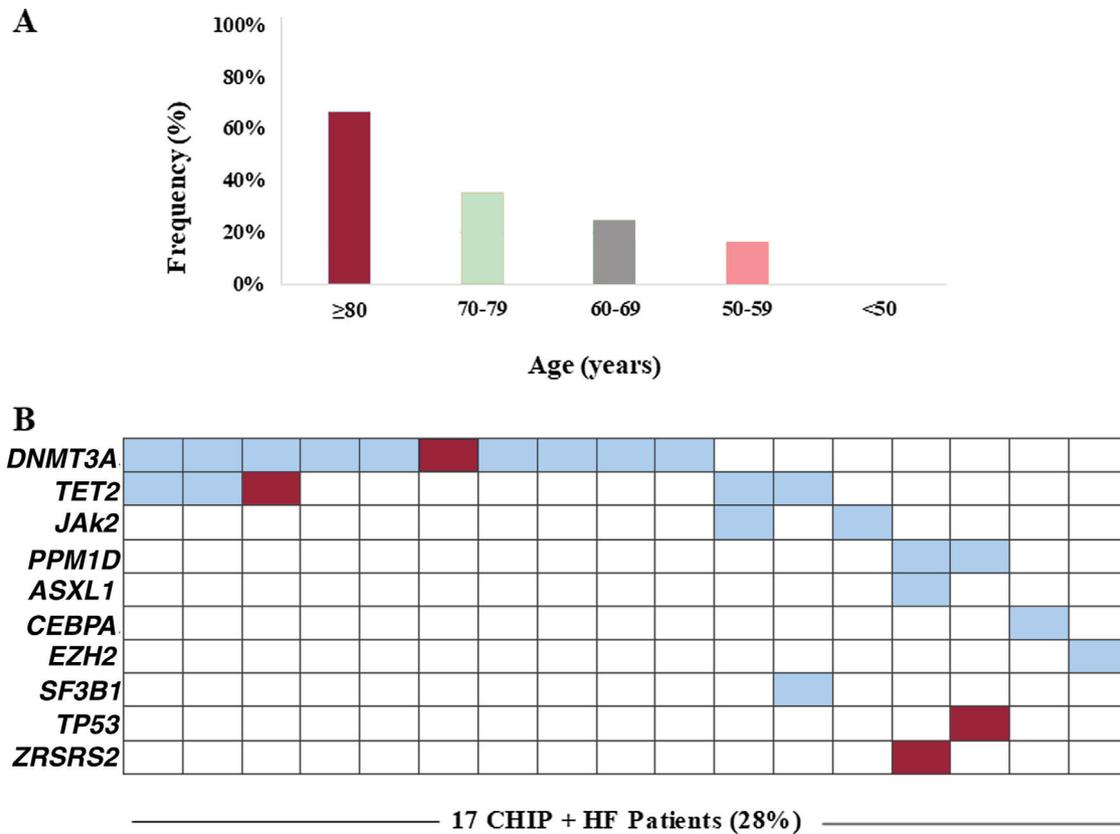
Clinical, biochemical, echocardiographic characteristics and treatment of the cohort (n=60) and between groups of patients presenting with CHIP (n=17) or without CHIP (n=43)

| Variable                | Total cohort (n=60) | Patients with CHIP (n=17) | Patients with no CHIP (n=43) | P    |
|-------------------------|---------------------|---------------------------|------------------------------|------|
| <b>Age, y</b>           | 64 ± 14             | 73 ± 11                   | 61 ± 14                      | .001 |
| <b>Sex</b>              |                     |                           |                              |      |
| Male                    | 45 (75)             | 12 (71)                   | 33 (77)                      | .620 |
| Female                  | 15 (25)             | 5 (29)                    | 10 (23)                      | .620 |
| <b>Etiology</b>         |                     |                           |                              |      |
| Ischemic                | 25 (41.7)           | 5 (29.4)                  | 20 (46.5)                    | .226 |
| Nonischemic             | 35 (58.3)           | 12 (70.6)                 | 23 (53.5)                    | .226 |
| <b>Comorbidities</b>    |                     |                           |                              |      |
| Hypertension            | 37 (61.7)           | 11 (64.7)                 | 26 (60.5)                    | .761 |
| Diabetes                | 29 (48.3)           | 10 (58.8)                 | 19 (44.2)                    | .307 |
| Peripheral disease      | 9 (15)              | 2 (11.8)                  | 7 (16.3)                     | .659 |
| COPD                    | 8 (13.3)            | 4 (23.5)                  | 4 (9.3)                      | .144 |
| <b>NYHA FC</b>          |                     |                           |                              |      |
| I                       | 12 (20)             | 3 (17.7)                  | 9 (20.9)                     | .958 |
| II                      | 38 (63.3)           | 11 (64.7)                 | 27 (62.8)                    | .958 |
| III                     | 10 (16.7)           | 3 (17.7)                  | 7 (16.3)                     | .958 |
| <b>AF/f</b>             | 10 (16.7)           | 4 (23.5)                  | 6 (14)                       | .245 |
| <b>Blood test</b>       |                     |                           |                              |      |
| Creatinine, mg/dL       | 1.2 ± 0.7           | 1.2 ± 0.5                 | 1.3 ± 0.8                    | .645 |
| NT-proBNP, pg/mL        | 1116 [443-2895]     | 1419 [747-3662]           | 1051 [343-2846]              | .976 |
| Hemoglobin, g/dL        | 13.3 ± 2.2          | 12.7 ± 2.6                | 13.6 ± 2                     | .135 |
| Ferritin, ng/mL         | 223 [84-411]        | 127 [62-408]              | 228 [87-411]                 | .594 |
| <b>Echocardiography</b> |                     |                           |                              |      |
| LVEF, %                 | 40.1 ± 13.4         | 44.2 ± 16.3               | 38.5 ± 11.8                  | .136 |
| LAD, mm                 | 46.6 ± 8.4          | 45.3 ± 10.9               | 47.1 ± 7.4                   | .464 |
| Mitral regurgitation    |                     |                           |                              |      |
| Mild                    | 33 (55)             | 9 (53)                    | 24 (55.8)                    | .854 |
| Moderate                | 14 (23.3)           | 5 (29.4)                  | 9 (21)                       | .854 |
| Severe                  | 1 (1.7)             | 0                         | 1 (2.3)                      | .854 |
| Diastolic dysfunction   |                     |                           |                              |      |
| I                       | 8 (13.3)            | 3 (17.7)                  | 5 (11.6)                     | .460 |
| II                      | 9 (15)              | 4 (23.5)                  | 5 (11.6)                     | .460 |
| III                     | 2 (3.3)             | 0                         | 2 (4.7)                      | .460 |
| <b>Treatments</b>       |                     |                           |                              |      |
| Beta-blocker            | 58 (96)             | 16 (94.1)                 | 42 (97.7)                    | .117 |
| ACEI or ARB             | 52 (86.3)           | 15 (88.2)                 | 37 (86)                      | .437 |
| Sacubitril/valsartan    | 4 (6.7)             | 1 (5.9)                   | 3 (7)                        | .878 |
| MRA                     | 49 (82)             | 14 (82.4)                 | 35 (81.4)                    | .429 |
| Loop diuretic agent     | 37 (61.7)           | 13 (76.5)                 | 24 (55.8)                    | .138 |
| Digoxin                 | 4 (6.7)             | 0                         | 4 (9.3)                      | .193 |
| Hidralazine             | 7 (11.7)            | 3 (17.6)                  | 4 (9.3)                      | .364 |
| Nitrates                | 8 (13.3)            | 4 (23.5)                  | 4 (9.3)                      | .144 |
| CRT                     | 4 (6.7)             | 1 (5.9)                   | 3 (7)                        | .878 |
| ICD                     | 5 (8.5)             | 2 (11.8)                  | 3 (7)                        | .545 |

ACEI, angiotensin-converting enzyme inhibitor; AF/f, atrial fibrillation/flutter; ARB, angiotensin II receptor blocker; COPD, chronic obstructive pulmonary disease; CRT, cardiac resynchronization therapy; ICD, implantable cardioverter-defibrillator; LAD, left atrial diameter; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA FC, New York Heart Association functional class. Data are expressed as No. (%), mean ± standard deviation, or median [interquartile range].

while individual mutations were identified in *ASXL1*, *CEBPA*, *EZH2*, *SF3B1*, *TP53*, and *ZRSR2* (figure 1B). Median VAF was 1.96% with values ranging from 1.05% to 37.25%. Most variants (77%) had VAF values < 5%. Interestingly, 3 patients carried a clone harboring known pathogenic myeloid mutations with a VAF larger than 20%, but showed no cytopenia or other hematological symptoms:

*DNMT3A* p.R882H mutation (VAF 37%); a clone harboring both the *DNMT3A* p.R882H mutation (VAF 30%) and a nonsense *TET2* mutation (VAF 30%); and a 27 bp deletion in *CEBPA* (VAF 27%). Baseline clinical characteristics, hematological profiles and treatment did not differ in HF patients between CHIP carriers and noncarriers (table 1). The presence of *DNMT3A* mutations was



**Figure 1.** Prevalence of CHIP. A, prevalence of CHIP according to age. B, distribution of genes mutated in the cohort. Each column represents a patient, and each row represents a gene. Boxes colored in blue indicate presence of CHIP (red color indicates the presence of 2 or 3 mutations, respectively, in the same gene). HF, heart failure.

associated with diastolic dysfunction (35% vs 16%,  $P = .031$ ). Time of follow-up was  $43 \pm 11$  months, without differences between patients with and without CHIP ( $P = .500$ ). There were four (23.5%) deaths in patients with CHIP and 7 (16.3%) in patients without CHIP ( $P = .513$ ). The combined endpoint (HF hospitalization or death) occurred in 7 (41.2%) patients with CHIP and in 9 (20.9%) patients without CHIP ( $P = .110$ ). CHIP was not related to death (hazard ratio [HR], 1.53; 95% confidence interval [95%CI], 0.45–5.24;  $P = .497$ ) or the combined endpoint (HR, 2.12; 95%CI, 0.79–5.71;  $P = .137$ ) in a Cox regression analysis with proportional hazards. In patients with CHIP, there were no differences in clinical outcomes between patients with and without ischemic etiology ( $P = .861$ ). There was no new-onset cancer during follow-up.

Contemporary registries show that currently a large percentage of HF patients die from noncardiac causes, mainly associated comorbidities and cancer. Moreover, recent studies indicate that HF patients are more prone to develop incident cancer,<sup>5</sup> and therefore there is a need for a better understanding of the causal relationship between HF and tumor growth. Similarly, recent studies suggest that CHIP is a new causal risk factor for HF and is associated with poor outcomes.<sup>6</sup>

## FUNDING

This work was supported in part by Generalitat de Catalunya (Departament de Salut) PERIS Acció Instrumental de Programes de Recerca Orientats (SLT002/16/00234) and AGAUR (2017-SGR288 and 2017-SGR-483) financial support from CERCA Programme/Generalitat de Catalunya, Fundació Internacional Josep Carreras, Societat Catalana de Cardiologia, “la Caixa” Banking Foundation,

and Red de Terapia Celular-TerCel (RD16/00111/0006), CIBER Cardiovascular (CB16/11/00403) projects, as a part of the Plan Nacional de I+D+I, and it was co-funded by ISCIII-Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional (FEDER). The research leading to this invention has received funding from “la Caixa” Foundation. The CNIC is supported by the Instituto de Salud Carlos III (ISCIII), the Ministerio de Ciencia e Innovación (MCIN) and the Pro CNIC Foundation.

## AUTHORS' CONTRIBUTIONS

L. Palomo performed sequencing studies, interpreted sequencing data and performed analysis; E. Santiago-Vacas collected samples, clinical data and performed analysis; D. Pascual-Figal and J.J. Fuster reviewed sequencing and statistical analyses; F. Solé and A. Bayés-Genis designed the study and provided samples. All authors reviewed the manuscript and gave final approval.

## CONFLICTS OF INTEREST

The authors report no conflicts of interest.

Laura Palomo,<sup>a,g</sup> Evelyn Santiago-Vacas,<sup>b,c,d</sup> Domingo Pascual-Figal,<sup>c,e,f,g</sup> José Javier Fuster,<sup>g</sup> Francesc Solé,<sup>a,g,\*</sup> and Antoni Bayés-Genís<sup>b,c,d</sup>

<sup>a</sup>Grup de Síndromes Mielodisplàsiques, Institut de Recerca Contra la Leucèmia Josep Carreras-Institut Català d'Oncologia, Hospital

Universitari Germans Trias i Pujol, Badalona, Barcelona, Spain

<sup>b</sup>Institut del Cor, Hospital Universitari Germans Trias i Pujol, Badalona, Spain

<sup>c</sup>Centro de Investigación Biomédica en Red Enfermedades Cardiovasculares (CIBERCV), Badalona, Barcelona, Spain

<sup>d</sup>Programa de Recerca “Insuficiència Cardíaca i Regeneració Cardíaca” (ICREC), Institut d’Investigació en Ciències de la Salut Germans Trias i Pujol, Badalona, Barcelona, Spain

<sup>e</sup>Servicio de Cardiología, Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar, Murcia, Spain

<sup>f</sup>Departamento de Medicina, Universidad de Murcia, Murcia, Spain

<sup>g</sup>Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

\* Corresponding author:

E-mail address: [fsole@carrerasresearch.org](mailto:fsole@carrerasresearch.org) (F. Solé).

Available online 11 June 2021

## REFERENCES

1. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med.* 2014;371:2488–2498.
2. Jaiswal S, Natarajan P, Silver AJ, et al. Clonal Hematopoiesis and Risk of Atherosclerotic Cardiovascular Disease. *N Engl J Med.* 2017;377:111–121.
3. Sano S, Oshima K, Wang Y, Katanasaka Y, Sano M, Walsh K. CRISPR-Mediated Gene Editing to Assess the Roles of Tet2 and Dnmt3a in Clonal Hematopoiesis and Cardiovascular Disease. *Circ Res.* 2018;123:335–341.
4. Sano S, Oshima K, Wang Y, et al. Tet2-Mediated Clonal Hematopoiesis Accelerates Heart Failure Through a Mechanism Involving the IL-1 $\beta$ /NLRP3 Inflammasome. *J Am Coll Cardiol.* 2018;71:875–886.
5. Hasin T, Gerber Y, McNallan SM, et al. Patients with heart failure have an increased risk of incident cancer. *J Am Coll Cardiol.* 2013;62:881–886.
6. Yura Y, Sano S, Walsh K. Clonal Hematopoiesis: A New Step Linking Inflammation to Heart Failure. *JACC Basic Transl Sci.* 2020;24:196–207.

<https://doi.org/10.1016/j.rec.2021.05.005>

1885-5857/ © 2021 Sociedad Española de Cardiología. Published by Elsevier España, S.L.U. All rights reserved.