

## Peer-Review Record

### Regulators of clonal hematopoiesis and physiological consequences of this condition

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by Eunbee Park, Megan A. Evans, Kenneth Walsh

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**Academic Editor:** Ali J. Marian

**Reviewer 1:** Anonymous

**Reviewer 2:** Anonymous

## Round 1

### Reviewer 1 Report

This is a wonderful and comprehensive review of the current state of clonal hematopoiesis in the cardiovascular area, mainly focusing on factors that promote the condition. The review covers the latest references, especially on the genetic risk of CH. The authors introduce clonal hematopoiesis in general, followed by a detailed description of factors that promote clonal hematopoiesis. The review is well-organized and valuable to the entire community, so it should be published with slight modifications.

I have a few comments for the author's consideration:

Although the abstract says that "we will focus on the association between extrinsic and intrinsic stressors and the development of CH," it is unclear what the authors mean by intrinsic stressors. Is the gene mutation (TET2, DNMT3A) an intrinsic stressor promoting CH? Do the authors represent Genetic risk (2.6. Part), including TERT and TCL1A germline mutation as intrinsic stressors? Please clarify this point.

The authors state in the 2.3 part that inflammation promotes CH. Since this review aims to potentially lead to new therapeutic avenues to treat individuals with CH, it is better to discuss the possibility of anti-inflammatory therapy to suppress clonal expansion of mutant HSPCs.

Minor points.

The sentence beginning on line 61, "standard NGS," might be vague. It is better to specify more.

The sentence beginning on line 113, CH-driver genes, looks unnecessary.

In the sentence beginning on line 124, words are missing after "can be found in."

In the author's contribution, Evans MA is not listed.

In the figure legend, although it has been said that "acquisition and expansion of CH mutant clones," in the manuscript, there is almost no mention of the acquisition. Maybe better remove "acquisition."

### **Author Response**

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Although the abstract says that "we will focus on the association between extrinsic and intrinsic stressors and the development of CH," it is unclear what the authors mean by intrinsic stressors. Is the gene mutation (TET2, DNMT3A) an intrinsic stressor promoting CH? Do the authors represent Genetic risk (2.6. Part), including TERT and TCL1A germline mutation as intrinsic stressors? Please clarify this point.

We appreciate your comment. CH denotes a condition characterized by the presence of genetically distinct blood cell populations. Common acquired mutations associated with CH include somatic gene mutations, notably TET2 and DNMT3A, which provide a selective advantage to the affected cells, leading to their increased presence in the blood cell population over time. As noted by the reviewer, the term "intrinsic stressor" may be unclear because it could suggest that various driver genes lead to different levels of expansion based on their inherent effects on HSCs or varying responsiveness to certain environmental stimuli.

However, our primary objective was to elucidate the stressors that propel the expansion of CH mutant clones subsequent to the acquisition of these somatic mutations. Inherent genetic risks, exemplified by TERT and TCL1A, were identified as inherited germline mutations, owing to their endogenous origin and their correlation with the expansion of CH mutant clone within the hematopoietic system. Acknowledging the potential for confusion arising from the phrase, "we will focus on the association between extrinsic and intrinsic stressors and the development of CH," we have refined the sentence to state, "we will focus on the association between environmental stressors and inherited genetic risk factors in the context of CH development." (Please see line 28-29). We also modified the previous sentence "Additional explorations of the intrinsic and extrinsic regulators of clone expansion will contribute to a deeper understanding of age-related diseases and aging *per se*." to "Additional explorations of regulators of clone expansion will contribute to a deeper understanding of age-related diseases and aging *per se*." (Please see line 604-606).

The authors state in the 2.3 part that inflammation promotes CH. Since this review aims to potentially lead to new therapeutic avenues to treat individuals with CH, it is better to discuss the possibility of anti-inflammatory therapy to suppress clonal expansion of mutant HSPCs.

We agree that this addition will enhance the comprehension of our readers. In the section on **Inflammation** (2.3), the following elucidation has been incorporated: *Clinical trials with anti-inflammatory agents*

Numerous inquiries underscore a correlation between elevated pro-inflammatory cytokine levels and the expansion of CH, suggesting that mitigating or reversing disease progression could be achievable through the antagonism of these inflammatory markers. Consequently, it could be hypothesized that individuals harboring CH clones may stand to benefit from anti-inflammatory interventions. Currently, three registered clinical trials are oriented towards addressing CH within the context of cardiovascular diseases. The first of these trials is a phase I study involving selnoflast, an NLRP3 inhibitor, designed to administer to patients with TET2-CH and concurrent coronary artery disease (ISRCTN10520571). The second is a phase II study centered on colchicine, to be administered to individuals with CH and chronic heart failure (EudraCT 2021-001508-13). Lastly, the third trial involves DFV890 (an NLRP3 inhibitor) and MAS825 (an anti-IL-1 $\beta$ /IL-18 agent), targeting patients with CH and coronary heart disease (NCT06097663). However, to draw definitive conclusions regarding the efficacy of anti-inflammatory therapies in the context of CH, large randomized studies will be imperative. Furthermore, a pivotal consideration arises concerning the capacity of anti-inflammatory therapies to regulate clone size. The prospect of discerning the feasibility of implementing this strategy in carriers of CH with preexisting associated pathologies remains an open question.

Minor points.

The sentence beginning on line 61, "standard NGS," might be vague. It is better to specify more.

Changed to "This threshold is a pivotal component within the analytical framework of NGS sequencing methods, including targeted sequencing, whole-exome, or whole-genome sequencing, which are commonly utilized for the detection of clonal hematopoiesis of indeterminate potential (CHIP).

The sentence beginning on line 113, CH-driver genes, looks unnecessary.

Removed.

In the sentence beginning on line 124, words are missing after "can be found in."

Changed to "A comprehensive review of these disease associations can be found in (1)."

In the author's contribution, Evans MA is not listed.

Modified to Park E prepared figures using biorender.com, Park E and Walsh K drafted manuscript; Park E, Evans MA, and Walsh K edited and revised manuscript.

In the figure legend, although it has been said that "acquisition and expansion of CH mutant clones," in the manuscript, there is almost no mention of the acquisition. Maybe better remove "acquisition."

Removed.

## **Reviewer 2 Report**

In this review, Park et al. extensively covered the outcomes and regulatory elements associated with CH. The authors explored the impact of aging, chemotherapy, radiation, inflammation, obesity/metabolic dysfunction/diet, lifestyle, and genetic risk on CH induction. Despite the well-written and thorough nature of the review, the inclusion of the following discussions could enhance the overall clarity of this review.

Major

1. While the authors extensively covered the potential role of inflammation, there is a notable absence of detailed discussion on reactive oxidative species (ROS).
2. The link between inflammasome activation and ROS stress has been firmly established. However, the correlation between inflammation and ROS remains unaddressed.
3. Recognizing the significant role of ROS in triggering DNA damage response and telomere dysfunction, it would be beneficial to explore the interplay among ROS induction, inflammation, DNA damage, and telomere dysfunction in the context of CH induction.
4. It would also be helpful to discuss the relationship between progeria and CH.
5. Finally, given the well-known pivotal role of mitochondrial dysfunction in all the stressors discussed as CH inducers in this review, it is imperative to delve into the contribution and role of mitochondrial dysfunction in the induction of CH or as a consequence thereof.

## **Author Response**

In this review, Park et al. extensively covered the outcomes and regulatory elements associated with CH. The authors explored the impact of aging, chemotherapy, radiation, inflammation, obesity/metabolic dysfunction/diet, lifestyle, and genetic risk on CH induction. Despite the wellwritten and thorough nature of the review, the inclusion of the following discussions could enhance the overall clarity of this review.

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dysfunction, it would be beneficial to explore the interplay among ROS induction, inflammation, DNA damage, and telomere dysfunction in the context of CH induction.

Thank you for your insightful comment. Although the commentary presents points 1-3 as discrete entities, our interpretation discerns a unifying question: an inquiry into the role of ROS in CH, particularly within the context of inflammation, including the DNA damage response. Consequently, we have incorporated the subsequent paragraph in the concluding part of section 2.3 **Inflammation** to address this concern. This new text:

#### *The role of ROS*

In addition to specific inflammatory mediators, HSCs may intricately engage with the age-associated inflammatory microenvironment. Factors such as DNA damage and replication stress, alterations in epigenetic profiles, and diminished autophagic activity contribute to the accumulation of mitochondrial stress and an augmented production of reactive oxygen species (ROS). For instance, a study has demonstrated the instrumental role of DNMT3A and TET2 in maintaining mitochondrial DNA integrity. The loss of function of these genes resulted in the activation of cyclic GMP-AMP synthase (cGAS) signaling and type I interferon pathway, indicating a potential association between mitochondrial dysfunction, inflammation and CH. Furthermore, macrophages with PPM1D mutations exhibited an impaired DNA damage response pathway, leading to increased production of ROS and IL-1 $\beta$  in response to lipopolysaccharide (LPS). The application of hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL), a ROS scavenger, rescued the elevated levels of ROS and IL-1 $\beta$ , suggesting an interplay between ROS, inflammation and CH. Despite the significance of these two findings, it is important to note that both studies were conducted *in vitro* using monocyte-derived macrophages, thereby deviating from the *in vivo* context of the CH phenomenon and lacking a pathological tissue environment. Therefore, the precise causal relationship between these determinants and CH necessitates further comprehensive investigative scrutiny in a physiological system.

Ref) Geiger, H., de Haan, G. & Florian, M. The ageing haematopoietic stem cell compartment. Nat Rev Immunol 13, 376 - 389 (2013). <https://doi.org/10.1038/nri3433>

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Li, X., Li, C., Zhang, W. et al. Inflammation and aging: signaling pathways and intervention therapies. Sig Transduct Target Ther 8, 239 (2023). <https://doi.org/10.1038/s41392-023-01502-8>

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<https://doi.org/10.1038/s41467-022-30440-2>

Amorim, J.A., Coppotelli, G., Rolo, A.P. et al. Mitochondrial and metabolic dysfunction in ageing and age-related diseases. *Nat Rev Endocrinol* 18, 243 – 258 (2022).

Cobo, Isidoro, et al. "DNA methyltransferase 3 alpha and TET methylcytosine dioxygenase 2 restrain mitochondrial DNA-mediated interferon signaling in macrophages." *Immunity* 55.8 (2022): 1386-1401.

Yura, Yoshimitsu, et al. "The cancer therapy-related clonal hematopoiesis driver gene Ppm1d promotes inflammation and non-ischemic heart failure in mice." *Circulation research* 129.6 (2021): 684-698.

4. It would also be helpful to discuss the relationship between progeria and CH.

Your insight is greatly appreciated. It is noteworthy that Hutchinson-Gilford progeria syndrome (HGPS) manifests various premature aging features and cardiovascular events, aligning with phenomenon observed in CH patients. This prompts speculation regarding the potential presence of CH in progeria patients. However, it is crucial to acknowledge that the existing literature is limited to a single report, wherein an analysis was conducted on 47 HGPS patients, 111 Healthy middle-aged individuals, and 62 early heart failure patients. Contrary to initial expectations, the study concluded that CH is not prevalent in HGPS patients. The authors offered two plausible explanations for this finding: firstly, the shortened lifespan of HGPS patients may not provide sufficient time for the expansion of CH mutant clones, and secondly, HGPS may carry a genetic mutant (lamin A protein: progerin) derived senescent phenotype that could impeded the expansion of CH clones. It is essential to recognize the inherent limitations of this study, such as the small sample size attributed to the rarity of HGPS and challenges in obtaining blood samples from young-aged controls. Thus, additional studies are warranted before forming conclusive interpretations. Moreover, considering that HGPS is primarily a genetic disease, delving into a discussion about this new ailment, which exhibits a partially negative association with CH, may introduce complexity and confusion to readers. As a result, it might be prudent to refrain from delving deeper into this new topic until more comprehensive investigations are conducted.

Ref) Díez-Díez, Miriam, et al. "Clonal hematopoiesis is not prevalent in Hutchinson-Gilford progeria syndrome." *Geroscience* 45.2 (2023): 1231-1236.

5. Finally, given the well-known pivotal role of mitochondrial dysfunction in all the stressors discussed as CH inducers in this review, it is imperative to delve into the contribution and role of mitochondrial dysfunction in the induction of CH or as a consequence thereof.



The pivotal role of mitochondria in the progression of age-related diseases is widely acknowledged. However, despite the intriguing nature of the association between mitochondrial dysfunction and CH, the extent of the literature on this subject remains limited.

In one study involving human macrophages, researchers delved into the intricate interplay between DNMT3A and TET2 with mitochondrial DNA-mediated interferon signaling. The findings indicated that both DNMT3A and TET2 are instrumental in maintaining mitochondrial DNA integrity. Loss-of-function mutations in these genes disrupt the expression of transcription factor A mitochondria (TFAM), a mitochondrial DNA-binding protein. Consequently, this dysregulation activates cyclic GMP-AMP synthase (cGAS) signaling and type I interferon pathway. The study is particularly significant as it identifies a shared mitochondrial DNA-mediated inflammatory pathway in two common CH-associated genes that demonstrates antagonistic regulation of DNA methylation: DNMT3A methylates CpG sites, leading to transcriptional repression, while TET2 catalyzes the hydroxylation of methylated cytosines, thereby reversing transcriptional repression at CpG sites. Furthermore, the study posits potential explanations for the increased risk of CVD in DNMT3A and TET2-CH carriers. Despite the significance of these findings, it is important to note that the study was conducted *in vitro* using human monocyte-derived macrophages, thereby deviating from the *in vivo* context of the CH phenomenon and lacking a pathological tissue environment. The field of mitochondrial research remains compelling, and we posit that further studies are warranted to elucidate the intricate relationship between mitochondrial dysfunction and CH.

As mentioned earlier, we have included the following paragraph in the concluding part of section 2.3.

In addition to specific inflammatory mediators, HSCs may intricately engage with the age-associated inflammatory microenvironment. Factors such as DNA damage and replication stress, alterations in epigenetic profiles, and diminished autophagic activity contribute to the accumulation of mitochondrial stress and an augmented production of reactive oxygen species (ROS). For instance, a study has demonstrated the instrumental role of DNMT3A and TET2 in maintaining mitochondrial DNA integrity. The loss of function of these genes results in the activation of cyclic GMP-AMP synthase (cGAS) signaling and type I interferon pathway, indicating a potential association between mitochondrial dysfunction, inflammation and CH. Furthermore, macrophages with PPM1D mutations exhibited an impaired DNA damage response pathway, leading to increased production of ROS and IL-1 $\beta$  in response to lipopolysaccharide (LPS). The application of hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL), a ROS scavenger, rescued the elevated levels of ROS and IL-1 $\beta$ , suggesting an interplay between ROS, inflammation and CH. Despite the significance of these two findings, it is important to note that both studies were conducted *in vitro* using monocyte-derived macrophages, thereby deviating from the *in vivo* context of the CH phenomenon and lacking a pathological tissue environment. Therefore, the precise causal relationship between these determinants and CH necessitates further comprehensive investigative scrutiny.

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