X Chromosome Inactivation Uncovers Age-Related Clonal Hematopoiesis and Hematologic Traits in Colombian Elderly Women

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Introduction

As people age, hematopoietic stem cells (HSCs) lose their ability to self-renew [1]. Hence, over time the HSCs pool declines. Somatic mutations associated with myeloid neoplasia are present in HSCs from 10–20% of healthy individuals over 70 years of age, and it can lead in some patients to clonal expansion [2-4].

Female primordial cells randomly inactivate one of the two X chromosomes. This pattern is maintained through subsequent mitotic divisions [5]. In theory, mature blood cells of an adult woman exhibit roughly equal proportions of paternal and maternal X chromosome inactivation (XCI). Skewed XCI in elderly women occur and it is likely due to reduced diversity from a shrinking HSCs pool or clonal expansion driven by mutations—both hallmarks of Age-related clonal hematopoiesis (ARCH) [6].

Inflammatory mediators in the bone marrow microenvironment also reduce HSCs diversity favoring the selection of specific clones [7,8]. ARCH has been linked to *inflammaging*, cardiovascular disease (CVD), and reduced overall survival [2,9].

Demographic shift will accelerate over the next decade in low- and middle-income countries like Colombia [10]. Therefore, healthcare efforts should prioritize elderly care. Although next-generation sequencing (NGS) is the gold standard for ARCH analysis, it remains difficult to access in our environment.

While ARCH appears to be less common in Hispanic populations [11], no data exist from our country. This pilot study is an effort to establish a cohort of elderly women with higher likelihood of presenting ARCH. In a subsequent phase, we aim to sequence these samples and compare results using complementary methodologies.

Aim

To determine XCI skewing in hematopoietic cells from elderly Colombian enabling the identification of individuals with a higher likelihood of ARCH.

Objectives

1. Quantify XCI skewing in peripheral blood whole samples from the study cohort using PCR-based HUMARA assay.
2. Investigate potential associations between XCI patterns and clinical/anthropometric/hematological parameters

Methods

Sample Collection

✔ Colombian women ≥ 65 years old from geriatric ambulatory care

Full medical history

Anthropometrics (body mass index, waist-hip ratio)

4mL EDTA peripheral blood (complete blood count)

XCI Skewing Analysis (HUMARA Assay)

HUMARA Protocol:

HpaII digestion (methylation-sensitive enzyme)

PCR amplification (35 cycles, 62°C)

Fragment analysis (3500 Genetic Analyzer)

Clonality Criteria:

✔ Method 1: Allele ratio cutoff (0.25-0.75) [12]

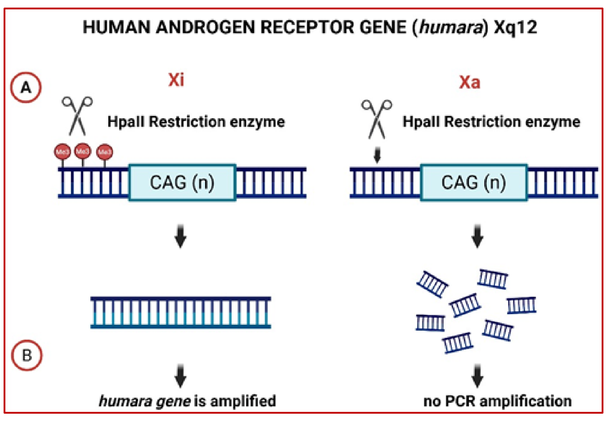
✔ Method 2: Absolute allele frequency difference (>50% = clonal) [13]

Statistical Analysis

- Data management: REDCap/R v4.4.2 for analysis
- Tests: Normality: Shapiro-Wilk - Non-parametric: Kruskal-Wallis/Wilcoxon Categorical: Chi-square/Fisher's exact - Regression models (adjusted for covariates)

HUMARA Assay Workflow

(A) CAG repeats in *the human androgen receptor (HUMARA)* gene distinguish parental alleles. HpaII digests unmethylated (active X) but not methylated (inactive X). (B) PCR post-digestion



Results

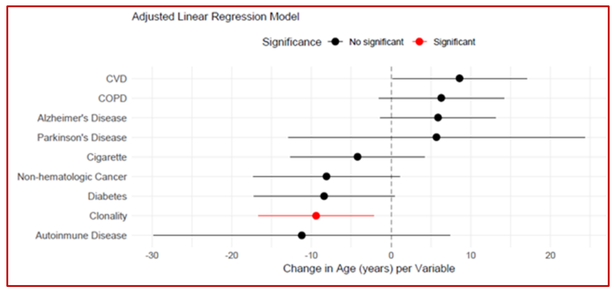
Of 37 elderly women included (mean age: 86 years), 13 (35.1%) were homozygous for *HUMARA* gene. Among the 24 heterozygous samples, we observed:

- 10 samples (41.7%) were consistently classified as potentially clonal (showing XCI skewing) by both analysis methods
- 2 discordant cases (8.3%) initially identified as polyclonal by Method 1 were reclassified as clonal by Method 2
- Overall concordance rate of 91.7% between the two analytical approaches

This resulted in a total of 12 potentially clonal samples (50%) among heterozygous cases.

Age & Clonality:

Clonal samples were on average 6.3 years younger than the polyclonal group (p=0.077). After adjusting for comorbidities, this difference increased to 9.4 years (p=0.025).



Hematologic Parameters:

Platelet Volume: Clonal samples showed 1.85 fL higher mean platelet volume (MPV) (p=0.026).

Hemoglobin: Homozygous patients had 1.17 g/dL lower hemoglobin (p=0.020), with smoking status confounding initial analyses (smokers: +1.19 g/dL, p=0.026).

Erythrocytes: Clonal cases exhibited 5.1% lower hematocrit (p=0.007) and 0.57×10^6/µL fewer erythrocytes (p=0.018).

Erythrocyte Indices: Homozygous patients had elevated mean corpuscular hemoglobin (MCH) (+7.8 pg, p=0.044) and mean corpuscular hemoglobin concentration MCHC (+2.09 g/dL, p=0.048).

Discussion

As a pilot study, our work has limitations, including a small sample size and an unexpectedly high rate of homozygous samples (35.1%). Despite this, we observed XCI skewing in 50% of heterozygous cases—a higher proportion than previously reported [12,14]. This may reflect our cohort's advanced mean age (86 years) and high comorbidity burden. While XCI skewing in elderly women likely represents ARCH, other causes must be considered [5]:

1. Stochastic or genetic skewing during XCI establishment
2. Somatic selection for/against germline X-linked mutations post-inactivation

Without additional somatic tissue samples, we could not confirm whether skewing was tissue-specific. Future whole-exome sequencing will help distinguish between clonal hematopoiesis driven by somatic mutations and cases caused by:

- HSCs depletion
- Extrinsic pressures (inflammation, oxidative stress)
- Epigenetic modifications (undetectable by NGS)

Key Findings

1. Age Association:

Clonal samples were associated with 9.4 years younger age (p=0.025) after comorbidity adjustment. This aligns with hypotheses that:

- Chronic conditions (e.g., CVD, diabetes) may suppress clones via proinflammatory microenvironments in advanced age [15].
- ARCH may emerge earlier as a compensatory mechanism to hematopoietic stress [16].

2. Hematologic Correlates:

- Elevated MPV (+1.85 fL, p=0.026) in clonal cases, possibly reflecting megakaryocytic dysregulation (e.g., as seen in DNMT3A-mutant clones [17]).
- Homozygosity-linked erythropoietic defects: Lower hemoglobin (−1.17 g/dL, p=0.020), hematocrit (−5.1%, p=0.007), and erythrocyte counts, but higher MCH/MCHC. This paradoxical pattern suggests ancestral influences (e.g., population bottlenecks [18], X-linked enzymatic deficiencies in glucose-6-phosphate dehydrogenase (G6PD) or 5'-aminolevulinate synthase 2 (ALAS2)), warranting germline sequencing.

Summary

This is the first characterization of XCI patterns in elderly Colombian women, revealing:

Unique age association: Clonality correlated with younger age (−9.4 years, p=0.025), potentially indicating that early clonal expansion precedes age-related hematopoietic decline.

Hematologic signatures: Elevated MPV (p=0.026) highlight potential biomarkers for clonal detection in resource-limited settings.

Despite limitations (small n=37, 35.1% homozygosity), our HUMARA assay demonstrated 91.7% inter-method concordance. Future exome sequencing will clarify genetic drivers and ancestral influences (e.g., **G6PD/ALAS2* variants).

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