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Age-related clonal haemopoiesis is associated with increased epigenetic age

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Age-related clonal haemopoiesis (ARCH) in healthy individuals was initially observed through an increased skewing in X-chromosome inactivation [1]. More recently, several groups reported that ARCH is driven by somatic mutations [2], with the most prevalent ARCH mutations being in the *DNMT3A* and *TET2* genes, previously described as drivers of myeloid malignancies. ARCH is associated with an increased risk for haematological cancers [2]. ARCH also confers an increased risk for non-haematological diseases, such as cardiovascular disease, atherosclerosis, and chronic ischemic heart failure, for which age is a main risk factor [3,4]. Whether ARCH is linked to accelerated ageing has remained unexplored. The most accurate and commonly used tools to measure age acceleration are epigenetic clocks: they are based on age-related methylation differences at specific CpG sites [5]. Deviations from chronological age towards an increased epigenetic age have been associated with increased risk of earlier mortality and age-related morbidities [5,6]. Here we present evidence of accelerated epigenetic age in individuals with ARCH.

The Lothian Birth Cohorts (LBCs) of 1921 and 1936 are two longitudinal studies of ageing [7]. Participants have been followed up every ~3 years, each for five waves, from the age of 70 (LBC1936) and 79 (LBC1921). Participants were community dwelling, relatively healthy, and mostly lived in the City of Edinburgh or its surrounding area when recruited.

Whole-blood DNA methylation levels were assessed using the Illumina HumanMethylation450 BeadChip (Supplemental Experimental Procedures). Genomic variants were determined in 1,136 LBC participants (n = 870 from wave 1 at mean age 70 years in LBC1936; n = 101 and n = 165 at mean ages 79 and 87, respectively, in LBC1921) with whole-genome sequencing (WGS) and methylation data. WGS data were aligned with Burrows-Wheeler Aligner and processed for duplicate mapping reads with samblaster (genome coverage of 34.3 reads). Single-nucleotide variants and short indels were called with MuTect (v3.8) before annotation using the Ensembl Variant Effect Predictor alongside the Cosmic database of coding mutations (v86). ARCH variants were classified as per Jaiswal *et al*. [2].

Epigenetic age acceleration was calculated online (https://dnamage. genetics.ucla.edu/home). We considered the Intrinsic Epigenetic Age Acceleration (IEAA, hereafter referred to as Horvath age acceleration) measure, which is an adapted version of the original Horvath clock that controls for white blood cell proportions [6]. Epigenetic age estimates were regressed on chronological age to yield age acceleration residuals. Linear regression adjusting for sex, imputed white blood cell proportions (monocytes, natural killer (NK), CD4+ T, CD8+ T, and B cells), and methylation processing batch was used to determine the association between ARCH status and Age Acceleration. All analyses were conducted in R v3.5.0.

Of the ten most prevalent ARCH mutations [2], we had sufficient sample size and sequencing depth to annotate the top six in the LBCs. We identified 73 participants (from 1,136) with ARCH (6%; Figure 1A). The gene-specific prevalence ranged between 1 and 36 cases with ARCH-variant allele frequencies ranging from 0.034 to 0.677 (Figure 1B). Mutations in *TET2* were exclusively frameshift and mutations detected in *JAK2* (all V617F), *SF3B1* and *TP53* were exclusively missense. ARCH status was associated with a significant increase in Horvath age acceleration: the increase was 4.5 (SE 0.9) years in LBC1936, and 3.7 (SE 1.2) years in LBC1921 (p = 2.3 x 10^-6 and 2.5 x 10^-3, respectively; Figure 1C and Table S1). Compared with non-ARCH carriers, those with *TET2* mutations had a 6.1 (SE 2.2) year and 6.4 (SE 1.9) year increase in Horvath age acceleration in LBC1936 and LBC1921 (p = 0.004 and p = 0.001), respectively. Those with *DNMT3A* mutations had 3.8 (SE 1.2) years increase in LBC1936, and 3.0 (SE 1.9) years in LBC1921 (p = 0.002 and p = 0.11), respectively (Figure 1D). These effect sizes are much larger than the sex differences in Horvath age acceleration, which were 1.8 (SE 0.4) years for men in LBC1936 (p = 5.1 x 10^-5), and 1.0 years (SE 0.8) in LBC1921 (p = 0.18) (Figure 1D and Table S1).

We also considered age acceleration estimates from four additional epigenetic clocks: Extrinsic (Hannum) Epigenetic Age (EEAA) [6], PhenoAge [8], GrimAge [9] and Zhang Age [10] (Figure 1E,F and Figure S1A–F). Briefly, ARCH status was linked to increased EEAA, PhenoAge, GrimAge and ZhangAge, acceleration in LBC1921 (effect sizes: 1.9 years, 3.7 years, 2.8 years and 0.8 years with p = 0.16, 0.014, 9.6 x 10^-4, and 3.5 x 10^-3, respectively). In LBC1936 there was a modest association between ARCH and increased EEAA and ZhangAge (2.3 years and 0.5 years, p = 0.012 and 4.4 x 10^-3) but no association with PhenoAge or GrimAge acceleration (p = 0.32 and 0.99, respectively). There was no consistent association between ARCH status and white cell count proportions across the two cohorts: a lower proportion of NK cells was linked with ARCH carrier status in LBC1936 (odds ratio per SD of cell counts, 0.57 95% CI [0.37, 0.84]), while a higher B cell proportion was associated with ARCH status in LBC1921 (OR 1.37 [1.01, 1.94]).

We observed associations between ARCH and epigenetic age acceleration in the independent LBCs of 1921 and 1936, where the WGS data and the DNA methylation data were processed together using identical protocols. Although we examined multiple epigenetic clocks in relation to ARCH status, it is possible that the effect sizes may vary by the quality control approach applied to the methylation data. Additional replication from other cohorts would further strengthen the magnitude and generalisability of the associations. Our results could indicate ARCH as an underlying cause for systemic ageing, explaining its link to non-haematological, age-related diseases.

SUPPLEMENTAL INFORMATION

Supplemental Information includes one figure, one table and experimental procedures and can be found with this article online at https://doi.org/10.1016/j.cub.2019.07.011.

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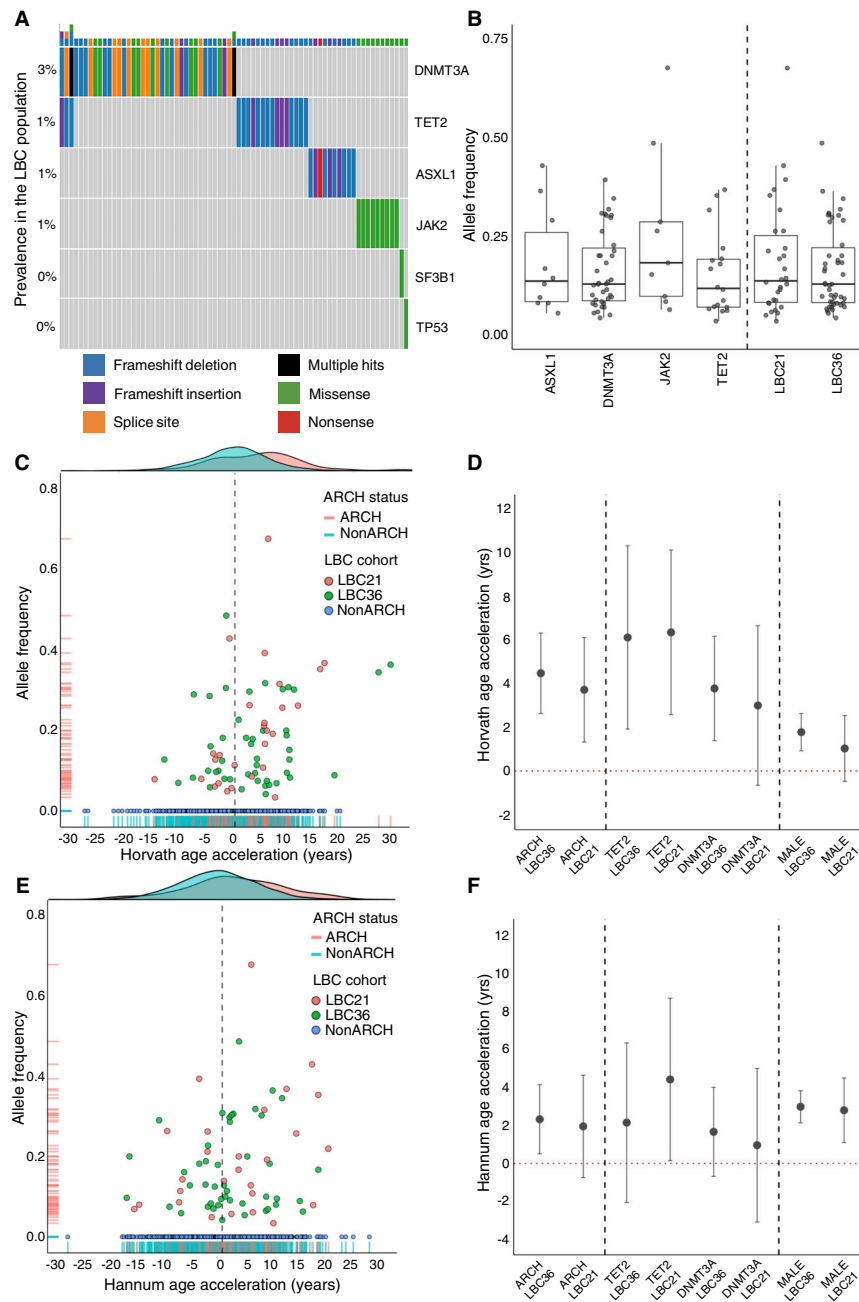
Figure 1. ARCH variants discovered in Lothian Birth Cohort (LBC) participants and their effects on epigenetic age as shown in the Horvath (IEAA) and Hannum (EEAA) clocks.
(A) Oncoplot showing variant types within the ARCH positive subset of the LBC. This subset represents 73 participants (6% of 1,136 total) where one or more described somatic variants were detected in the six most prevalent ARCH-associated genes. (B) Box plot describing the distribution of allele frequencies in all detected somatic ARCH variants. Genes with a single variant not shown are *TP53* and *SF3B1* (allele frequencies of 0.089 and 0.257, respectively). The overall distribution of allele frequencies by LBC cohort (LBC1921/LBC1936) is also displayed. (C) Scatter plot of Horvath age acceleration (IEAA; years) for individual LBC participants against the allele frequency of their somatic ARCH variant in both LBC1921 (orange dots, net 3.7 years; $p = 2.5 \times 10^{-3}$) and LBC1936 (green dots, net 4.5 years; $p = 2.3 \times 10^{-6}$) cohorts. Density plot highlighting the shift in distribution of Horvath age acceleration between ARCH-positive (orange) and -negative participant (turquoise) groups. Non-ARCH carriers (blue dots). (D) Plot showing net IEAA in ARCH (with 95% confidence intervals). The effect of sex (male versus female) on epigenetic ageing within the LBC is shown for comparison. (E) Scatter plot showing the Hannum age acceleration (EEAA; years) against the allele frequency of ARCH variants in both LBC1921 (orange dots, net 1.9 years;

p = 0.16) and LBC1936 (green dots, net 2.3 years; p = 0.01) cohorts. Density plot highlighting shift in distribution of EEAA between ARCH-positive (orange) and -negative participant (turquoise) groups. Non-ARCH carriers (blue dots). (F) Plot showing the net EEAA in ARCH (with 95% confidence intervals). The effect of sex (male versus female) on epigenetic ageing within the LBC is shown for comparison.