Increased hippocampal epigenetic age in the Ts65Dn Mouse Model of Down Syndrome

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Running Title: Higher hippocampal epigenetic age in Ts65Dn

Abstract

Down syndrome (DS) is a segmental progeroid genetic disorder associated to multi-systemic precocious ageing phenotypes, which are particularly evident at the immune and nervous systems. Accordingly, people with DS show an increased biological age as measured by epigenetic clocks. Ts65Dn trisomic mouse, which harbors extra-numerary copies of Hsa21-syntenic regions, was shown to recapitulate several progeroid features of DS, but no biomarkers of age have been applied to it so far. Here we used a mouse specific epigenetic clock to measure epigenetic age of hippocampi from Ts65Dn and euploid mice at 20 weeks. Ts65Dn mice showed an increased hippocampal epigenetic age respect to controls, and the observed changes in DNA methylation partially recapitulated those observed in hippocampi from people with DS. Collectively, our results support the use of the Ts65Dn model to decipher the molecular mechanisms underlying the progeroid DS phenotypes.

Keywords
Down Syndrome, Epigenetic clock, Ts65Dn, Aging

Abbreviations
DS – Down Syndrome
Hsa21 – Human Chromosome 21
DNAm – DNA methylation

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Down Syndrome (DS) is a common genetic disorder caused by complete or segmental triplication of chromosome 21 (Hsa21) and is the most frequent genetic cause of intellectual disability. DS is considered a segmental progeroid syndrome, characterized by a precocious aging-like deterioration which is particularly evident at the immune system and brain level. This view, originally proposed by George Martin on the basis of the analysis of DS phenotypic traits (Martin 1978), has been further refined in the last two decades through physiological and molecular analyses that explored similarities and differences between the pillars of aging and alterations occurring in DS (Franceschi et al. 2019; Chen et al. 2021; Zigman 2013). In this framework, several biomarkers of age have been explored in people with DS, including those based on telomere length (Gimeno et al. 2014; Holmes et al. 2006), magnetic resonance neuroimaging (brain-age) (Cole et al. 2017), serum proteins glycosylation (GlycoAge) (Borelli et al. 2015) and DNA methylation (DNAm) (epigenetic clocks) (Horvath, Garagnani, et al. 2015). These studies concordantly suggest that people with DS are older than their chronological age.

Murine models are largely employed in the study of ageing and age-related disease (Palliyaguru et al. 2021) and mouse epigenetic biomarkers of age have been developed (Coninx et al. 2020; Lu et al. 2023; Han et al. 2018; Zhou et al. 2022; Wang & Lemos 2019). So far, however, these epigenetic clocks have been applied to a limited extent and, to the best of our knowledge, no data are available for mouse models of DS.

The Ts65Dn mouse strain is the most common model for the study of DS. These mice are segmentally trisomic for a region of chromosome 16 that is homologous to part of Hsa21. Ts65Dn mice were shown to recapitulate a wide range of DS-specific behavioral, physiological and neuroanatomical features such as reduced brain size, neuronal density (Lorenzi & Reeves 2006; Baxter 2000; Insausti et al. 1998; Stagni et al. 2018), altered neuronal function (Kleschevnikov et al. 2004; Siarey et al. 1997) and altered dendrite architecture in hippocampal regions (Uguagliati et al. 2022) as well as spatial learning and memory deficits. The Ts65Dn mouse, in addition, shares with the DS human condition multi-systemic premature aging associated with early alterations in mitochondrial function, DNA damage response, proteostasis and early neurodegeneration (Cisterna et al. 2020; Vacano et al. 2012; Puente-Bedia et al. 2022; Mollo et al. 2020; Holtzman et al. 1996; Kirstein et al. 2022).

In this study, we aimed to evaluate hippocampal epigenetic age in the Ts65Dn model. We used a hippocampus-specific mouse epigenetic clock developed by Zymo Research (referred as DNAge®) which is based on deep bisulfite sequencing of 300 target regions containing 2045 CpG sites. Using this clock, Coninx and colleagues previously reported an increase in epigenetic age in the triple transgenic AD mouse model (Coninx et al. 2020).

We applied the DNAge® clock to 6 Ts65Dn mice (age: 20 weeks; 5 males and 1 female) and 7 euploid mice (age: 20 weeks, 6 males and 1 female) (Supplementary information). As shown in Figure 1A, the hippocampal epigenetic clock model tended to underestimate the age of euploid mice (mean epigenetic age: 4.7 weeks instead of 20), an effect that is in line with the original publication (Coninx et al. 2020). With respect to the estimated epigenetic age of euploid controls, Ts65Dn mice were significantly epigenetically older (Mann-Whitney test p-value=0.0047). This result indicates for the first time that the Ts65Dn murine model mimics the increase in epigenetic age previously described in the brain and blood of subjects with DS (Horvath, Garagnani, et al. 2015; Do et al. 2017). Ts65Dn mice also showed a higher variance compared to euploid mice, although not reaching statistical significance (F-test p-value = 0.1231). This trend in higher variance can be related to the progeroid

phenotype, as an increase in epigenetic variability has been described during aging (BIOS consortium et al. 2016), although we cannot exclude that it is the result of the phenotypic drift observed in the Ts65Dn model (Shaw et al. 2020).

To get further insights into the DNAm differences that contribute to the increased epigenetic age of Ts65Dn mice, we applied the Mann-Whitney test to each CpG site that makes the epigenetic clock. We found 27 differentially methylated CpGs (nominal p-value<0.01), of which 21 were hypermethylated and 6 hypomethylated in Ts65Dn compared to euploid mice (Supplementary Table 1). Furthermore, we identified 5 genomic regions that contained at least two CpG sites having a nominal p-value<0.01 (Figure 1B). These differentially methylated regions (DMRs) annotated to *Bin1*, *Ajm1*, *Hsf4, Gm2662* and *Gm26576* genes. *Bin1* is a ubiquitously expressed gene which is known to modulate tau processing as well as to be involved in vesicle trafficking, inflammation and apoptosis (GERAD consortium et al. 2013; Thinakaran & Koo 2008; Galderisi et al. 1999). *Ajm1* seems to be involved in cell-to-cell organization, while *Hsf4* is a transcription factor known to act upstream of several processes, including DNA damage repair (Cui et al. 2012), and is central in the development of the eye (Fujimoto et al. 2004). *Gm2662* and *Gm26576* functions are not known.

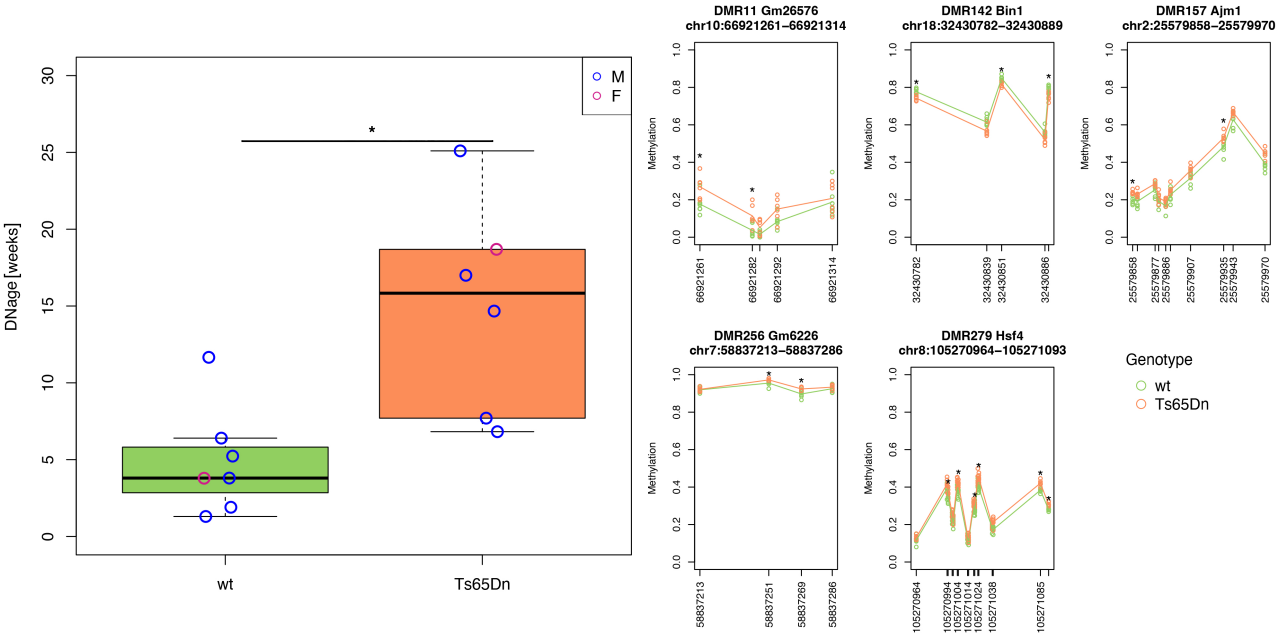

Figure 1. Increased epigenetic age of hippocampi in Ts65Dn mice. Boxplots showing epigenetic age predicted using ZymoResearch DNAge® predictor algorithm in Ts65Dn and wild-type euploid (wt) mice. Blue and red circles indicate data from male and female mice, respectively. *: p < 0.01, Mann-Whitney test). B) Lineplots of DNA methylation profiles in Ts65Dn mouse hippocampi for differentially methylated regions with at least two significant CpG sites. *: p < 0.01, Mann-Whitney test).

Previous studies using whole-genome bisulfite sequencing (WGBS) on whole cerebral hemispheres of newborn Dp(16)1Yey and Dp(10)1Yey mice highlighted similarities in DNAm profiles between trisomic humans and mice (Mendioroz et al. 2015). We therefore performed a cross-species analysis to check whether the DNAge® CpG sites differentially methylated in Ts65Dn mice showed altered methylation in hippocampi from subjects with DS. We searched Gene Expression Omnibus (GEO) repository and found a small dataset (GSE63347) containing DNAm data from hippocampi from 2 subjects with DS (age:42-57 y.o., 2 males) and 7 euploid controls (age:38-64, 2 males and 5 females), generated by the *Illumina Infinium HumanMethylation450K* microarray (Horvath, Garagnani, et al. 2015). Using UCSC *lift-over* tool (Hinrichs 2006), the genomic coordinates of 15 out of the 27 CpG

sites identified above were lifted from mouse (mm10 genome assembly) to human (hg19 genome assembly). We analyzed all the human microarray probes mapping between 250bp upstream and 250bp downstream of the 15 lifted CpG sites (a total of 19 probes). No probe was significantly differentially methylated between subjects with DS and controls (Mann-Whitney test p-value>0.05), possibly due also to the small sample size of the dataset; however, we found a CpG probe (cg04235075) mapping within *HSF4* gene which showed a trend towards hypermethylation in DS (Figure 2A), concordantly with what observed in mouse. Interestingly, DNAm of this probe was also found to be positively associated with age in healthy human hippocampi, as resulting from the analysis of GSE129428 (age 34-78, 13 females and 19 males) (Fries et al. 2020) and GSE64509 (age 38-114, 17 females and 8 males) (Horvath, Mah, et al. 2015) datasets (Figure 2B and Figure 2C). This concordance in DNAm changes observed in trisomic mice and humans as well as in human aging is of interest, as it suggests the presence of cross-species conserved epigenetic mechanisms that can contribute to the progeroid phenotype of DS.

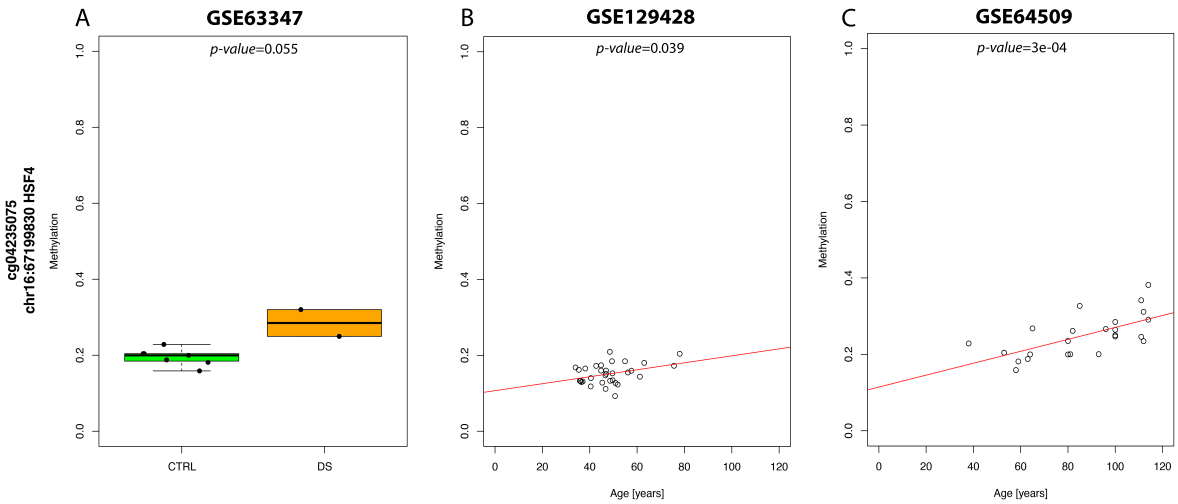

Figure 2. DNAm profiles of *HSF4* CpGs in human hippocampi. A) DNAm profiles of Illumina Infinium 450k probes cg03140421 and cg04235075, mapping within human Hsf4 orthologous region, in hippocampus from subjects with DS (GSE63347) (*p-value* calculated with Mann-Whitney test). B, C) DNAm profiles of Illumina Infinium 450k probes cg03140421 and cg04235075 in hippocampus from subjects without overt pathologies at different ages (GSE129428, GSE64509) (*p-values* calculated with linear model).

Collectively, our results show an increased hippocampal epigenetic age in the Ts65Dn mouse model. This is fully in line with its progeroid phenotypes (see above) and the notion that epigenetic alterations represent a pillar of aging (Mendioroz et al. 2015). The analysis of a small DNAm dataset of hippocampi from DS subjects revealed that some DNAm changes found in Ts65Dn mice were also present in humans. Although larger samples are needed, our study supports the use of the Ts65Dn model to decipher the molecular mechanisms underlying the progeroid DS phenotype. Finally, our study supports the use of the DNAge® clock and, possibly, other recently developed mouse epigenetic clocks (Lu et al. 2023; Zhou et al. 2022), as biomarkers of biological age. Such tools might be exploited to monitor the impact of disease and disease-modifying interventions in DS. It is worth to note that the DNAge® epigenetic clock is based on deep bisulfite sequencing of few genomic regions and therefore it is not informative of genome-wide epigenetic remodeling occurring in Ts65Dn mice. The recent release of *Illumina Infinium Mouse Methylation* microarray will allow investigation of genome wide DNAm profiles of DS models in a cost-effective manner and to get further insights into the epigenetic basis of the progeroid phenotype of DS.

Authors' Contributions

Maria Giulia Bacalini, Renata Bartesaghi and Fiorenza Stagni conceptualized and designed the study; Fiorenza Stagni and Sandra Guidi provided tissue samples; Francesco Ravaioli and Maria Giulia Bacalini performed experimental procedures and statistical analysis; Maria Giulia Bacalini, and Francesco Ravaioli drafted the manuscript; Maria Giulia Bacalini, Renata Bartesaghi, Paolo Garagnani, Sandra Guidi, Chiara Pirazzini, Alessandro Silvani; Fiorenza Stagni and Giovanna Zoccoli contributed to the interpretation of results and the review of the manuscript. All authors approved the final version of the manuscript.

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Conflict of Interest Statement

The authors state no conflict of interest.

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