Clinical Epigenetics

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Epigenetic age acceleration in Turner and Klinefelter syndrome: Correlations with clinical aging markers

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Abstract

Background The sex chromosome aneuploidies Turner syndrome (45,X; TS) and Klinefelter syndrome (47,XXY; KS) are associated with aging-related comorbidities, reduced life expectancy and genome-wide DNA methylation changes. This indicates that biological aging, reflecting physiological function rather than chronological age, is increased in both syndromes. To investigate whether DNA methylation patterns linked to physiological decline could contribute to the comorbidity patterns and reduced lifespan in TS and KS, we applied so-called epigenetic clocks to DNA methylation data from cohorts of TS (n = 57) compared to female controls (n = 33) and KS (n = 65) compared to male controls (n = 63). Additionally, we evaluated correlations between epigenetic age and clinical variables, aiming to identify clinical aging markers in TS and KS.

Results Comparing TS to female controls, all epigenetic clocks indicated advanced biological aging. Comparing KS to male controls, less evidence was observed although some epigenetic clocks indicated accelerated biological aging. Considering estrogen replacement therapy in TS, some epigenetic clocks found that treatment reduced biological age in TS. Correlating epigenetic clocks to clinical variables, several unfavorable outcomes—mainly related to body composition—correlated with age in controls. In TS, and in some cases KS, these correlations were diminished. In TS and KS, we instead found correlations between body composition and the rate of aging.

Conclusion We demonstrated that biological aging was clearly increased in sex chromosome aneuploidies, especially TS, potentially contributing to the severely reduced lifespan. Additionally, unfavorable changes in body composition, common in both TS and KS, and in particular in the presence of hypogonadism, could result in accelerated aging—or be the result thereof.

Keywords Turner syndrome, Klinefelter syndrome, Aging, Epigenetic age, DNA methylation

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Background

The sex chromosome aneuploidies Turner syndrome (45, X; TS) and Klinefelter syndrome (47, XXY; KS) are associated with increased morbidity and a reduced life expectancy [1–5]. Both TS and KS are affected by hypergonadotropic hypogonadism and routinely treated with sex hormone replacement therapy (HRT) to alleviate the effects hereof. Treatment reduces morbidity [5, 6] and has the potential to reduce mortality in both TS [6] and KS [5]. However, both syndromes present with an unfavorable body composition, which is not fully reversible with appropriate sex hormone replacement therapy [7–9]. Both syndromes are associated with increased incidence of aging-related comorbidities such as osteoporosis, sarcopenia, adiposity, diabetes, metabolic syndrome, as well as cardiovascular disease [10–16]. The average loss in lifespan is substantial for both syndromes, amounting to about 13–15 years for TS and 5–6 years for KS [4, 17], partly explained by hypogonadism [18–20], but other syndrome-specific characteristics may also be at play.

In recent years, the cause of these syndrome-specific characteristics has been investigated through genetic studies, primarily focusing on gene expression and DNA methylation (DNAm), a dynamic epigenetic mechanism governing gene expression [21]. These studies found that both gene expression and whole-genome DNAm profiles were altered in TS and KS, independent of sex hormone levels, with general hypomethylation in TS and hypermethylation in KS [22–26].

Aging is accompanied by a progressive decline in physiological functioning, which may vary between individuals of the same chronological age (cAge), defined as the time passed since birth. Thus, the degree of aging is better captured by the concept of biological age [27]. It is well recognized that DNAm is influenced by age [28], being acknowledged as a "hallmark of aging," and thus, suggested as a marker of biological age [29, 30]. Therefore, specific patterns of DNAm have been shown to accurately predict age [31], which led to the development of "epigenetic clocks" based on associations between DNAm and cAge [32, 33]. More recent "second-generation" epigenetic clocks have incorporated clinical measures of biological age, aiming to predict lifespan and health span. These include the PhenoAge clock, based on DNAm-based markers of clinical measures of health [34], and the GrimAge clock that is based on a composite biomarker consisting of DNAm-based surrogates of certain plasma protein levels, sex, and smoking pack-years [35, 36]. From these clocks, epigenetic age acceleration can be determined, estimating the difference between biological age and cAge by obtaining the residuals of regressing epigenetic age on cAge. Most recently, longitudinal data of physiological decline have been incorporated in the "third-generation" epigenetic clock DunedinPACE to estimate an "aging pace," quantifying the increase in biological age per year of cAge from DNAm-based indicators of organ system integrity across four time points spanning two decades. The model thus encompasses measures from the cardiovascular, metabolic, renal, hepatic, immune, periodontal, and pulmonary systems, adding incremental prediction beyond previous clocks [37]. Another well-recognized predictor of biological age is telomere length (TL). Telomeres are repetitive nucleotide sequences at the ends of chromosomes that shorten with replication of somatic cells, thereby being negatively correlated with age [38]. Adding to the aforementioned epigenetic clocks, a DNAm estimator of TL has been developed, which is associated with lifespan and health span [39].

Accelerated aging could help explain the reduced life expectancy in TS and KS; however, this has not been investigated in the cohorts studied so far [40–42]. Here, we set out to study epigenetic aging using a collection of epigenetic clocks, hypothesizing that TS and KS would present with accelerated biological aging compared to controls.

Methods

Dataset description

We combined data from two previously published studies of DNAm (Infinium HumanMethylation450k, Illumina, US) in peripheral blood samples of individuals with TS compared to age-matched female controls (TS cohort) [25] and KS compared to age-matched male controls (KS cohort) [26]. cAge was available for all subjects.

In addition, clinical variables were available for a subset of the TS cohort (54 of 57 individuals with TS, 25 of 33 female controls) and the entire KS cohort (described in [7, 43–45]). We included the following standard measurements from the clinic: triglycerides, total and high- and low-density lipoprotein (HDL, LDL) cholesterol to monitor lipids, in addition to BMI, weight, hip–waist ratio, and hip and waist circumferences to monitor body composition. Moreover, body fat percentage was available for the KS cohort. Additional variables measured in the TS cohort included body surface area (BSA)-adjusted creatinine level reflecting muscle mass, HbA1c reflecting long-term blood glucose, and systolic and diastolic 24-h blood pressure, length of the QT-interval (ECG-QTc), left ventricular mass, ejection fraction, coronary plaques, and the E/A ratio (indicating ventricular function), monitoring cardiovascular health (Supplementary Table 1). Medical usage (antihypertensive, antidiabetic, and statins) was available for the individuals with TS (Supplementary Table 1). For both TS and KS groups, treatment status (estrogen

replacement therapy (ERT) and testosterone replacement therapy (TRT)) was available (Supplementary Table 2).

Extraction of CpG methylation beta-values

The raw intensity values for all CpG sites, from all individuals, were imported and processed using the Bioconductor package Minfi in R [46]. Probes identified as cross-reactive or with a detection p-value below 0.01 were removed. Next, the CpG intensity values were normalized using the preprocess Funnorm method [47], and the methylation data for each CpG site were then converted to beta-values and used for downstream analysis. The two datasets (TS cohort and KS cohort) were merged by common CpG sites (n = 435,163).

Measures of epigenetic age

As multiple types of epigenetic clocks have been developed with different features, strengths, and applications, we chose to use an array of epigenetic clocks to reach a more nuanced understanding of epigenetic aging in TS and KS. We employed the Online DNAmAge Calculator (https://dnamage.genetics.ucla.edu) to calculate epigenetic age from an array of epigenetic clocks, including PhenoAge [34], GrimAge (DNAmGrimAge2BasedOnRealAge) [35, 36], and the Telomere Length's clock [39], predicting telomere length from DNAm (DNAmTL). From PhenoAge and GrimAge, we included measures of age acceleration, ageAccPheno and ageAccGrim, respectively.

Finally, we included the third-generation clock DunedinPACE via the DunedinPACE R-package (version 0.99.0) [37]. Beta-values were used as input for all epigenetic clocks.

Statistical analysis

Linear regression models were constructed for all measures of epigenetic age.

PhenoAge, GrimAge, and DNAmTL were adjusted for cAge. We evaluated cAge-adjusted pair-wise comparisons between groups within each cohort using the following model:

$$\text{Epigenetic age} \sim \text{cAge} + \text{group}.$$

For ageAccPheno, ageAccGrim, and DunedinPACE, pair-wise comparisons of groups were performed for each cohort using the following model:

$$\text{Epigenetic age acceleration} \sim \text{group}.$$

The influence of group on the association between epigenetic age and cAge was assessed using a model with an interaction term:

$$\text{Epigenetic age} \sim \text{cAge} + \text{group} + \text{cAge} : \text{group}.$$

We compared females with TS receiving estrogen replacement therapy (ERT) and those not receiving ERT, and males with KS receiving testosterone replacement therapy (TRT) and those not receiving TRT, adjusting for cAge. For this purpose, we applied the following model:

$$\text{Epigenetic age} \sim \text{cAge} + \text{treatment}.$$

Moreover, an adjustment for BMI in addition to cAge was performed. We evaluated BMI and cAge-adjusted pair-wise comparisons between groups within each cohort using the following model:

$$\text{Epigenetic age} \sim \text{BMI} + \text{cAge} + \text{group}.$$

We used the emmeans function of the emmeans R-package (version 1.10.3) on our models, setting group as the predictor to compute estimated marginal means with 95% confidence intervals of the estimate, setting a pair-wise contrast for within-cohort comparisons.

Correlations between clinical variables and epigenetic age measures were calculated with the rcorr function of the Harrell Miscellaneous (Hmisc) R-package (version 5.1–3).

$P < 0.05$ was used as significance threshold. All statistical analyses were conducted using R (version 4.3.3) (Vienna, Austria).

Results

Cohort description

The TS cohort included 57 individuals diagnosed with TS (cAge: 45.5 ± 9.79, 26–71) compared to 33 female controls (cAge: 42.6 ± 12.33, 22–65). Of these, clinical data were available from 54 individuals with TS and 25 female controls (Fig. 1A). The KS cohort included 65 individuals diagnosed with KS (cAge: 36.8 ± 10.54, 18–59) compared to 63 age-matched male controls (cAge: 36.4 ± 10.28, 19–59), all of which had clinical data available (Fig. 1). Inherent to the age-based matching applied in both cohorts, no differences in cAge were seen between TS and female controls or between KS and male controls (Table 1).

Epigenetic age, age acceleration, and aging pace

Comparing TS to female controls, we found a consistent and highly significant increase across epigenetic age, age acceleration, and aging pace (Table 1).

To investigate lifespan and health span, we employed the second-generation epigenetic clocks GrimAge and PhenoAge. Both were increased in TS compared to female controls, with the PhenoAge (7.14 years, $p < 0.001$) estimate exceeding the GrimAge estimate (2.81 years,

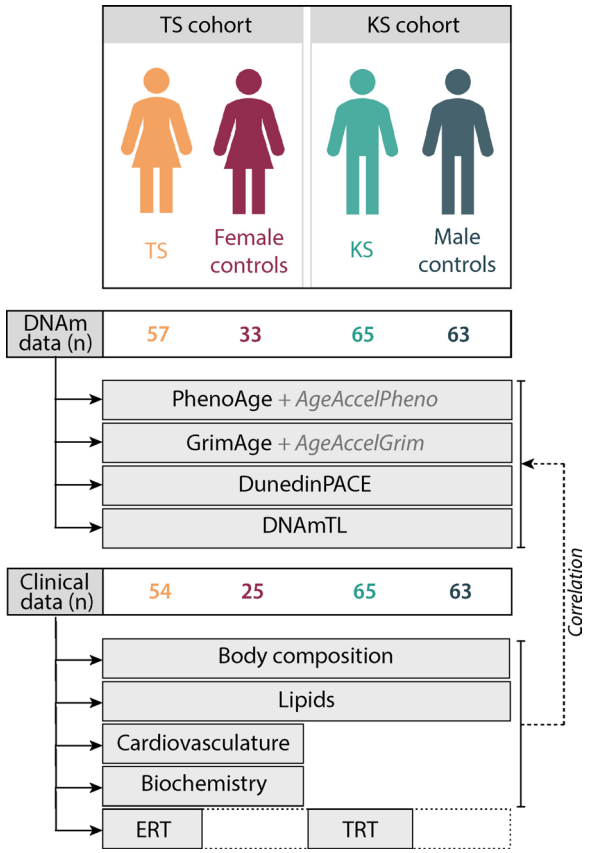

Fig. 1 Study overview DNAm data were available from 57 individuals with TS and 33 female controls (TS cohort) in addition to 63 male controls and 65 individuals with KS (KS cohort). We evaluated epigenetic age and age acceleration from the epigenetic clocks PhenoAge and GrimAge, pace of aging from the epigenetic clock DunedinPACE, and telomere length from the Telomere Length's Clock (DNAmTL). Clinical data were available from a subset of the TS cohort and the entire KS cohort, including treatment status (ERT for the TS cohort and TRT for the KS cohort) and measures of body composition and lipids, and cardiovascular function and biochemistry for the TS cohort. These were correlated to the predictions of the epigenetic clocks. TS, Turner syndrome; KS, Klinefelter syndrome; DNAm, DNA methylation; ERT, estrogen replacement therapy; and TRT, testosterone replacement therapy

$p < 0.01$) (Fig. 2A–B). By performing a regression analysis, we investigated if TS impacted the relationship between epigenetic age and cAge, comparing the intercept and slope between TS and controls. We found an increased PhenoAge throughout the measured span of cAge (estimated intercept + 11.69, $p = 0.015$) and an increased GrimAge early in life (estimated intercept + 12.37, $p < 0.001$), seemingly diminished with cAge (estimated interaction − 0.20, $p = 0.005$) (Fig. 2A–B; Table 2). As expected from an increased epigenetic age, we found an accompanying increase in age acceleration, the PhenoAge estimate (AgeAccelPheno estimate: 6.65 years, $p < 0.001$) again surpassing the GrimAge estimate (AgeAccelGrim estimate: 1.65 years, $p = 0.019$) (Fig. 2C–D; Table 1).

Based on our findings of increased age acceleration, we included the third-generation epigenetic clock DunedinPACE to estimate aging pace, quantifying how much faster or slower an individual is aging relative to the normative rate of 1 year of cAge. Rather than estimating epigenetic age relative to cAge, this measure estimates the rate of biological aging independent of cAge, linking DNAm profiles to aging trajectories [37]. Aging pace was increased by 0.12 years in TS compared to female controls ($p < 0.001$) (Fig. 2E; Table 1), meaning that the TS population ages 0.12 years faster than female controls per year of cAge. As expected, aging pace increased with cAge [37], their relationship indicating an increase in TS throughout the measured span of cAge (estimated intercept + 0.23, $p = 0.008$) (Fig. 2F; Table 2).

Comparing KS to male controls, our findings were less conclusive. While no differences in PhenoAge or GrimAge were observed for the KS cohort (Fig. 2G–I), age acceleration based on GrimAge was increased by ~ 2 years ($p = 0.015$), indicating aging acceleration in KS (Fig. 2J). Additionally, a slight, though insignificant, increase in aging pace was found for KS compared to male controls (DunedinPACE estimate: 0.04 years, $p = 0.061$) (Fig. 2K; Table 1).

Telomere length

We found that individuals with TS had 0.14 kb shorter DNAmTL compared to female controls when adjusting for cAge ($p < 0.001$) (Fig. 2F), while individuals with KS had 0.08 kb longer DNAmTL compared to male controls ($p = 0.009$) (Fig. 2L). As expected, DNAmTL was negatively correlated with age. TS had a weaker negative correlation between DNAmTL and cAge (0.01, $p = 0.013$) and a lower intercept (− 0.49, $p < 0.001$) (Table 2), indicating that telomeres were shorter in young individuals with TS, while the difference diminishes over time (Fig. 2F).

Treatment effects

A comparison of epigenetic age between treated and untreated individuals with TS (ERT vs. no ERT) and KS (TRT vs. no TRT) was also carried out (Supplementary Table 2). However, the analysis was complicated by significant differences in chronological age, particularly in TS, where untreated individuals were an average of 10 years older. Although adjustments for cAge eliminated statistically significant differences in most measures, these comparisons remain difficult to interpret due to the inherent biological differences between treated and untreated groups, making direct comparisons less meaningful. After adjusting for cAge, a significant difference was only observed between treated and untreated TS

Table 1 Age measures across patient groups

	TS versus Female controls				KS versus Male controls			
	TS (n = 57)	Female controls (n = 33)	Effect size	*p*-value	KS (n = 65)	Male controls (n = 63)	Effect size	*p*-value
cAge (years)	45.5 (26.6–71.7)	42.6 (22.0–65.4)	2.9	0.222	36.8 (18.1–59.8)	36.4 (19.4–59.3)	0.39	0.834
PhenoAge (years)	42.04[40.66;43.42]	34.91 [33.09;36.72]	7.14	**< 0.001**	29.71 [28.28; 31.13]	27.99 [26.54; 29.43]	1.72	0.096
GrimAge (years)	58.04 [57.10;58.98]	54.23 [52.99;55.47]	3.81	**< 0.001**	49.59 [48.33; 50.85]	48.16 [46.88; 49.44]	1.43	0.117
ageAccelPheno (years)	3.71 [2.27; 5.16]	− 2.94 [− 4.84; 1.04]	6.65	**< 0.001**	− 0.05 [− 1.46; 1.36]	− 1.78 [− 3.22; 0.33]	1.73	0.093
ageAccelGrim (years)	− 0.08 [− 0.91; 0.76]	− 1.72 [− 2.82; 0.63]	1.65	**0.019**	1.47 [0.33; 2.61]	− 0.55 [− 1.71; 0.61]	2.02	**0.015**
DunedinPACE	1.08 [1.06; 1.11]	0.96 [0.93; 1.00]	0.12	**< 0.001**	0.98 [0.95; 1.01]	0.94 [0.91; 0.97]	0.04	0.061
DNAmTL (kb)	7.01 [6.97; 7.05]	7.15 [7.09; 7.20]	-0.14	**< 0.001**	7.27 [7.23; 7.31]	7.19 [7.15; 7.23]	0.08	**0.009**

Data are presented as means (total range) or estimated marginal means [95% confidence intervals] for each patient group, with *p*-values of two-sample t-tests for each cohort. Significant p-values ($p < 0.05$) are highlighted in bold. PhenoAge, GrimAge, and DNAmTL are adjusted for cAge. TS, Turner syndrome and KS, Klinefelter syndrome

from the GrimAge clock ($p = 0.005$), with untreated TS being predicted 3.2 years older than treated TS, and with GrimAge acceleration bordering significance ($p = 0.061$), untreated TS exhibiting 0.36 years accelerated GrimAge (Fig. 3A–B, Supplementary Table 2).

Correlation between epigenetic age and clinical variables

Next, we set out to identify correlations between epigenetic age and clinical variables (Supplementary Table 1). We observed vastly different correlation profiles between TS and female controls (Fig. 3C, see supplemental Fig. 1 for *p*- and correlation values). Most strikingly, non-acceleration parameters (cAge, PhenoAge, GrimAge, and DNAmTL) were positively correlated (negative for DNAmTL) to measures of body composition (hip and waist circumference, BMI, and weight) for female controls, but not TS, where positive correlations were instead found for GrimAge acceleration and in some cases aging pace. In addition, the HbA1c concentration, a risk indicator of diabetes, was more strongly correlated to cAge, epigenetic age, and aging pace in TS than controls. A negative correlation between cAge/PhenoAge/GrimAge and body surface area (BSA)-adjusted creatinine, reflecting muscle mass, was evident from female controls but diminished in TS.

Correlation profiles between KS and male controls showed closer similarity; however, discrepancies were present (Fig. 3D, see supplemental Fig. 1 for p- and correlation values). While non-acceleration parameters were, again, positively correlated to measures of body composition, correlations to body fat percentage and triglyceride levels were only evident from the controls and not KS. In addition, correlations to aging pace were consistent for all measures of body composition in KS, but only waist circumference, waist-to-hip ratio, and body fat percentage for controls. Levels of triglyceride and body fat percentage were increased in KS compared to controls (Supplementary Table 1), suggesting that a higher baseline results in a slower cAge-dependent increase.

As body composition seemed clearly linked to both cAge and epigenetic age and BMI was significantly increased in TS (Supplementary Table 1), we adjusted for BMI. After this adjustment, differences in epigenetic age generally became less pronounced, yet all but one of the previously significant increases remained significant (Supplementary Table 3).

Discussion

In the first study of its kind, we demonstrated pervasively increased epigenetic age in TS compared to age-matched female controls, suggesting that TS is indeed affected by increased biological aging. We also found indications of advanced epigenetic aging in KS, though these findings were less conclusive. We further demonstrated that epigenetic aging in both TS and KS was linked to the syndrome-specific clinical phenotype, arguing that distinct aging mechanisms may affect the clinical presentation of both syndromes throughout the lifespan.

Increased epigenetic age acceleration has been associated with poor health in other cohorts, including an unfavorable body composition with reduced muscle mass, decreasing cardiovascular and metabolic health [48]. We found that age acceleration and pace were uniquely positively correlated to altered body composition in TS. We speculate if changes in body composition in youth and mid-life are more detrimental to the aging process in TS than in other women, or if accelerated biological aging causes unfavorable changes in body composition or are the result of combined effects of aging together with body composition changes. We found that measures of body

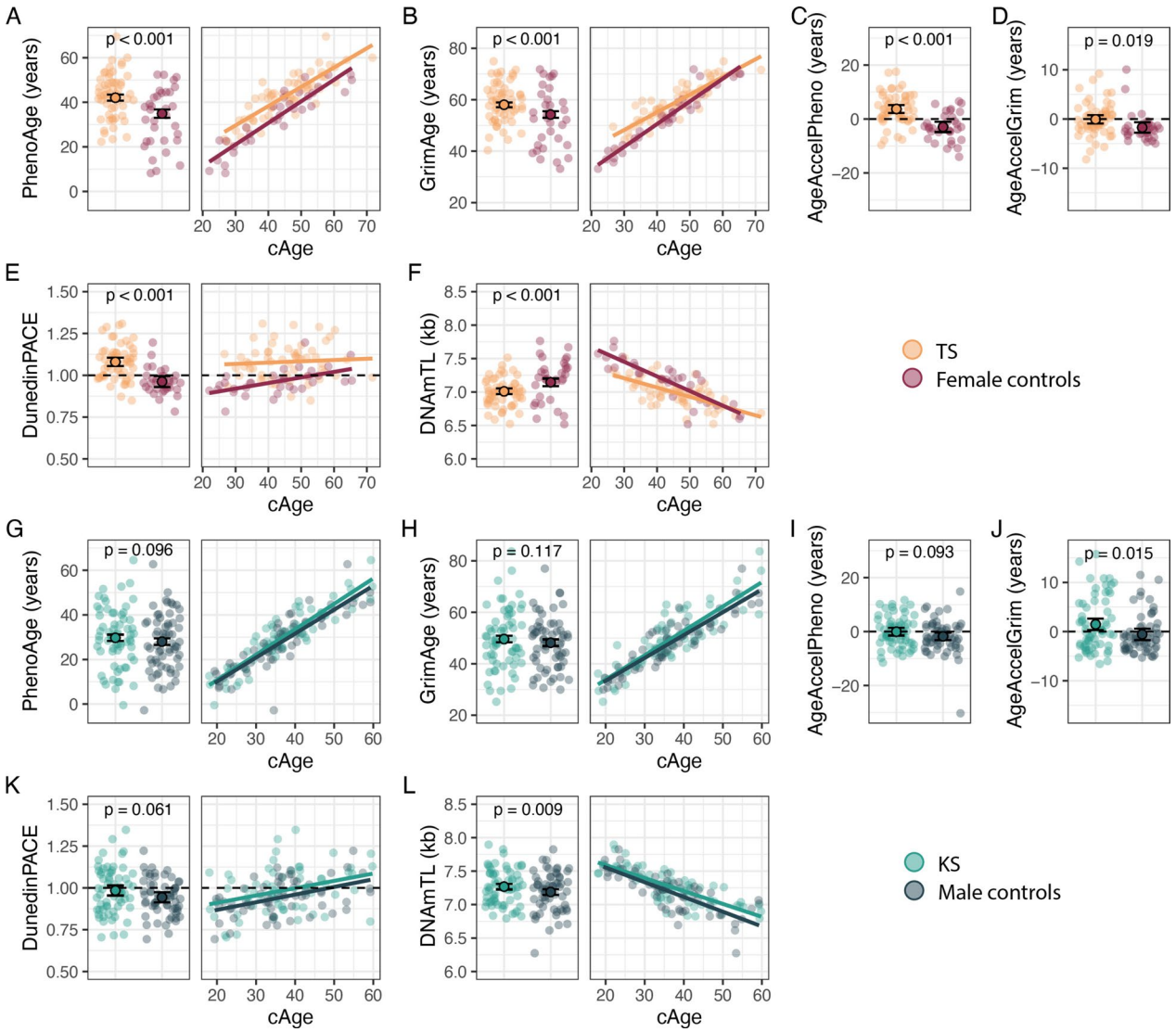

Fig. 2 Epigenetic age, age acceleration, aging pace, and telomere length Epigenetic age was determined from the PhenoAge (TS cohort, A; KS cohort, G) and GrimAge (TS cohort, B; KS cohort, H) clocks, as well as age acceleration from AgeAccelPheno (TS cohort, C; KS cohort, I) and AgeAccelGrim (TS cohort, D; KS cohort, J). Aging pace was determined from the DunedinPACE clock (TS cohort, E; KS cohort, K) and telomere length (DNAmTL) from the Telomere Length's Clock (TS cohort, F; KS cohort, L). Means adjusted for cAge are presented with 95% confidence intervals of the estimates, with pair-wise p-values between groups. Non-acceleration measures are further presented with linear regressions against cAge. Turner syndrome; KS, Klinefelter syndrome and cAge, chronological age

composition were more consistently correlated to aging pace in KS and speculate if this reflects changes in body composition leading to immediate aging processes that are more unfavorable than in other males or vice versa. As epigenetic age remained increased despite adjustment for BMI, it is suggested that the higher BMI observed in TS partly influences epigenetic aging; however, several comparisons remained significant, indicating that body composition does not fully explain the observed differences and that other factors contribute to the accelerated epigenetic aging.

One study found a significant association between epigenetic age acceleration and early menopause in women, with hormone treatment reducing epigenetic age [49]. Another study found that increased age acceleration was associated with low testosterone in prepubertal boys [50], and we theorize that the same is true for adults, with hypogonadism being the primary driver of increased epigenetic age in TS and KS. We speculate if the increased epigenetic age acceleration and correlations to clinical traits observed in both syndromes are a product of hypogonadism and could be alleviated

Table 2 Chronological age regressed on epigenetic age across cohorts

		TS versus Female controls		KS versus Male controls	
		Estimate	*p*-value	Estimate	*p*-value
PhenoAge (years)	(Intercept)	− 8.01	**0.018**	− 11.41	**< 0.001**
	cAge	0.99	**< 0.001**	1.08	**< 0.001**
	Group	11.69	**0.015**	− 0.69	0.856
	cAge:Group	− 0.10	0.319	0.07	0.510
GrimAge (years)	(Intercept)	15.27	**< 0.001**	15.59	**< 0.001**
	cAge	0.88	**< 0.001**	0.89	**< 0.001**
	Group	12.37	**< 0.001**	− 0.76	0.821
	cAge:Group	− 0.20	**0.005**	0.06	0.498
DunedinPACE	(Intercept)	0.82	**< 0.001**	0.78	**< 0.001**
	cAge	0.00	**0.014**	0.01	**0.001**
	Group	0.23	**0.008**	0.05	0.529
	cAge:Group	0.00	0.165	0.00	0.914
DNAmTL (kb)	(Intercept)	8.27	**< 0.001**	8.16	**< 0.001**
	cAge	− 0.02	**< 0.001**	− 0.02	**< 0.001**
	Group	− 0.48	**< 0.001**	− 0.02	0.876
	cAge:Group	0.01	**0.013**	0.00	0.365

Controls as reference levels. Significant p-values ($p < 0.05$) are highlighted in bold. TS, Turner syndrome and KS, Klinefelter syndrome

with sufficient hormone replacement therapy [51]. We did compare TS receiving ERT with those not receiving ERT; however, this comparison is confounded by the fact that most of those not receiving ERT had passed the normal age of menopause, where other factors affecting aging come into play. We did observe a significantly higher GrimAge in women with TS when left untreated, likely linked to this epigenetic clock being the only one to directly include CpGs associated with sex, thereby catching DNAm changes associated with changes in sex hormones. Similarly, the comparison between KS receiving TRT and those not receiving TRT was confounded by a difference in age, and in addition, it is well known that TRT induces large changes in body composition in KS [7]. As our findings can only be regarded as explorative, we recommend future longitudinal studies of HRT in both syndromes to investigate the impact on biological aging. Investigating epigenetic age in untreated individuals who are starting HRT with follow-up would be beneficial to disclose the impact of ERT and TRT on biological aging.

A larger increase in epigenetic age in TS compared to KS coincides with a markedly larger loss in lifespan [4, 17]. Predictors of lifespan and health span suggested that DNAm patterns linked to morbidity and mortality are evident in TS but not KS, indicating that differences in lifespan arise from variations in "classic" aging mechanisms captured by these clocks. Based on regression analyses, differences in intercept and slope suggested more pronounced differences of epigenetic age in TS earlier in life. This potentially indicates that TS is subjected to aging mechanisms, or phenotypic characteristics leading to accelerated biological aging, prior to adulthood. These changes may then be diminished by a healthy survivor bias, illustrated here by the fact that you could only participate in this study if you had survived into your thirties or forties.

We showed that telomeres were predicted to be shorter in TS compared to female controls, the shortening being more pronounced in the younger TS individuals. We have previously compared telomere restriction fragment lengths in 30 TS cases and 30 age-matched female controls [42], finding no difference in a younger (20–30 years) or an older (40–50 years) age group. However, this study investigated telomere restriction fragment length from leukocytes using southern blotting, while the present study utilized 140 DNAm markers. The latter method has previously been shown to be more strongly associated with cAge than southern blot-based leukocyte telomere length [39]. Thus, it is plausible that the DNAm-based predictions used in this study are more accurate.

We found slight increases in telomere length for KS compared to male controls, the increase being more pronounced with increased cAge. Previously, a study used pyrosequencing to investigate age-associated DNA methylation markers and relative telomere lengths, finding significantly longer telomeres in KS [41]. However, this was restricted to younger men with KS (18–24 years). The discrepancy between our findings and those of the previous study is likely attributable to inclusion of younger subjects and the use of a less advanced methodology in the previous study, which only included three DNA methylation markers [41].

It is well known that females, on average, have longer telomeres than men, with the difference being present from birth and corresponding to the difference in life expectancy [52, 53]. This could be caused by limited telomerase activity in embryonic stem cells in males [53]. For a short time during early embryonic life, the X-linked gene *DKC1*, encoding the protein dyskerin, has monoallelic expression in males but biallelic expression in females due to delayed X chromosome inactivation [53]. Linking this to our findings of shorter telomeres in TS and longer telomeres in KS, it is likely that the number of X chromosomes influences telomere length in a similar fashion in sex chromosome aneuploidies. A second X chromosome could cause longer telomeres in KS from birth due to delayed X chromosome inactivation, comparable to females, while the single X chromosome in TS results in shorter telomeres, comparable to males. As a

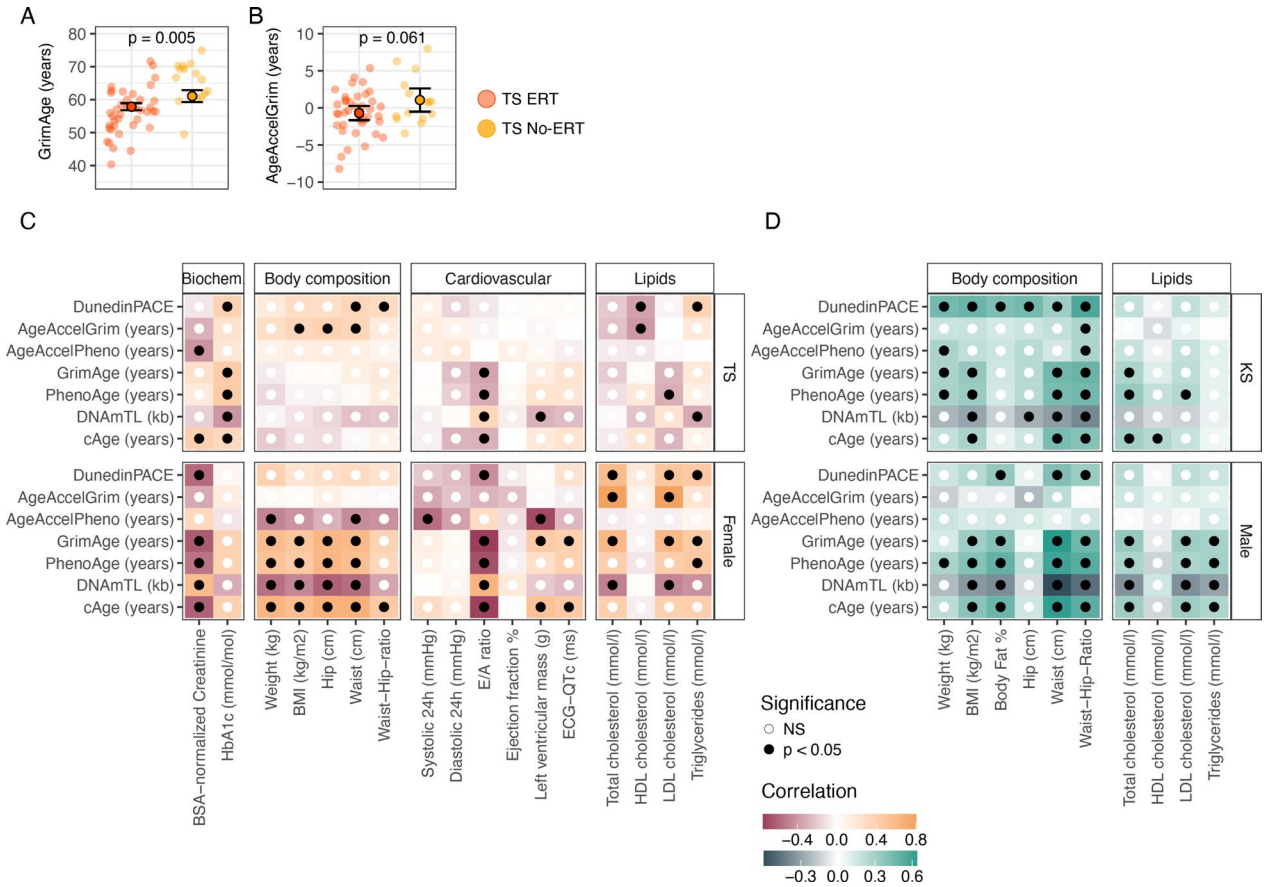

Fig. 3 Treatment effect and correlations to clinical variables (**A**–**B**) GrimAge (**A**) and AgeAccelGrim (**B**) comparing ERT-treated (TS ERT) and untreated (TS No-ERT) TS subgroups. Means adjusted for cAge are presented with 95% confidence intervals of the estimates, with pair-wise p-values between groups. **C**–**D**) Correlations between clinical variables and measures of age in the TS cohort (**C**) and KS cohort (**D**). Clinical variables include measures of biochemistry for the TS cohort (BSA-adjusted creatinine, HbA1c), body composition (weight, BMI, hip circumference, waist circumference, hip–waist ratio, and body fat percentage for the KS cohort), cardiovascular function for the TS cohort (systolic and diastolic 24h blood pressure, ECG-QTc, left ventricular mass, ejection fraction, and E/A ratio), and lipids (HDL cholesterol, total cholesterol, and triglycerides). Measures of age include cAge, PhenoAge, GrimAge, AgeAccelPheno, AgeAccelGrim, DNAmTL, and DunedinPACE. Color scales indicate the strength of the trait-clock correlation. Dots indicate p-value, $p < 0.05$ in black and non-significant findings in white. BSA, body surface area; ERT, estrogen replacement therapy; KS, Klinefelter syndrome; and TS, Turner syndrome

result, individuals with KS would have a more advantageous baseline in comparison with TS. This could explain, at least in part, the less severe increase in epigenetic age and epigenetic age acceleration, as well as the shorter loss in lifespan.

In recent years, epigenetic clocks have attracted attention for their potential to predict biological aging, often outperforming other biomarkers [48, 54]. We employed multiple epigenetic clocks to reach a more refined understanding of aging in TS and KS, as different generations of epigenetic clocks are based on different measures of age, thereby complementing one another [37]. The clocks agreed on increased age and age acceleration in TS. The most pronounced variability was, however, noted from the correlations to clinical variables. This is not surprising, considering the variability in clinical markers on which the clocks were based. We acknowledge that the epigenetic clocks applied in these analyses were developed on euploid populations, per se not designed for aneuploid populations such as TS and KS, in which we see global hypo- and hypermethylation, respectively. We currently have no way of testing the impact of the global methylation changes in these syndromes on epigenetic clocks—to distinguish syndromic patterns vs true biological aging. Nevertheless, these patterns are an integral part of the syndromes themselves, and thus, we believe that it would not be appropriate to view the methylation profiles and syndromes as separate entities.

A strength of the present study is the availability of both DNAm and relevant clinical data from the same

cohorts, allowing correlations between epigenetic age measures and clinical variables. The study could be limited by the available sample size. Still, we succeeded in showing increased epigenetic age and age acceleration in TS, and the cohort is of a considerable size in view of the rarity of the syndromes. Some of the TS participants also suffered from comorbidities and were receiving treatment for hypertension, type 2 diabetes, or hypercholesterolemia, while none of the controls received such treatment. How, if at all, such treatment affects measures of aging is difficult to say. Likewise, this study is cross-sectional, and hence, it would increase the strength of the observed associations to include a follow-up part in the future. Moreover, it would be of interest to study TS and KS cohorts from childhood, prior to the development of age-related disease.

In conclusion, we consistently show accelerated aging, possibly explaining part of the loss in lifespan in TS, indicating that the generalized hypomethylation associated with TS may have actual clinical consequences. Whether this may be amenable to clinical intervention is currently unclear. Unfavorable changes in body composition seem to be important for biological aging in both syndromes.

Abbreviations

cAge	Chronological age
DNAm	DNA methylation
DNAmAge	DNA methylation age
ERT	Estrogen replacement therapy
HDL	High-density lipoprotein
KS	Klinefelter syndrome
LDL	Low-density lipoprotein
TL	Telomere length
TRT	Testosterone replacement therapy
TS	Turner syndrome

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13148-025-01963-4.

Supplementary Figure 1: Correlations to clinical variables Correlations between clinical variables and measures of age in the TS cohort (A) and KS cohort (B). Clinical variables include measures of biochemistry for the TS cohort (BSA-adjusted creatinine, HbA1c), body composition (weight, BMI, Hip circumference, waist circumference, hip–waist ratio, and body fat percentage for the KS cohort), cardiovascular function for the TS cohort (systolic and diastolic 24 h blood pressure, ECG-QTc, left ventricular mass, ejection fraction, and E/A ratio), and lipids (HDL cholesterol, total cholesterol, and triglycerides). Measures of age include cAge, PhenoAge, GrimAge, AgeAccelPheno, AgeAccelGrim, DNAmTL, and DunedinPACE. Correlation and p-values are given for each trait-clock correlation, the color indicating significance; black if $p < 0.05$. Color scales indicate the strength of the trait-clock correlation. BSA, body surface area; ERT, estrogen replacement therapy; KS, Klinefelter syndrome; and TS, Turner syndrome.

Supplementary file 2

Author contributions

Conceptualization by CHG, EBH and JJ, data analysis and visualization by EBH, interpretation of data by EBH, JJ, CHG, SC and AS, drafting of the manuscript by EBH, CHG, and JJ, review and editing by EBH, JJ, CHG, SC and AS.

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Data availability

Methylation data are available from the European Genome-phenome Archive (EGA) under accession numbers EGAS00001002190 and EGAS00001002797. R scripts are available in GitHub (https://github.com/EmmaBJohannsen/Epi-Age_TS_KS). The clinical data is not publicly available due to them containing information that could compromise the research participants' privacy. Access to the data requires that the Danish National Committee on Health Research Ethics ethically approve the requestors' intended use of the data, and that the legal entity of the data requestor enters into a data protection agreement with the Danish data controller, the Central Denmark Region.

Declarations

Ethics approval

All DNAm data used in this study have previously been published (ClinicalTrials.gov NCT00624949, NCT00999310, NCT02526628, and NCT01678261). All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Competing interests

The authors declare no competing interests.

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