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Epigenetic clocks indicate that kidney transplantation and not dialysis mitigate the effects of renal ageing

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Background. Chronic kidney disease (CKD) is an age-related disease that displays multiple features of accelerated ageing. It is currently unclear whether the two treatment options for end-stage kidney disease (dialysis and kidney transplantation [KT]) ameliorate the accelerated uremic ageing process.

Methods. Data on clinical variables and blood DNA methylation (DNAm) from CKD stage G3–G5 patients were used to estimate biological age based on blood biomarkers (phenotypic age [PA], $n = 333$), skin autofluorescence (SAF age, $n = 199$) and DNAm (Horvath, Hannum and PhenoAge clocks, $n = 47$). In the DNAm cohort, we also measured the change in biological age 1 year after the KT or initiation of dialysis. Healthy subjects recruited from the general population were included as controls.

Results. All three DNAm clocks indicated an increased biological age in CKD G5. However, PA and SAF age tended to produce implausibly large estimates of biological age in CKD G5. By contrast, DNAm age was 4.9 years ($p = 0.005$) higher in the transplantation group and 5.9 years ($p = 0.001$) higher in the dialysis group compared to controls. This age acceleration was significantly reduced 1 year after KT, but not after 1 year of dialysis.

Conclusions. Kidney failure patients displayed an increased biological age as estimated by DNAm clocks compared to population-based controls. Our results suggest that KT, but not dialysis, partially reduces the age acceleration.

Keywords: ageing, chronic kidney disease, dialysis, DNA methylation, epigenetic clock, kidney transplantation

Introduction

Chronic kidney disease (CKD) is part of a collection of age-related 'burden of lifestyle' diseases termed the 'diseasome' of ageing, which includes cardiovascular disease (CVD), neurodegenerative diseases, osteoarthritis, chronic obstructive pulmonary disease, type-2 diabetes, obesity and osteoporosis [1, 2]. Albeit with different clinical phenotypes and aetiologies, these age-related diseases share a number of features that are characteristic of a dysregulated ageing process: epigenetic alterations, increased oxidative stress, mitochondrial dysfunction, dampening of Nrf2-mediated cytoprotection and microbial dysbiosis [1, 3]. As such, CKD presents with multiple features of an accelerated ageing phenotype [4]. In particular, the uremic milieu drives early vascular ageing and thus contributes to systemic accelerated biological ageing [4].

In CKD, the diminished clearance of uremic toxins leads to an accumulation of toxic solutes, which contributes to a chronic inflammatory burden, increased oxidative stress and endothelial

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dysfunction [2, 4, 5]. An additional contributor to the accelerated ageing in CKD is the disruption of Klotho homoeostasis and phosphate toxicity [6]. Around 3 million people globally are receiving treatment for kidney failure, and this number is expected to double or triple over the next decade [7–9]. Although the current best available treatment for kidney failure is kidney transplantation (KT) [10], this therapy has drawbacks, including a limited kidney lifespan, risk of organ rejection or dysfunction, necessity for immunosuppression with subsequent risk of cancer and infectious complications. Organ shortage is another limitation which causes prolonged dialysis vintage in some patients [2, 11]. Dialysis has several drawbacks, such as low efficacy, lowered quality of life, being costly, time-consuming and causing additional oxidative stress, inflammation and increased risk of infection [2, 4]. On top of this, patients receiving dialysis have an expected remaining lifespan that is approximately 50% lower compared to transplant recipients of similar age [12, 13].

It remains to be determined whether kidney replacement therapy (KRT), in the form of KT or dialysis, can decelerate or reverse the accelerated biological ageing associated with CKD. This task has been complicated by difficulties in identifying suitably robust biomarkers of biological age and distinguishing between normative and premature ageing [14, 15]. One novel approach is the use of 'ageing clocks'. There is a growing number of models that estimate (biological) age based on DNA methylation (DNAm), gene expression, the proteome, the gut microbiome, skin autofluorescence (SAF) (which correlates with the presence of advanced glycation end-products, or AGEs) and other features [15–18]. Epigenetic clocks based on DNAm are the most well established [15, 19]. These clocks correlate well with chronological age and capture some important aspects of biological ageing, including its association with morbidity and mortality [15, 20–23]. The first generation of DNAm clocks, which includes the original Horvath and Hannum clocks, were built using chronological age to train the model [15, 20, 21]. Although this method is straightforward, the biological and chronological ages are not perfectly correlated, so training the model on the latter can reduce its ability to predict the former [23]. The second generation of DNAm clocks, which includes the PhenoAge clock, attempts to improve upon the first generation by training the model, not on chronological age, but on an estimate of biological age [22]. In the case of PhenoAge, this estimate of biological age, termed phenotypic age (PA), is based on nine whole blood parameters plus chronological age and is an ageing clock in its own right [22].

In the present pilot study, we aimed to estimate the biological age of CKD patients at stages G3 (moderate reduction of kidney function) to G5 (kidney failure) and controls using three different approaches: PA, SAF age, and DNAm age. In the DNAm age cohort, we further compared three groups of subjects (CKD G5 patients receiving dialysis or KT and population-based controls), using three different clocks (Horvath, Hannum and PhenoAge). Finally, we investigated if CKD G5 patients display increased DNAm age before initiation of therapy and, if they do, whether available KRT ameliorates this age acceleration.

Materials and methods

Patient cohorts

Three different cohorts of CKD patients were included in this study, drawn from ongoing studies that have been previously described [24]. The PA cohort ($n = 333$) was composed of 78 population-based controls (randomly selected by Statistics Sweden, SCB), patients with CKD G3–4 ($n = 64$) and CKD G5 ($n = 191$). The SAF cohort was composed of 199 end-stage kidney disease (ESKD) patients (prevalent peritoneal dialysis, $n = 50$; incident dialysis patients, $n = 62$; and patients receiving a living donor KT, $n = 87$). Median dialysis vintage was significantly lower in the IDP group (0.9 months, interquartile range [IQR] 0.6–2.0) compared to the PPT (13.3 months, 6.4–31.1) and KT (11.4 months, 5.1–18.5) groups. Subsets of 39 patients were part of both the PA and the SAF cohorts (PA × SAF cohort); thus, both measurements were available for these subjects. The DNAm age cohort ($n = 47$) was composed of CKD G5 patients initiating dialysis or KT ($n = 23$) and population-based controls ($n = 24$).

Biochemical analyses

Venous blood samples were taken after an overnight fast at baseline (immediately before dialysis initiation for prevalent dialysis or before KT surgery for kidney transplantation patients). The Karolinska University Laboratory analysed concentrations of serum creatinine, serum albumin, high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), haemoglobin, leukocyte count,

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Table 1. *Variables and coefficients used to calculate phenotypic age (PA) and number of missing (imputed) values in our PA cohort (N = 333).*

Variable	Units	Weight	# Imputed
Albumin	g/L	−0.0336	0
Creatinine	μmol/L	0.0095	0
Glucose, serum	mmol/L	0.1953	113
C-reactive protein (log)	mg/dL	0.0954	0
Lymphocyte per cent	%	−0.012	182
Mean (red) cell volume	fL	0.0268	43
Red cell distribution width	%	0.3306	333
Alkaline phosphatase	U/L	0.0019	260
White blood cell count	1000 cells/μL	0.0554	0
Age	Years	0.0804	0
Constant		−19.9067	
Gamma		0.0077	

Source: Adapted from Levine et al. 2018 [22].

cholesterol, triglycerides, calcium, phosphate and intact parathyroid hormone (iPTH).

Framingham CVD risk score

We applied the Framingham CVD risk score to account for the following risk factors: age, sex, diabetes, systolic blood pressure, anti-hypertensive medication, total cholesterol, HDL-cholesterol and smoking [25, 26].

Phenotypic age

In 333 subjects (CKD G3–4, $n = 64$; CKD G5, $n = 191$; population-based control subjects, $n = 78$), PA was calculated based on chronological age and 9 blood biomarkers: albumin, serum glucose, creatinine, CRP (log), lymphocyte per cent, mean red blood cell (RBC) volume, RBC distribution width (RDW), alkaline phosphatase and white blood cell count [22]. In the case of missing data (Table 1), the mean value of the biomarker in each group was imputed. As RDW data were not available, the reference value (13%) for a normal population was used for all. To confirm that our imputation method did not introduce bias in our results, some of the analyses were replicated in a subset of patients excluding those who had more than two imputed parameters (restricted PA cohort, $N = 150$). PA was calculated according to the formula:

$$PA = 141.50225 + \frac{\ln(-0.00553 \times \ln(1 - MortalityScore))}{0.090165}$$

where *MortalityScore* is

$$MortalityScore = 1 - e^{-e^{xb}(\exp(120\times\gamma)-1)/\gamma}$$

and *xb* is the linear combination of each biomarker multiplied by its coefficient [22]. Table 1 lists all variables, coefficients and gamma.

Skin autofluorescence

SAF was measured at the time of blood draw in 199 CKD G5 patients (KT, $n = 87$; incident dialysis patients, $n = 62$; prevalent PD patients, $n = 50$) using the Autofluorescence AGE reader (DiagnOptics Technologies BV) as previously described [27]. By using a spectrometer, excitation light (wavelength between 300 and 420 nm) is reflected in the skin and measured as emitted fluorescence (wavelength between 420 and 600 nm), guarded from surrounding light. The ratio between the average intensity of the emitted light and the average intensity of the excitation light is calculated and multiplied by 100. The value is expressed in arbitrary units. Patients with dark skin or tattoos were not investigated. All measurements were performed at room temperature in a semi-dark environment. Biological age was calculated by the following formula [27–29]: SAF age = (SAF − 0.83)/0.024.

DNA methylation analysis

Patients and DNAm analysis have been described previously [7]. In brief, 11 patients undergoing either dialysis (peritoneal dialysis $n = 7$, haemodialysis $n = 4$) or KT ($n = 12$) were recruited

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from the Karolinska University Hospital's Departments of Renal Medicine and of Transplantation Surgery, respectively. In the KT group, 7 of the 12 patients received dialysis for 0.2–4 months before KT and 5 underwent pre-emptive KT. An age- and sex-matched control group of 24 individuals was drawn from the population-based cohort.

Whole blood samples were taken after an overnight fast from kidney failure patients at baseline (i.e. immediately before dialysis initiation or before KT surgery) and 1 year after KT or dialysis initiation. Blood samples from control subjects were only taken at baseline. DNA was isolated using QIAamp DNA Blood Maxi Kit (Qiagen), and DNA concentration and integrity were assessed by NanoDrop ND-1000 (NanoDrop). All procedures were performed in accordance with the manufacturer's protocols.

A fixed amount of DNA (500 ng) for each sample was bisulphite-treated using the EZ-96 DNA Methylation kit (Zymo Research). Whole-genome DNAm was measured using the Illumina Infinium HumanMethylation450K BeadChip (Illumina). All procedures were performed in accordance with the manufacturer's protocols. Beta values (per cent methylation at each locus) were calculated from the Illumina IDAT files using R v.4.0.0 (and RStudio v.1.2.5042 as interface) and following the method described by Maksimovic et al. with minor modifications [30].

DNAm age

DNAm age was calculated from the beta values using three previously described DNAm clocks: the Horvath, Hannum and PhenoAge clocks [20–22]. The Horvath DNAm age was calculated following the method described in the original article [20], including the recommended beta value normalization step. The Hannum and PhenoAge epigenetic ages were calculated following the same procedure, but replacing the list of CpG loci and coefficients, and skipping the beta value normalization step due to the necessary information being unavailable. A non-normalized output for the Horvath DNAm age was also calculated, yielding largely similar results to the normalized output, and was therefore excluded from the subsequent analysis. In addition, in order to reduce the statistical noise of each individual clock, a novel composite clock was evaluated and calculated for each sample as the average of the Horvath, Hannum and PhenoAge DNAm ages for that sample. For each DNAm clock, the age acceleration (difference between DNAm and chronological age) was also calculated. A positive age acceleration implies that a person is epigenetically older than their chronological age; vice versa, a negative age acceleration implies that a person is epigenetically younger than their chronological age [23].

Statistical analysis

Results are expressed as median and IQR unless otherwise indicated, with $p < 0.05$ considered statistically significant. *P*-values were not adjusted for multiple comparisons and are therefore intended as descriptive when comparing sets of clinical and biochemical characteristics between patient groups. Differences between the groups were analysed with the non-parametric Kruskal–Wallis analysis of variance (ANOVA). Comparisons between groups for nominal variables were made using the Chi-squared test. The patients were followed from the inclusion date until KT, death and the completion of 60 months of follow-up. The cause of death was established through a death certificate issued by the attending physician. Correlations between estimated PA and estimated SAF age compared to chronological age were evaluated by Spearman´s rank correlation test (rho). These statistical analyses were performed with SAS statistical software (Version 9.4; SAS Institute, Inc.) and Stata version 17.0 (Stata Corporation). Data analysis for DNAm age was carried out using Microsoft Excel 365, R v.4.0.0, and Minitab 20.3. *P*-values and adjusted *p*-values for differences between transplant, dialysis and control patients were calculated based on one-way ANOVA with Tukey's test. Normality of the age acceleration distribution was confirmed by the Shapiro–Wilk test. A paired *t*-test was used when comparing values for the same patients across two timepoints.

Results

Phenotypic age

We analysed PA in 333 subjects. Clinical and biochemical characteristics of the population-based control subjects and patients with CKD G3–4 and CKD G5 are shown in Table S1. The restricted PA cohort ($N = 150$) had similar overall characteristics but did not include any population-based controls (Table S2). Median age was 56 (IQR 47–66) years; overall, 56.5% were males, 24.0% had diabetes and 24.6% had CVD. Clinical and biochemical characteristics in relation to tertiles of estimated

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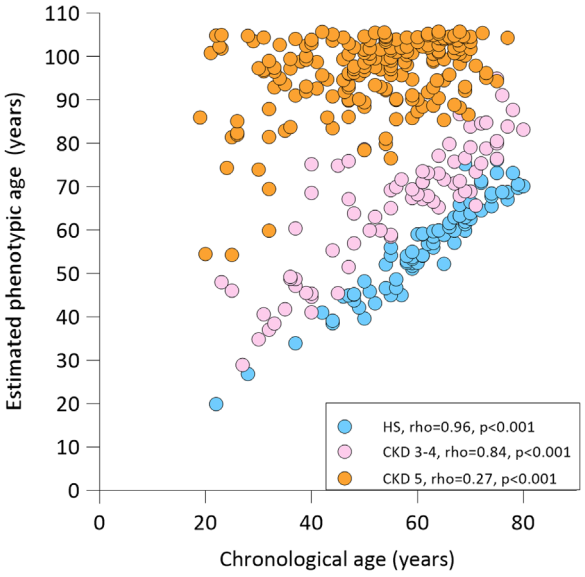

Fig. 1 *Estimated phenotypic age versus chronological age in population-based control subjects (HS, healthy subjects), n = 78 (blue); chronic kidney disease (CKD) G3-4 patients, n = 64 (pink); and CKD G5 patients, n = 191 (orange).*

PA are presented in Table S3. When subgroup analysis was performed (Fig. 1), associations between PA and chronological age in the control group (rho = 0.96; $p < 0.001$) and CKD G3–4 (rho = 0.84; $p < 001$) were found. By contrast, estimated PA in the CKD G5 group was very high, and the correlation with chronological age was low, but significant (rho = 0.27; $p < 0.001$) (Fig. 1). In univariate analysis (Table S3), higher PA was associated with lower hand grip strength (HGS), albumin, haemoglobin, estimated glomerular filtration rate (eGFR), HDL and higher systolic blood pressure, hsCRP, triglycerides, IL-6, iPTH ($p < 0.001$) and higher prevalence of diabetes mellitus (DM) and CVD ($p < 0.001$). All-cause mortality (adjusted for gender, CKD stage and DM) was lowest in the highest tertile of PA acceleration and highest in the middle tertile, though only the difference between middle and high tertiles was statistically significant (Shr = 2.96, $p = 0.002$) (Fig. 2). We observed similar results in the restricted PA cohort (Fig. S1).

SAF age

Table S4 presents clinical and biochemical characteristics of the patients in the SAF cohort ($n = 199$). Median age was 56 (43–66) years; overall, 65.3% were males, 20.6% had diabetes and 19.6% had CVD. Clinical and biochemical characteristics in relation to tertiles of estimated SAF age are presented in Table S5. The correlation between SAF age and chronological age in CKD G5 was moderate (rho = 0.48; $p < 0.001$) (Fig. 3). In the univariate analysis, higher SAF age was associated with higher chronological age, hsCRP, IL-6, Framingham risk score, prevalent DM and CVD and lower HGS and albumin (Table S5). We observed a significant correlation between PA and SAF age (rho = 0.18, $p = 0.02$) in the PA × SAF cohort ($N = 39$, Fig. 4), despite the unrealistically high age estimates generated by these two clocks.

Epigenetic age

Patient characteristics at baseline and after 1 year of KRT are shown in Table S6. There was no baseline difference in the burden of comorbidities between the KRT groups, measured by the Charlson Comorbidity Index ($p = 0.39$, data not shown). At baseline, the average chronological age of the control group was 49.7 (SD = 9.1), whereas the average DNAm age was 50.0 (SD = 8.7), 52.8 (SD = 9.6) and 44.7 (SD = 10.2) years according to the Horvath, Hannum and PhenoAge clocks, respectively. All three clocks showed good accuracy, with a median absolute error (MAE) in the control group of 4.1, 4.7 and 4.4 years for Horvath, Hannum and PhenoAge, respectively (Fig. 5), consistent with values previously reported [20–22]. The average composite DNAm age (calculated as the average of the Horvath, Hannum and PhenoAge ages) was 49.2 (SD = 9.2) years with an MAE of 3.6 years.

Figure 6 shows chronological versus DNAm age for each clock at baseline. We observed a weaker correlation between DNAm and chronological age at baseline in the KT group (0.31, 0.47, −0.05 and 0.27 for Horvath, Hannum, PhenoAge and composite ages, respectively) compared to both the dialysis (0.71, 0.86, 0.86 and 0.92) and the control group (0.80, 0.85, 0.89 and 0.88). All three clocks showed larger average age acceleration in the KT and dialysis groups compared to controls, although the difference was not statistically significant in the case of the Horvath clock (Fig. 7). The mean age acceleration difference between KT and control group was 1.6 (adj. $p > 0.5$), 4.5 (adj. $p = 0.025$), 8.5 (adj. $p < 0.001$) and 4.9 years (adj. $p = 0.005$) according to the Horvath, Hannum, PhenoAge and composite clock, respectively. In the dialysis group, the differences were 2.1 (adj. $p > 0.5$), 6.0 (adj. $p = 0.003$),

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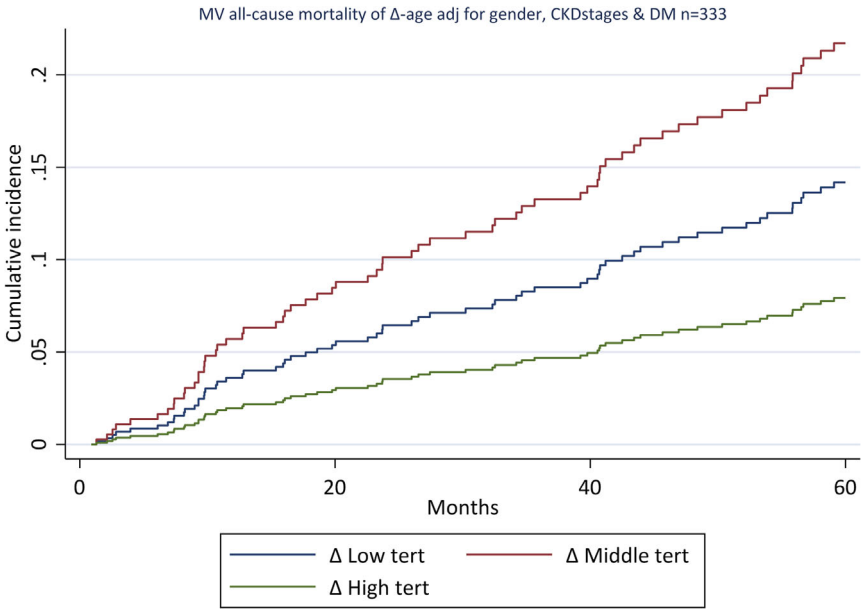

Fig. 2 *All-cause mortality in the phenotypic age (PA) cohort (N = 333), stratified by tertile of PA acceleration (delta age), adjusted for gender, chronic kidney disease (CKD) stage and diabetes mellitus (DM). S-HR compared to high tertile: low = 1.85 [0.57 – 5.94], p = 0.30; middle = 2.96 [1.47 – 5.94], p = 0.002.*

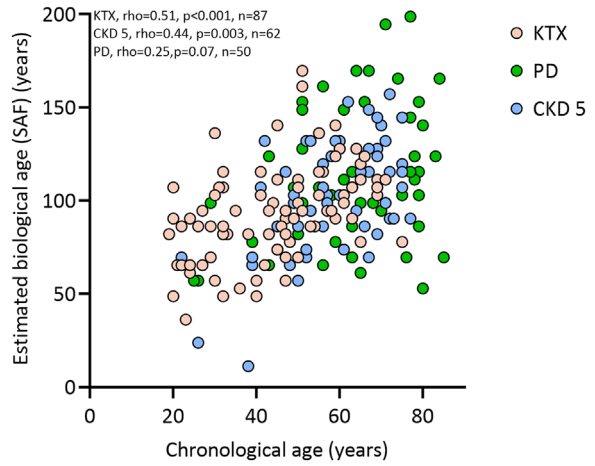

Fig. 3 *Estimated biological age (by skin autofluorescence [SAF]) versus chronological age in the SAF cohort, n = 199: kidney transplant recipients (KTX), n = 87; incident dialysis chronic kidney disease (CKD 5), n = 62; prevalent PD, n = 50.*

9.7 (adj. $p < 0.001$) and 5.9 (adj. $p = 0.001$) years, respectively (Fig. 7).

In the longitudinal part of the study, we studied the effect of KRT on age acceleration. We observed a statistically significant reduction in PhenoAge acceleration (−4.4 years, $p = 0.016$) and in composite age acceleration (−2.5 years, $p = 0.009$) 1 year after KT compared to baseline; there was a reduction also according to the Horvath and Hannum clocks, but these were not statistically significant ($p = 0.173$, $p = 0.158$, respectively). Notably, no significant differences were observed between the control group at baseline and the KT group 1 year after transplant, with only PhenoAge displaying a trend to significance (adj. $p = 0.076$) (Fig. 7). No significant changes in age acceleration were observed after 1 year of dialysis (0.4, −0.5, −0.7 and −0.3 years for Horvath, Hannum, PhenoAge and Composite age, respectively; $p > 0.6$). When comparing peritoneal dialysis versus haemodialysis patients, we observed no statistically significant difference between groups at any timepoint or with any clock, nor any change within either subgroup after 1 year. It should be noted that our sample size (PD $N = 7$, HD $N = 4$) would have only allowed the detection of a large difference. As expected, after 1 year, the KT group showed an improvement in key measures of kidney function (eGFR and serum creatinine, as well as serum phosphate and PTH), whereas the dialysis group did not.

Discussion

Our results support the concept that CKD promotes accelerated ageing, and recent studies report an association between poor renal function and age acceleration [31–34]. Indeed, CKD patients had the highest age acceleration in a

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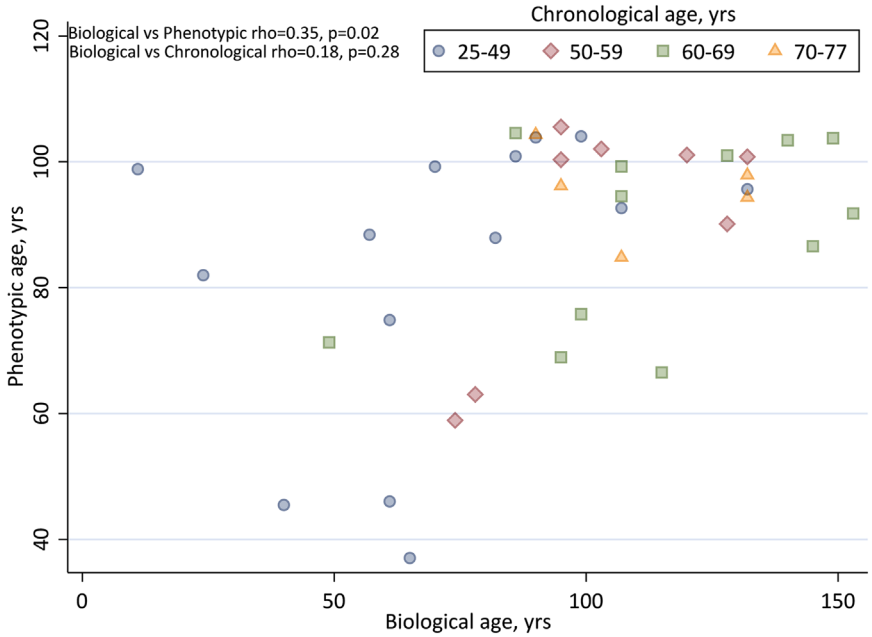

Fig. 4 *Estimated biological age (by skin autofluorescence [SAF]) versus phenotypic age in the subset of patients for which both were available, by chronological age group (N = 39).*

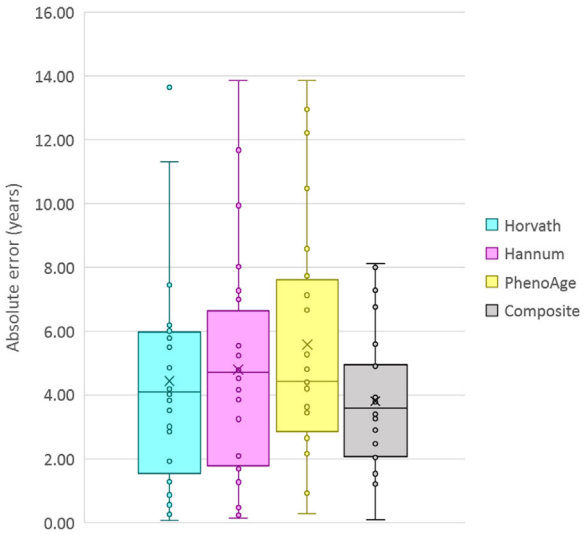

Fig. 5 *Box plot of the absolute errors (absolute difference between DNA methylation [DNAm] age and chronological age) for the control group according to each clock. The median absolute error (MAE) is between 4.1 and 4.7 years depending on the individual clock. The maximum error is under 14 years for all clocks. The composite clock has reduced error compared to each individual clock, with an MAE of 3.6 years and a maximum error of 8.1 years.*

study comparing 16 chronic diseases [34]. They also highlight the utility of DNAm clocks to assess this. As the uremic milieu promotes accelerated biological ageing, it is of interest to establish whether KRT slows down or reverses accelerated ageing. Identification of a robust biomarker for biological ageing may help nephrologists identify CKD patients that are on 'fast track ageing' and tailor the most appropriate nutritional, lifestyle and therapeutic interventions [34, 35]. Our study suggests that neither PA nor SAF alone are suitable biomarkers of ageing in advanced kidney disease, as they produce implausibly high estimates of biological age. The poor performance of PA in CKD patients is likely due to the large increase in serum creatinine observed in these patients, well outside of normal range, even for very old people. Indeed, we calculated that a creatinine increase from 70 to 700 μm (typical values in healthy controls and CKD G5 patients, respectively) causes a PA increase of 65 years. Our all-cause mortality analysis yielded inconsistent and counter-intuitive results, with both the upper and lower tertiles of PA acceleration showing reduced risk, suggesting that this biomarker may have limited predictive power after adjustment for confounding factors. The poor

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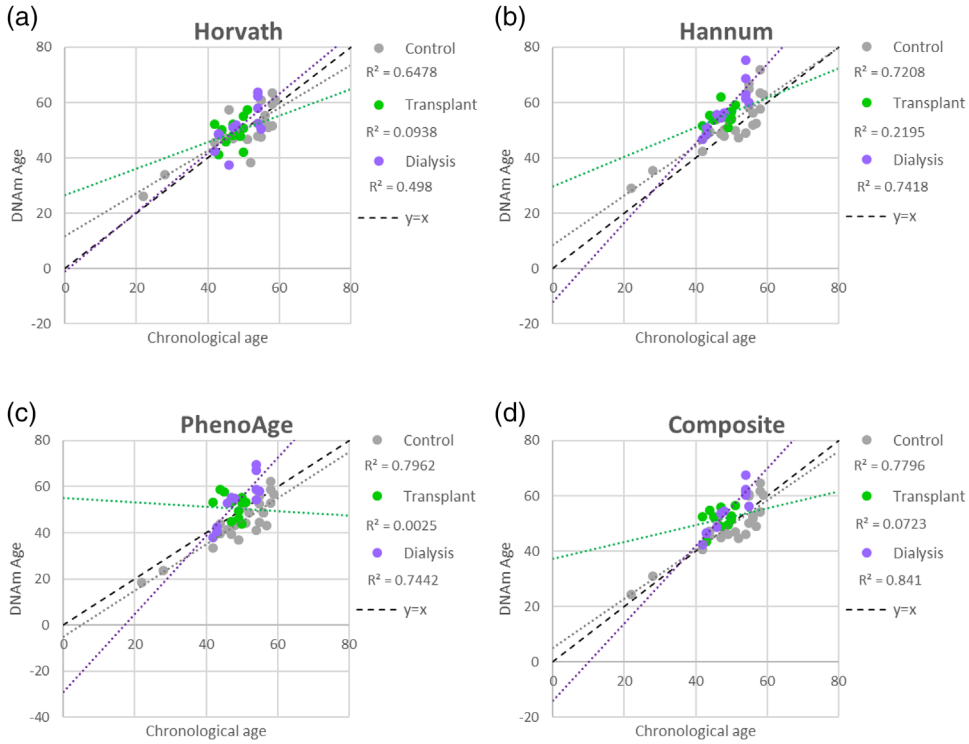

Fig. 6 *Estimated DNA methylation (DNAm) age compared to chronological age in the DNAm cohort at baseline according to the Horvath (A), Hannum (B), PhenoAge (C) and Composite (D) clocks. Dotted coloured lines represent linear regressions for each respective group.*

performance of the SAF clock is likely explained by the fact that AGEs accumulate at an accelerated rate in CKD patients as a result of metabolic stress, CVD, oxidative stress and uraemia, reaching levels beyond the 'normal' range [36, 37]. Interestingly, SAF has been found to be lower in KT recipients than in dialysis patients and comparable to CKD G3 patients, consistent with our DNAm clock findings and clinical observations of a partial, but not the complete amelioration of the disease following KT [38]. Thus, although both PA and SAF correctly indicate that CKD patients are at increased risk of morbidity and mortality, they arguably overestimate the magnitude of this risk increase as a result of their focus on cardiovascular health biomarkers, which are more impaired in CKD than other physiological functions. We found PA and SAF age to correlate with each other better than with chronological age. The clear link between CKD stage and PA indicates that it would be possible to use PA to estimate kidney biological age or function. Construction and validation of such a tool are beyond the scope of this pilot study. In addition, given that a sufficient measure of kidney function already exists (eGFR), it is not clear that such a tool would have a meaningful contribution in terms of risk prediction and patient stratification on top of eGFR. A previous study using an ageing clock based on inflammatory/immunological markers in blood (ipAGE) has similarly obtained large age acceleration estimates and a weakened correlation between biological and chronological ages compared to controls [33]. However, it should be noted that in our cohorts, there is still a correlation between biological and chronological ages after adjusting for CKD stage, as well as association with several clinical parameters. Thus, these biomarkers may have utility after appropriate adjustment, for example, for CKD stage.

DNAm clocks, on the other hand, appear to perform better in the context of CKD. The DNAm-based PhenoAge clock produces more biologically plausible and clinically meaningful values than the blood biomarker-based PA, upon which PhenoAge itself is trained. This suggests that DNAm may be a

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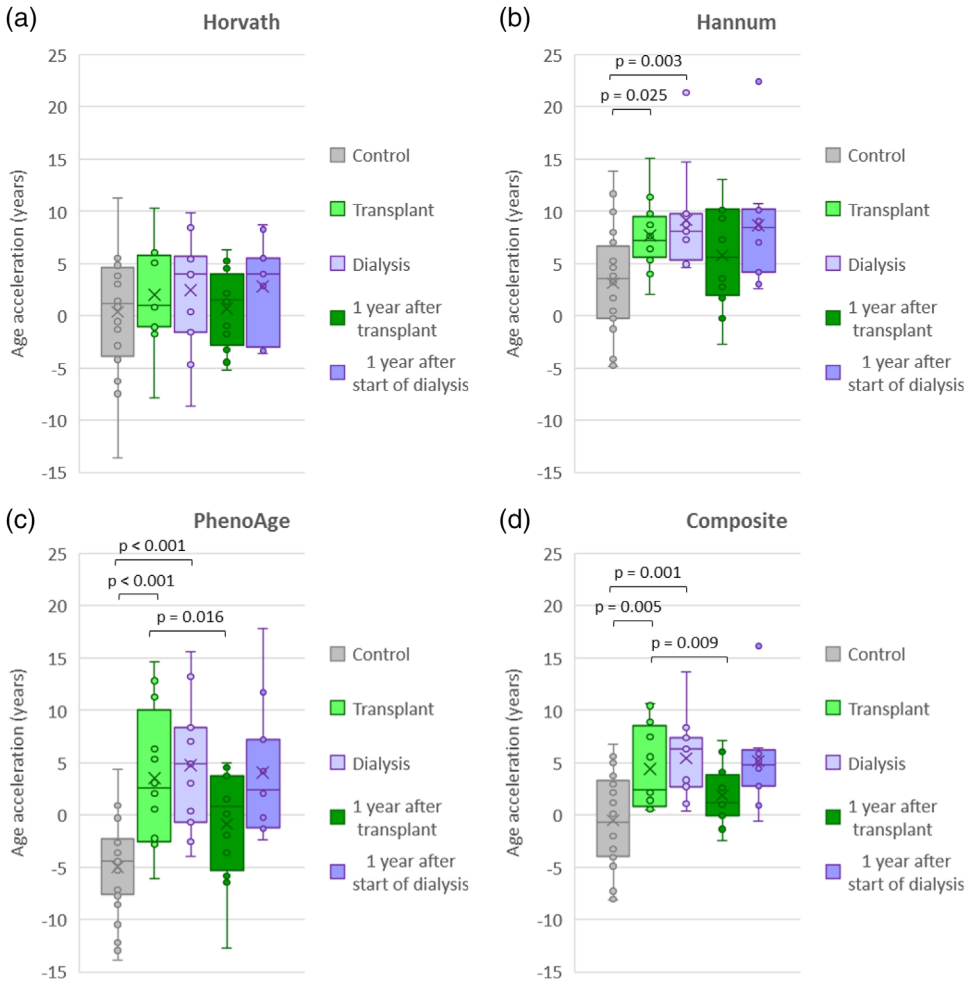

Fig. 7 *Box plot of age acceleration by treatment group and timepoint according to the Horvath (A), Hannum (B), PhenoAge (C) and Composite (D) clocks. Both the transplant and the dialysis groups have increased age acceleration compared to controls, with this difference being statistically significant in all cases except the Horvath clock. All clocks show a reduction in age acceleration at year 1 compared to baseline in the transplant group, but this difference reaches statistical significance only in the PhenoAge and composite clocks. There is no significant reduction of age acceleration in the dialysis group. P-values calculated from analysis of variance (ANOVA) with Tukey's test or paired t-test.*

superior biomarker for the purpose of biological age estimation in CKD and is consistent with previous research that found PA acceleration to be larger than PhenoAge acceleration in ESKD [33]. By using three different epigenetic clocks (Horvath, Hannum and PhenoAge), as well as a composite average of the three, we confirm that ESKD patients display epigenetic age acceleration. The DNAm age acceleration values are both biologically plausible and not dissimilar to previously reported values [20–22]. The weaker correlation that we observed between DNAm and chronological age in the KT group may be due to the limited sample size and the reduced range of chronological ages in the KT group compared to the other two.

We also report that the age acceleration is reduced 1 year after KT. Our results are consistent with clinical observations that KT leads to greater physiological improvement, better quality of life and increased life expectancy compared with dialysis. They are consistent with a recent publication reporting increased age acceleration upon physiological stress and amelioration of this age acceleration after recovery [39]. As vascular calcification and immune ageing are not reversed by KT, this

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may contribute to why we observe a limited amelioration of the age acceleration in the KT group [31, 40, 41]. Importantly, KT recipients have reduced life expectancy compared to the general population [12]. Therefore, either the DNAm clocks we used do not fully capture biological ageing, or there are additional risk factors in CKD that are not abolished by KT and are not part of the biological ageing process per se. Dialysis treatment for 1 year, on the other hand, did not have a significant effect on age acceleration. This may be due to treatment-associated increase in inflammation and oxidative stress [2, 4]. It is worth noting that, in spite of differences in effect size, statistical significance and the limited sample size, all DNAm clocks showed the same core results: increased age acceleration in CKD patients and reduced age acceleration after 1 year of KT, but not after 1 year of dialysis.

There are limitations in our study that are worth noting. First, we encountered a relatively large number of missing values in our dataset when calculating PA, including one parameter that was missing entirely (RDW). Therefore, we could only compute an approximation of this ageing clock. Greater RDW has been associated with reduced kidney function and CKD stage progression [42, 43]. Therefore, our estimate of PA for the CKD patients is likely an underestimate of the values we would have obtained if RDW values had been available. In addition, even an RDW of 0%, the lowest theoretically possible value and biologically highly implausible, would only reduce PA by 47 years, still not sufficient to explain the 80+ years of PA acceleration measured in some of the younger CKD G5 patients. Notably, our calculated PA accelerations for the population-based controls were reasonably small, in spite of the fact that all of these subjects had at least three imputed parameters; this confirms that the large PA accelerations observed in the CKD subjects were not due to missing values.

Second, it remains to be determined if changes in DNAm are causal for the ageing process or simply reflect changes in the underlying process [17]. Third, even though we did not find any difference in the baseline Charlson Comorbidity Index between patients receiving dialysis versus KT, there might be residual unaccounted bias. Further research, ideally with larger sample size or additional/longer timepoints, would be beneficial in order to confirm our results. Our findings strongly concur with our previous report indicating that acceleration of biological age in CKD, as determined using telomere length, was mitigated by KT but not by dialysis [31]. We have also previously highlighted a possible interplay among telomere attrition, folate and homocysteine levels and immunosuppressant drug treatment [31]. The role of folate (and other methyl donors such as betaine) could be especially relevant to DNAm, given its role in nucleotide biosynthesis and as a methyl donor, as well as the fact that haemodialysis patients can suffer from low serum levels due to insufficient intake or increased loss caused by the dialysis [44]. Thus, it would be of interest to further explore the possible roles of diet and different immunosuppressant treatments with respect to epigenetic age in CKD. An additional limitation of DNAm clocks is the relatively high degree of variability between technical replicates [45]. Thus, although DNAm clocks can yield useful results when comparing large enough groups, they are still poorly suited for use on individuals, unless improved or paired with additional diagnostic criteria [15, 45]. Finally, the fact that we tested different clock types in different patient cohorts prevented us from being able to directly compare these clocks.

In conclusion, our study confirms accelerated ageing in patients with kidney failure by all used methods in the study. KT, but not dialysis, appears to partially mitigate this age acceleration. Given the opportunities of a robust estimation of biological age in ESKD, further studies are warranted.

Author contributions

Peter Stenvinkel and Paul G. Shiels conceived and designed the study. Clinical and biochemical data were collected and compiled by Helen Erlandsson, Anna Witasp, Louise Nordfors, Ken Iseri and Hokuto Morohoshi. Ognian Neytchev, Colin Selman and Paul G. Shiels conducted DNAm clock analysis. Ognian Neytchev and Abdul Rashid Qureshi performed statistical analysis. Ognian Neytchev, Helen Erlandsson, Peter Stenvinkel and Paul G. Shiels wrote the manuscript, and all authors contributed to and revised the manuscript.

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Conflict of interest statement

PS has received consultancy fees, research grants and speaker's honoraria from Baxter Healthcare, Astra Zeneca, Vifor, Fresenius Medical Care, Pfizer, Astellas, Novo Nordisk and Bayer. HE received funds from ALF and consultancy fees from TEVA. PGS has received consultancy fees and Industrial PhD Partnership grants from Mars UK Ltd, 4D Pharma and Constant Pharma. TE has received grants from Karolinska Institutet Research Foundation, Novo Nordisk postdoctoral fellowship and Astra Zeneca. KK received research grants from the Swedish Research Council. The other authors declare no conflicts of interests.

Data availability statement

Data can be shared upon request.

Ethics statement

The studies were approved by the Swedish Ethical Review Authority in Stockholm and adhered to the Helsinki and Istanbul declarations.

Patient consent statement

All subjects included in the study were >18-year old and provided written informed consent.

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