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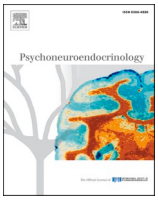

HPA-axis dysregulation is not associated with accelerated epigenetic aging in patients with hypersexual disorder

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ABSTRACT

Background: Hypersexual disorder (HD) - a nonparaphilic sexual desire disorder with impulsivity component - was evaluated for inclusion as a diagnosis in the DSM-5 and the diagnosis compulsive sexual behavior disorder is included as an impulse control disorder in the ICD-11. Hypothalamic-pituitary-adrenal (HPA)-axis hyperactivity is believed to affect cellular senescence and has been implicated in HD. No previous study investigated HD or HPA-axis dysregulation in relation to measures of epigenetic age (EA) acceleration.

Methods: This study reports on a case-control study set-up from a well-characterized cohort, contrasting EA predictors in relation to 60 HD patients and 33 healthy volunteers (HV) and 19 mixed HD/HV exhibiting dexamethasone suppression test (DST) non-suppression to 73 mixed HD/HV DST controls. The genome-wide methylation pattern was measured in whole blood from 94 subjects using the Illumina Infinium Methylation EPIC BeadChip and preprocessed according to specialized protocols suitable for epigenetic age estimation. The online DNAm Age Calculator (https://dnamage.genetics.ucla.edu/) was implemented to retrieve various EA predictors, which were compared between the in-silico generated subgroups.

Results: Quality control analyses indicated strong correlations between the EA measure DNA methylation GrimAge (DNAm GrimAge – the EA clock most reliably associated with mortality risk) and chronological age in all sub-groups. The study was adequately powered to detect differences of 2.5 and 3.0 years in DNAm GrimAge minus age in relation to both HD and HPA-axis dysregulation, respectively. Baseline DNAm GrimAge exceeded chronological age by 2.8 years on average across all samples. No EA acceleration marker was associated with HD or DST suppression status ($p > 0.05$).

Conclusion: EA acceleration markers shown to be strongly predictive of physiological dysregulation and mortality-risk, are not related to HD or DST non-suppression status (measured after 0.5 mg dexamethasone). The independency of HPA-axis dysregulation to EA acceleration does not support the biological relevance of this dosage-regimen when applied to patients with HD. These findings do not support the notion of accelerated cellular senescence in HD. Studies stratifying DST non-suppressors according to established dosage-regimens in somatic settings are needed to fully elucidate the putative contribution of HPA-axis dysregulation to EA.

1. Introduction

Hypersexual disorder (HD) is a diagnostic concept originally suggested for inclusion in the Diagnostic and Statistical Manual of Mental Disorders: DSM-5, describing a nonparaphilic sexual desire disorder with an impulsivity component. It entails persistent and excessive

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sexual behavior associated with distress and functional impairment, loss of control and engagement in repetitive sexual activity as a response to dysphoric mood states and stressful life events (Kafka, 2010). Although HD was ultimately not included in the DSM-5 (Kafka, 2010), Compulsive Sexual Behavior Disorder (CSBD) is a similar diagnostic entity included as an impulse control disorder in the eleventh edition of the International classification of Diseases (ICD-11)(ICD-11 for Mortality and Morbidity Statistics., 2022). Prevalence rates of HD of 3–6% have been reported, with men strongly overrepresented amongst those afflicted (Chatzittofis et al., 2022). Commonly occurring comorbid conditions include mood and anxiety disorders, substance use, and impulse control disorders (Chatzittofis et al., 2022). Sexual desire dysregulation, impulsivity, addiction and compulsivity have been proposed as important aspects of pathophysiology in different conceptualizations of HD (Chatzittofis et al., 2021).

In a recently published review on the neuroendocrinology of CSBD, Chatzittofis et al. highlight the importance of three neuronal circuits for the understanding of compulsive sexual behavior; the hypothalamic-pituitary-gonadal (HPG) axis, the hypothalamic-pituitary-adrenal (HPA) axis and the oxytonergic system (Chatzittofis et al., 2022). The associations between androgens produced by the HPG-axis and sexual behavior have received substantial previous interest (Jordan et al., 2011). A study on HPG-axis functioning in men with HD showed no differences in plasma testosterone in comparisons to controls. However, men with HD had elevated levels of luteinizing hormone (LH), an important regulator of HPG-axis hormone, although LH-levels were still in the reference range in the HD-group, limiting generalizability of results (Chatzittofis et al., 2020).

HPA-axis activity is assessed by administering the steroid medicine dexamethasone (DST) in the evening, followed by a subsequent measurement of plasma cortisol in the morning. Dexamethasone normally exerts an inhibitory influence on cortisol secretion. Thus, elevated levels of cortisol in the morning after consumption (non-suppression) is considered indicative of a hyperactive HPA-axis. As a clinical tool, the DST is used to diagnose Cushing's disease, but has also been applied in research of associations between hypercortisolism and various psychiatric diagnoses (Lindholm, 2014). Alterations in functioning in the HPA-axis, demonstrated by the dexamethasone test (DST), has been previously associated with several psychiatric conditions such as borderline personality disorder (Carrasco et al., 2007), suicidality, depression and addiction (Sher, 2006; Jussi Jokinen et al., 2007). In regard to the latter, research has indicated that substance abuse can cause hyperactivity in the HPA axis, suggested to play a role in maintaining addictive behavior by increasing cravings and relapses (Koob et al., 2014). Analogously to this, it has been demonstrated that HD is associated with higher likelihood of non-suppressor status on the DST, and elevated levels of adrenocorticotropic hormone, independent of potential cofounders (childhood trauma and PTSD), suggesting an association between HD and HPA-axis hyperactivity (Chatzittofis et al., 2016). Reduced DNA-methylation levels at a CpG-site related to corticotropin-releasing factor (CRH) have also been demonstrated in relation to HD and data from an independent control group showed a positive relationship between this methylation pattern and CRH gene expression, indicative of an influence on HPA-axis activity (J. Jokinen et al., 2017). Interpretation of these studies are, however, complicated by the lack of consensus on dexamethasone dosage-regimens to reliably measure HPA-axis dysregulation in psychiatric cohorts and the methodological heterogeneity amongst studies purporting to identify such subjects in psychiatric populations (DST dosages used for other psychiatric disorders range from 0.25 mg (Carrasco et al., 2007) to 1.5 mg (Schubert et al., 2019)). Notably, previous studies examining the neurobiological underpinnings of CSBD in the vast majority pertain exclusively to male subjects, and may thus not be generalizable to female populations (Chatzittofis et al., 2022).

Research with populations with HD have also shown alterations in the activity of the oxytocinergic system. In a study evaluating effects of a psychological treatment, plasma oxytocin levels were shown to be elevated in men with HD and positively correlated with symptom severity. Further strengthening these findings, Cognitive Behavior Therapy (CBT) completers had reduced oxytocin levels and a positive correlation was found for reductions in plasma oxytocin and HD symptoms post-treatment (Flanagan et al., 2022) Furthermore, a genome-wide study of methylation patterns in subjects with HD showed changes in methylation on CpG sites associated with a decreased transcription of a microRNA with putative effects on the oxytocin signaling system (Boström et al., 2020). One suggested explanation for the association between increased oxytocinergic activity and HD is the modulatory role increased oxytocinergic activity can play in the context of HPA-axis activity, as shown in several previous studies (Cera et al., 2021).

A recently introduced method to study the impact of intrinsic and extrinsic factors on health is the concept of epigenetic aging (EA) (Liu and Zhu, 2021), utilizing DNA-methylation data to predict biological age. EA has been shown to outperform chronological age in predicting physiological dysregulation and time-to-death. Different methodologies exist to measure age related changes to the epigenome, with intrinsic epigenetic age acceleration (*IEEA*) and extrinsic epigenetic age acceleration (*EEAA*) commonly used, differing in that the latter incorporates blood cell proportions as well as cell-intrinsic measures in the calculation of epigenetic age (EA) (Chen et al., 2016). Methylation data has also been used to estimate telomere length, an indication of biological strain – DNA methylation telomere length (*DNAmTL*). Measurements of EA describe a subjects "biological age" and is directly interpreted as a number corresponding to biological age, whereas epigenetic age acceleration (EAA) assesses the biological aging process in relationship to a subjects chronological age. Correspondingly, age-adjusted DNA methylation telomere length (*DNAmTLadjAge*) relates a subject's telomere length to chronological age. These methods have been described in greater detail elsewhere (Beynon et al., 2020). Several composite measures, so called "epigenetic clocks" have been previously presented (*Horvath's Clock*, *Hannum Age*, *DNAmPhenoAge*). Several of these have been evinced to be predictive of chronological age, morbidity and excess mortality (Zuyun Liu, 2021). However, the predictors show weak correlations amongst themselves, suggesting that each estimator of biological aging measures a different aspect of a pluralistic aging process. Lu et al. recently presented the DNA methylation clock GrimAge (*DNAm GrimAge*). This measure was formed by consolidation of surrogate DNA methylation levels used to estimate plasma protein levels, previously presented as a powerful predictor of lifespan. DNAm GrimAge has shown superiority to other measures of epigenetic aging in predicting time-to-death, time-to-cancer and time-to-coronary disease, as well as strong relationships to measures of excess visceral fat and a comorbidity index. DNA methylation GrimAge age acceleration (*GrimAgeAccel)* – a measure of accelerated aging obtained by adjusting DNAm GrimAge to chronological age – has demonstrated high predictive power in regards to ageing-associated diseases including physical functioning, time-to-coronary heart disease, time-to-congestive heart failure, hypertension and type 2 diabetes mellitus (Lu et al., 2019).

Currently, there are no studies of biological aging in individuals afflicted by HD. Considering the above-described comorbid symptomatology and putative associations to HPA-axis hyperactivity, it seems reasonable to suspect altered biological aging processes in HD. In addition, associations between HD, health-related behaviors and events (e.g. smoking, substance use, contraction of sexually transmittable disease) and decreased subjective satisfaction with health have been previously demonstrated (Långström and Karl Hanson, 2006), further underscoring the potential relevance of HD to somatic health status and biological aging processes. Thus, the purpose of the current study was to compare EA in subjects with HD to healthy controls and investigate the relationship of EA to HPA-axis dysregulation using a 0.5 mg dosage-regimen in individuals with HD. Post-hoc analyses pertaining exclusively to male subjects were performed, to account for the relative

scarcity of research in support of the above discussed neurobiological underpinnings in female subjects.

2. Methods

2.1. Study population and ethics

Detailed descriptions of the samples included in this study has been previously published (Chatzittofis et al., 2016; JussiJokinen et al., 2017; Boström et al., 2020; Chatzittofis et al., 2021). The Regional Ethical Review Board in Stockholm authorized the study under Dnrs: 2013/1335–31/2. Informed consent was provided in writing from all study subjects. The study included both HD patients and healthy volunteers. In brief, 74 adult patients seeking treatment (medical or psychotherapeutic) at the Center for Andrology and Sexual Medicine (CASM) were recruited in the HD arm. HD participants were included if fulfilling proposed DSM-5 criteria for HD (Kafka, 2010). Exclusion criteria included, among others, ongoing substance use, psychotic disorder, serious somatic disorders (i.e., severe renal or hepatic disease) or other psychiatric disorders requiring immediate attention (i.e., schizophrenia, bipolar disorder, substance withdrawal or major depressive episode with high suicide risk). Psychiatric diagnoses were evaluated by a structured clinical interview (Mini International Neuropsychiatric Interview; MINI) and face-to-face interviews with qualified psychiatrists and psychologists. In the control arm, the Karolinska Trial Alliance (KTA) database was utilized to recruit 39 healthy volunteers, paired with HD samples according to age and time of blood collection (spring or fall). Exclusion criteria for this arm of control subjects included positive screening for pedophilic disorder, first-degree heredity for any serious mental disorder (schizophrenia, bipolar disorder or completed suicide), experiences of previous severe traumatic events or exhibiting any past or ongoing psychiatric disorder. The following assessments were performed on all study participants (HD and healthy volunteers): MINI 6.0, the self-rating version of the Montgomery-Åsberg Depression Rating Scale (MADRS-S), the Childhood Trauma Questionnaire (CTQ), the Sexual Compulsivity Scale (SCS) and the Hypersexual Disorder Screening Inventory (HDSI)(Chatzittofis et al., 2016) The dexamethasone suppression test (DST) was conducted by oral administration of 0,5 mg dexamethasone at 23:00 p.m. Post DST blood samples were collected at 08:00 a.m. the next day and immediately analyzed at Karolinska University Hospital using a chemiluminescence immunoassay with sensitivity for cortisol 15 nmol/l with normal range 200–800 nmol/l. Inter assay and intra assay coefficients of variation were 1.3% and 1.5%. Participation of patients and controls were scheduled in order to achieve equal distribution of both groups between spring and fall (Chatzittofis et al., 2016).

2.2. Blood sample collection, DNA methylation profiling and preprocessing

Participants were non-fasting at the collection time of blood samples, which took place in morning and by routine procedures. DNA was recovered and bisulfite converted (EZ DNA Methylation – GoldTM kit (ZymoResearch, USA)) from 94 subjects. Bisulfite converted DNA was hybridized to the Illumina Infinium Methylation EPIC BeadChip and imaged by Illumina iScan system (Illumina, San Diego, CA, USA). Preprocessing of DNA methylation specimens adhered to provided recommendations for EA estimation as has been conducted in previously published EA studies (Roshandel et al., 2020). Quality control and normalization of methylation IDAT files were performed in using the R package meffil (https://github.com/perishky/meffil/). One sample was identified as an outlier in predicted median methylated vs unmethylated signal and was excluded from the analysis. No outlier samples were identified by sex prediction or detection. Thus, 93 subjects remained to be included in the subsequent analysis.

2.3. DNAm age calculation

The online DNAm Age Calculator (https://dnamage.genetics.ucla.edu/) was implemented to retrieve various measures of epigenetic aging from subject DNA methylation signatures. This tool was designed for the Illumina 450k platform for measuring DNA methylation, which differ slightly from the array used in the present manuscript (EPIC BeadChip). Specifically, 2562 EA-specific DNA methylation probes are unaccounted for on the EPIC BeadChip, resulting in 27,253 methylation probes being uploaded to the site for the 'Advanced analysis' with 'Normalization' in addition to data pertaining to subject age, gender and sampled tissue (whole blood). The procedure of using Illumina EPIC BeadChip data for this analysis has been deemed reliable by the authors of the online web tool and EA studies using this approach has also been previously published (Roshandel et al., 2020). Intrinsic EA acceleration (IEAA and IEAAHannum) and extrinsic EA acceleration (EEAA, AgeAccelPheno and AgeAccelGrim – the two latter based on independent estimators (Zuyun Liu, 2021) were thus retrieved for all 93 samples. Several additional measurements of EA acceleration were also extracted (i.e., IEAA, DNAmTLadjAge and GrimAgeAccel). Measurements of EA correspond to the assessed 'biological age' of the subject and can be directly interpreted in number of years, whereas EA acceleration estimates can be interpreted as assessing the dynamic quality of EA in that a positive value (exceeding zero) implicates accelerated aging and vice versa. DNAmTL corresponds to telomere length. As such, these measures are to be interpreted as inverse to the other EA acceleration clocks (i.e. positive value implicating deccelerated aging and vice versa). The above referenced epigenetic age predictors have been previously described in great detail (Beynon et al., 2020).

2.4. Statistical analysis

Chronological age and all EA predictors were each tested for normality by Shapiro-Wilk tests. In these analyses, assumption of normality could not be supported in the case of chronological age, or the following meaurements of EAA described in greater detail elsewhere (Beynon et al., 2020): DNAm GrimAgeAdjAge, DNAm PhenoAgeAdjAge, AgeAccelGrim and AgeAccelPheno. To allow for normality, these measures were all subjected to Blom-transformation. Shapiro-Wilk tests supported the assumption of normality for all other included EA predictors. The validity of key EA predictors was investigated by studying Pearson correlations, assessing associations between chronological age and several EA estimators (in the subsequent analyzes, also separately for the full cohort and each group). For the full study sample (including both HD and healthy volunteers), all demographic and clinical variables presented in Table 1 were investigated for an association with key EA predictors (EEAA, IEAA, IEAA.Hannum, DNAmTLAdjAge, AgeAccelPheno, AgeAccelGrim) by Pearson correlations for continuous variables and Welch two sample *t*-tests for dichotomous variables.

Participants were stratified into two groups depending on their clinical characteristics (HD patients and healthy volunteers) (Boström et al., 2020) and, subsequently, depending on their DST suppression status (Chatzittofis et al., 2021). The below described method was hence implemented in the same manner for each group stratification, separately. Clinical variables were assessed by Shapiro-Wilks tests and evaluated for between-group differences by Welch two sample *t*-tests, Mann-Whitney U-tests and chi-squared tests. Detailed descriptions of this analysis has been previously published (Boström et al., 2020). Mean difference between GrimAge and chronological age was visualized in violin plots, separately for each group. To assess and communicate power to detect meaningful differences in EA between HD and healthy volunteers, we made use of the R-function 'power.t.test'. As no clinical variable correlated significantly ($p > 0.05$) with the EA acceleration predictor of interest (EEAA, IEAA, IEAA.Hannum, DNAm TLAdjAge, AgeAccelPheno, AgeAccelGrim) potential differences in EA predictors between the groups were compared by Welch two sample *t*-tests.

Table 1
Clinical characteristics of patients with hypersexual disorder and healthy volunteers.

	Patients	Healthy volunteers	Statistics (t-test, Kruskall-Wallis, Chisq. test), p value
N	60	33	
Age (years)	39.4 (11.9)	37.4 (11.3)	*ns*
Men:Women, (n (%))	54 (90.0): 6 (10.0)	33 (100.0): 0 (0.0)	*ns*
Scandinavian descent (n (%))*	43 (71.7)	23 (69.7)	*ns*
Diagnosis depression (n (%))	9 (15.0)	0 (0.0)	**4.83E-02**
DST non-suppressors (n (%))	16 (26.7)	3 (9.1)	5.78E-02
CTQ Total	40.42 (11.89)	32.85 (9.39)	**2.22E-04**
TSH (mE/L)/T4 (nmol/L)	0.019 (0.0098)	0.027 (0.033)	*ns*
HBA1C (mml/mol)	32.97 (5.70)	32.82 (3.96)	*ns*
Cortisol (nmol/L)	467.85 (132.33)	474.49 (148.32)	*ns*
DST Cortisol (nmol/L)	100.4 (101.3)	62.54 (48.54)	*ns*
ACTH (pmol/L)	6.36 (3.05)	5.82 (2.99)	*ns*
DST ACTH (pmol/L)	2.11 (1.58)	1.26 (0.90)	**8.10E-03**
Testosteron (nmol/L)	14.02 (5.86)	14.24 (4.37)	*ns*
TNF-alpha (ng/L)	7.26 (1.83)	5.84 (2.41)	**4.39E-06**

Values are shown as mean (SD) unless otherwise specified. P-values were calculated by means of unpaired t-tests, Kruskall-Wallis' test or chi-squared tests, contrasting values for patients with hypersexuality disorder and healthy volunteers. A one-tailed p-value < 0.05 was considered significant.
*As a proxy for ethnicity, subjects born in a Scandinavian country (e.g., Denmark, Finland, Iceland, Norway or Sweden) and with biological parents born in the same country, were stratified as being of 'Scandinavian descent'.
Abbreviations: CTQ, childhood trauma questionnaire; DST, dexamethasone suppression test; ACTH, ACTH levels after the dexamethasone suppression test; DST Cortisol, cortisol levels after the dexamethasone suppression test; DST non-suppressors, non-suppression status defined as DST cortisol levels > 138 nmol/l

All demographic and clinical variables presented in Table 1 (including, for example, depression status) were again investigated for an association with key EA predictors (EEAA, IEAA, IEAA.Hannum, DNAm TLAdjAge, AgeAccelPheno, AgeAccelGrim) in each respective group (HD or healthy volunteer) by Pearson correlations for continuous variables and Welch two sample *t*-tests for dichotomous variables. Given the relatively small sample size and large number of potential covariates for multivariate analyses, a calculation of optimal co-variates was conducted to reduce the risk of overfitting the model from including too many co-variates. On the association analysis between EA and EA acceleration to group, clinical variables with near-significant between-group differences ($p < 0.10$) were incrementally added to a binary logistic regression model of the variable of interest (EA or EA acceleration). To deduce whether a single co-variate significantly improved the model, each co-variate was incrementally tested in using the R "anova" command. Co-variates were regarded as 'optimal' if their addition to the regression model resulted in a better model fit ($p < 0.05$) (Boström et al., 2020; Zaghlool et al., 2015). In the case of the HD stratification, the best model included the following co-variates: depression (Yes/No), DST suppression status (suppressor vs non-suppressor), CTQ total score and tumor necrosis factor alpha (TNF-alpha). Implementing the same analysis on the DST stratified groups did not result in any co-variate which significantly improved the model. Between-group differences in EA and EA acceleration predictors were thus evaluated by both univariate and multivariate analyses (binomial logistic regression models including optimal co-variates) in the case of HD group stratification, and only by univariate analyses in the case of the DST group stratification. To avoid multi-collinearity from highly correlated EA markers and over-fitting of the binomial logistic regression model, each individual EA and EA acceleration marker was assessed separately in relation to HD group stratification taking the optimal co-variates into account - resulting in several independent regression models being run. Lastly, post-hoc analyses pertaining exclusively to male subjects were performed by excluding 6 female subjects and performing identical analysis to that described above (evaluating between-group differences in EA and EA acceleration predictors).

3. Results

3.1. Baseline descriptives: HD patients vs healthy volunteers

The sample included 60 HD subjects and 33 healthy volunteers. There were in the majority male subjects (90% and 100%, respectively). There were no significant ($p > 0.05$) between-group differences with regards to age, gender, DST suppression status, TSH, HbA1C, baseline and post-DST plasma cortisol, or baseline plasma ACTH levels. The HD group exhibited higher frequencies of depressed cases (15% vs 0%), greater scores on the CTQ, higher post-DST plasma ACTH levels and higher TNF-alpha levels (Table 1).

3.2. Baseline descriptives: DST suppressors vs non-suppressors

This group stratification pertained to 19 DST non-suppressors and 73 control subjects (DST suppressors). Aside from DST associated metrics, there were no significant between-group differences in any of the clinical variables, including depression status. DST non-suppressors exhibited nominally higher frequencies of HD patients (85% vs 58.9%), albeit not reaching the threshold for unadjusted statistical significance ($p = 0.0578$).

3.3. Interrelatedness between epigenetic clocks and their association to chronological age

For the full cohort (including all 93 samples), there was a significant correlation of moderate strength between chronological age (blom-transformed) and Horvath Age ($r = 0.630, p < 0.001$), Hannum Age ($r = 0.626$, $p < 0.001$) and DNAmTL ($r = -0.558$, $p < 0.001$). DNAm GrimAge was very strongly correlated to chronological age ($r = 0.917, p < 0.001$). In the subgroup analyses, DNAm GrimAge demonstrated very strong correlations with age in all subgroups (HD patients, healthy volunteers, DST-suppressors, and DST non-suppressors)($r_{absolute} > 0.9, p < 0.001$), indicating a valid high accuracy of this epigenetic estimator. All EA predictors were very strongly correlated with chronological age in HD patients ($r_{absolute} > 0.8$, $p < 0.001$), but these were, except for DNAm GrimAge, weakly correlated to age in the group of healthy volunteers ($r_{\text{absolute}} < 0.25$, $p > 0.05$). Excluding DNAm GrimAge, all EA predictors were strongly correlated to age in the DST suppressor group ($r_{absolute} > 0.7, p < 0.001$), and strongly to moderately correlated in the non-suppressor group ($r_{absolute} > 0.51, p < 0.001$)(Figs. 1 and 3).

In the full cohort, AgeAccelGrim demonstrated a moderate strength correlation to AgeAccelPheno, EEAA and the EAA measure derived from the Hannum age clock (AgeAccelerationResidualHannum) ($r > 0.54$, $p < 0.001$), not unlike previous findings in psychiatric cohorts (Lu et al., 2019; Yang et al., 2020). AgeAccelPheno exhibited correlations of medium strength to AgeAccelGrim, IEAA and IEAA.Hannum clocks ($r > 0.54$, $p < 0.001$), and very strong correlations to the EEAA and AgeAccelerationResidualHannum predictors ($r > 0.80$, $p < 0.001$). Horvath and Hannum clocks were moderate to strongly correlated amongst themselves, including both intrinsic and extrinsic measures of

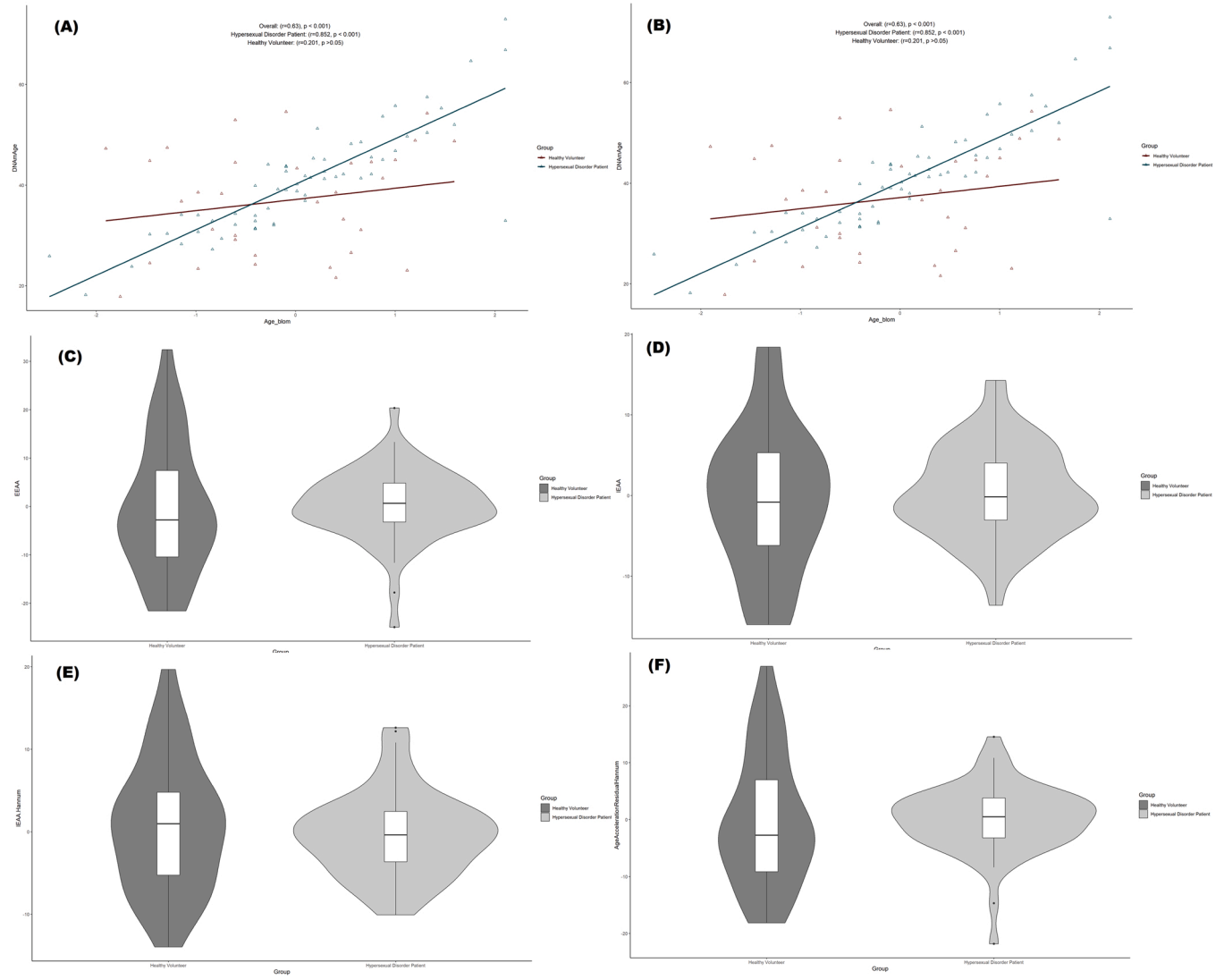

Fig. 1. Horvath and Hannum epigenetic age acceleration – HD patients vs healthy volunteers, a, b Scatterplots show Horvath or Hannum Age vs chronological age. Pearson's correlation analysis indicated all EA predictors were very strongly correlated with chronological age in HD patients ($r_{absolute} > 0.8$, $p < 0.001$), but these were, with the exception of *GrimAge*, weakly correlated to age in the group of healthy volunteers ($r_{absolute} < 0.25$, $p > 0.05$). c-f Violin plot with boxplots show Horvath EAA, IEAA, Hannum EAA, or EEAA. Between-group comparisons were conducted using a Student's *t*-test. No significant between group differences were revealed ($p > 0.1$). EAA, epigenetic age acceleration; IEAA, intrinsic epigenetic age acceleration; EEAA, extrinsic epigenetic age acceleration.

epigenetic age acceleration (Fig. 2).

3.4. No epigenetic age acceleration clock predictor distinguishes HD patients from healthy volunteers

The baseline mean difference between DNAm GrimAge and chronological age for the full cohort averaged 2.8 years (SD = 4.41 years). In the case of HD, mean EA increases were comparable between HD patients (mean difference = 2.77 years, SD = 4.22 years) and healthy volunteers (mean difference = 2.75 years, SD = 4.79 years). The percentage change in baseline DNAm GrimAge compared to chronological age averaged 9.7% and 10.8% in the HD patient and control group, respectively (Supplementary Fig. 1). The study achieved sufficient power to detect differences of 2.5 in DNAm GrimAge measurements between groups using a desired power of 0.8 in Welch two sample *t*-tests, as demonstrated by power-analysis (Supplementary Figure 2.).

Differences in EA acceleration was compared between HD patients and healthy volunteers by Welch two sample *t*-tests and binomial logistic regression models with optimally calculated co-variates. Multivariate analyses were run separately for each EA and EA acceleration marker contrasting HD group (HD or healthy volunteer) to the EA/EA acceleration predictor of interest and adjusting for optimally determined co-variates, i.e., depression (Yes/No), DST suppression status (suppressor vs non-suppressor), CTQ total score and tumor necrosis factor alpha (TNF-alpha). By these analyses, all EA acceleration predictors were fully independent of HD disease status (HD patient or control) pertaining to EEAA, IEAA, Hannum IEAA, AgeAccelerationResidualHannum, AgeAccelGrim and DNAm TLadjAge ($p > 0.05$)(Figs. 1 and 3).

3.5. DST suppression status is independent of all epigenetic age acceleration clock predictors

In the case of DST group stratification, mean EA increases were comparable between DST non-suppressors (mean difference = 2.7 years, SD = 4.41 years) and DST suppressor controls (mean difference = 2.76 years, SD = 4.55 years). The percentage change in baseline DNAm GrimAge compared to chronological age averaged 9.8% and 11.4% in the DST non-suppressor and control group, respectively (Supplementary Fig. 3). The study achieved sufficient power to detect differences of ~3.0 years in DNAm GrimAge measurements between groups using a desired

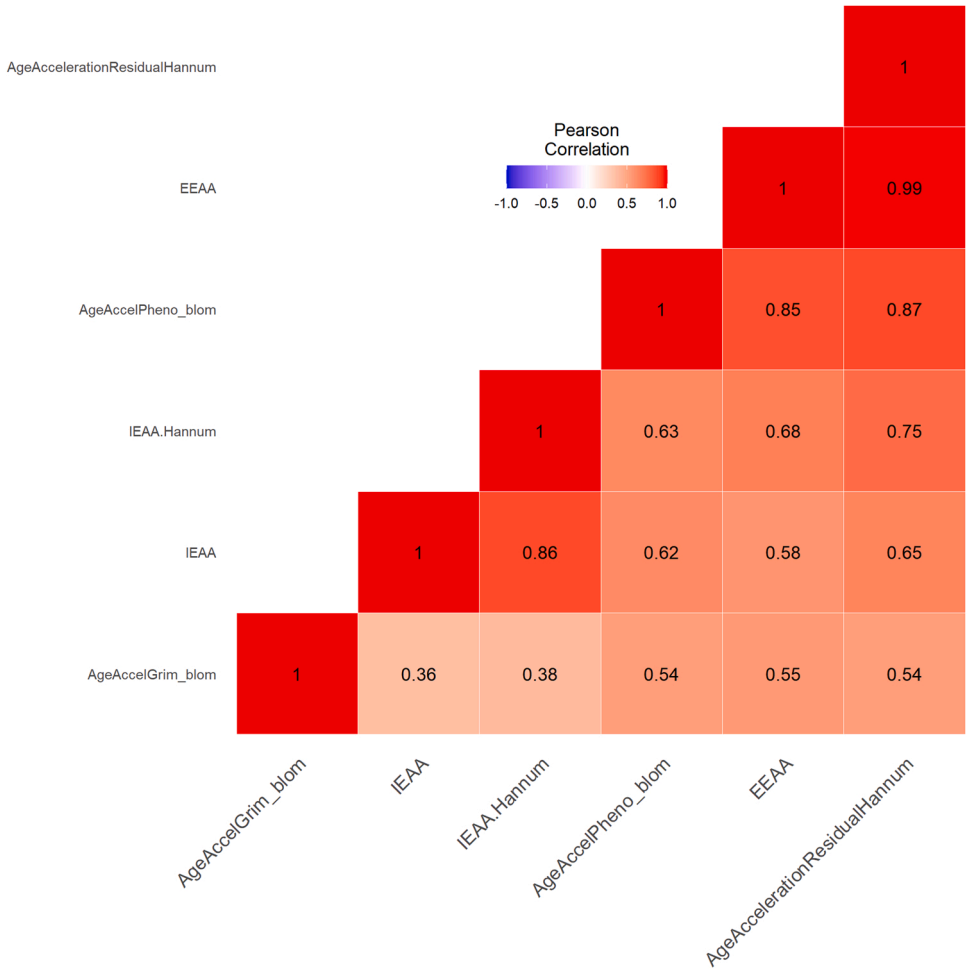

Fig. 2. Correlations of AgeAccelGrim with other epigenetic age clocks, The plot visualizes the inter-correlations between seven DNAm clocks. The deeper color indicates stronger correlations. AgeAccelGrim was relatively independent to other clocks, while AgeAccelPheno was correlated of medium strength with the acceleration of Horvath and Hannum clocks.

power of 0.8 in Welch two sample *t*-tests, as demonstrated by power-analysis (Supplementary Figure 4.).

Differences in EA acceleration was compared between DST non-suppressors and DST suppressor controls by Welch two sample *t*-tests. By these analyses, all EA acceleration predictors were fully independent of HD disease status (HD patient or control) pertaining to EEAA, IEAA, Hannum IEAA, AgeAccelerationResidualHannum, AgeAccelGrim and DNAm TLadjAge ($p > 0.05$)(Figs. 4 and 5).

3.6. No epigenetic age acceleration clock predictor distinguished DST suppression status or separated hypersexual disorder patients from healthy volunteers in a post-hoc analysis pertaining exclusively to male subjects

Post-hoc analyses pertaining exclusively to male subjects were performed, to account for the relative scarcity of research in support of the above discussed neurobiological underpinnings in female subjects. Six female subjects were thus excluded from the sample, resulting in a data set pertaining exclusively to male subjects. In this post-hoc analysis, no individual EA acceleration marker was associated with HD or DST suppression status in this post-hoc analysis exclusively pertaining to male subjects ($p > 0.05$, data not shown).

4. Discussion

We present the first study to unconditionally investigate epigenetic age acceleration predictors in relation to HD patients and DST suppression status in a well-characterized sample including both HD patients and healthy volunteers. DNAm GrimAge, a reliable estimator of biological aging, exceeded chronological age by 2.8 years on average for the full cohort, but did not differ significantly between HD patients and healthy volunteers or between DST non-suppressors and DST suppressor controls, respectively. These results were consistent in a post-hoc analysis pertaining exclusively to male subjects. Novel EA algorithms (Lu et al., 2019) were applied and the study was sufficiently powered to detect differences of EA in relation to chronological age exceeding 2.5 and 3.0 years for HD and DST non-suppression, respectively. This study uniformly implicates that HD and DST non-suppression is not associated with epigenetic age acceleration markers previously shown to be strongly associated with mortality risk and physiological dysregulation. Moreover, baseline plasma levels of several hormonal and inflammatory markers were independent of EAA. Importantly, plasma levels of TNF-alpha – distinguishing HD patients from controls – was not associated with EAA, reducing confidence in the biological relevance of this observation. Taken together, the findings presented herein could not contribute to provide objective evidence of accelerated cellular senescence in relation to HD, which separates HD from other psychiatric disorders such as depression (Han et al., 2018), PTSD (Yang et al., 2020), or schizophrenia (Teeuw et al., 2021). Importantly, the unexpected independency of EA acceleration to HPA-axis dysregulation as measured by a low-dose regimen of Dexamethasone (0.5 mg) implicates higher dosage-regimens may be needed to infer biologically relevant results when applied to psychiatric patients with HD. Studies stratifying DST

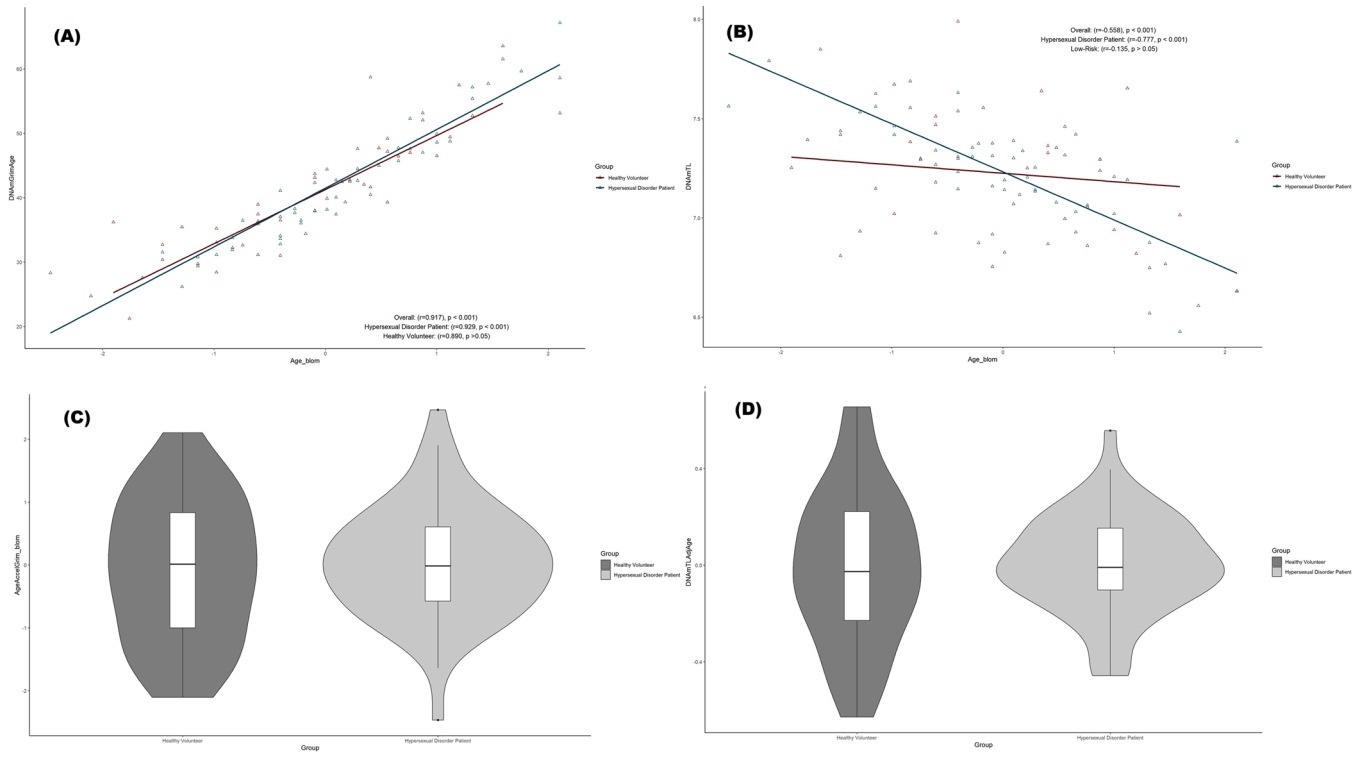

Fig. 3. Grim epigenetic age acceleration and DNA methylation-based telomere length – HD patients vs healthy volunteers, a, b Scatterplots show GrimAge or DNAmTL vs. chronological age. Pearson's correlation analysis indicated a significant correlation between GrimAge/DNAmTL and chronological age in both groups. c, d Violin plot with boxplots shows Grim EAA or DNAmTLAdjAge. Student's *t*-test showed no significant between-group differences. EAA, epigenetic age acceleration; DNAmTL, DNA methylation-based telomere length; DNAmTLAdjAge, age-adjusted DNAmTL.

non-suppressors according to established dosage-regimens in somatic settings are needed to fully elucidate the putative contribution of HPA-axis dysregulation to EA. In a wider sense, these findings warrant further consideration of the biological correlates of HD.

Much is unknown about the long-term health effects of hypersexual behavior. Previous research indicates a bidirectional relationship between sexual behavior and neuroendocrine activity (Cera et al., 2021). In the case of oxytocin, studies with human subjects have indicated a role for this neuropeptide in stress regulation (Heinrichs et al., 2009), prosocial (Ross and Larry, 2009b) and affiliative behavior (Heinrichs et al., 2009; Ross and Larry, 2009a). A potential therapeutic role has also been suggested in addiction, due to indications of a modulatory role for oxytocin on reward related pathways (Sanna, Maria Antonietta, 2021). Oxytocin has also been shown to be involved in reproductive behavior (Burri et al., 2008) and animal models have demonstrated increased release during male sexual activity (Yanagimoto et al., 1996). In humans, previous research has shown associations between oxytocin and sexual satiety, orgasm intensity and increased release during orgasm or ejaculation have been demonstrated (Thackare et al., 2006; Cera et al., 2021). Regarding potential health effects, animal models have shown that oxytocin can have a protective effect against premature sarcopenia (Elabd et al., 2014) and associations have been shown between social enrichment, oxytocin levels and telomere length in female rats (Faraji et al., 2018). In humans, several health promoting effects of oxytocin have been documented and therapeutic applications in a wide variety of conditions have been suggested (Carter et al., 2020). Oxytocin has also been suggested to promote human longevity suggested in part to be explained by associations between oxytocin and sexual intimacy (Benameur et al., 2021). Regarding the HPA-axis, sexually induced longevity in rodents have been shown to be associated to transcriptional changes in related genes (Sahm et al., 2021). Dysregulation of the HPA-axis in humans has also been implicated in sarcopenia (Gupta and John, 2014), longevity (Debono et al., 2014) and prospectively associated with mortality, mainly due to cardiovascular disease and infections (Debono et al., 2014; Jussi Jokinen et al., 2007; Jussi Jokinen and Nordström, 2009). Among inpatients with mood disorders, DST non-suppression predicted all-cause mortality in a long-term follow-up study of almost 20 years. It should however be mentioned that these relationships are believed to be complicated and other studies have suggested a non-relationship between resting state HPA-axis activity and familial longevity in non-clinical populations (Jansen et al., 2015). In regard to the potentially health-promoting effects of sexual behavior briefly outlined above, it should also be mentioned that support exists for behavior specific effects, with health promoting effects conferred by penile-vaginal intercourse and adverse health effects promoted by masturbation and/or anal intercourse (Brody, 2010). It has also been suggested that conflict in the context of sexual relations can have negative effects on health and longevity (Adler and Bonduriansky, 2014). In addition, as previously mentioned clinical studies have demonstrated that HD is associated with other behaviors detrimental to health (e.g. smoking, substance use) and decreased satisfaction with somatic and psychological health (Långström and Karl Hanson, 2006).

Considering the symptom burden of people afflicted with HD, in conjunction with frequently occurring co-morbidities and previously shown associations between HD and unhealthy life-style behaviors such as smoking and substance use, the absence of indication of accelerated aging on several indices is arguably somewhat puzzling. Previous studies with other clinical psychiatric populations have indicated substantial premature mortality due to natural causes and in some cases positive relationships to indices of accelerated epigenetic aging (Bersani et al., 2019). This raises the question of what differentiates mental pathology that is associated to accelerated aging from that which is not. One possible explanation for the results of this study is that potentially negative health consequences of the neuroendocrinal and behavioral aspects of HD are offset by other balancing factors, such as health promoting effects of frequent engagement in sexual behavior, potentially

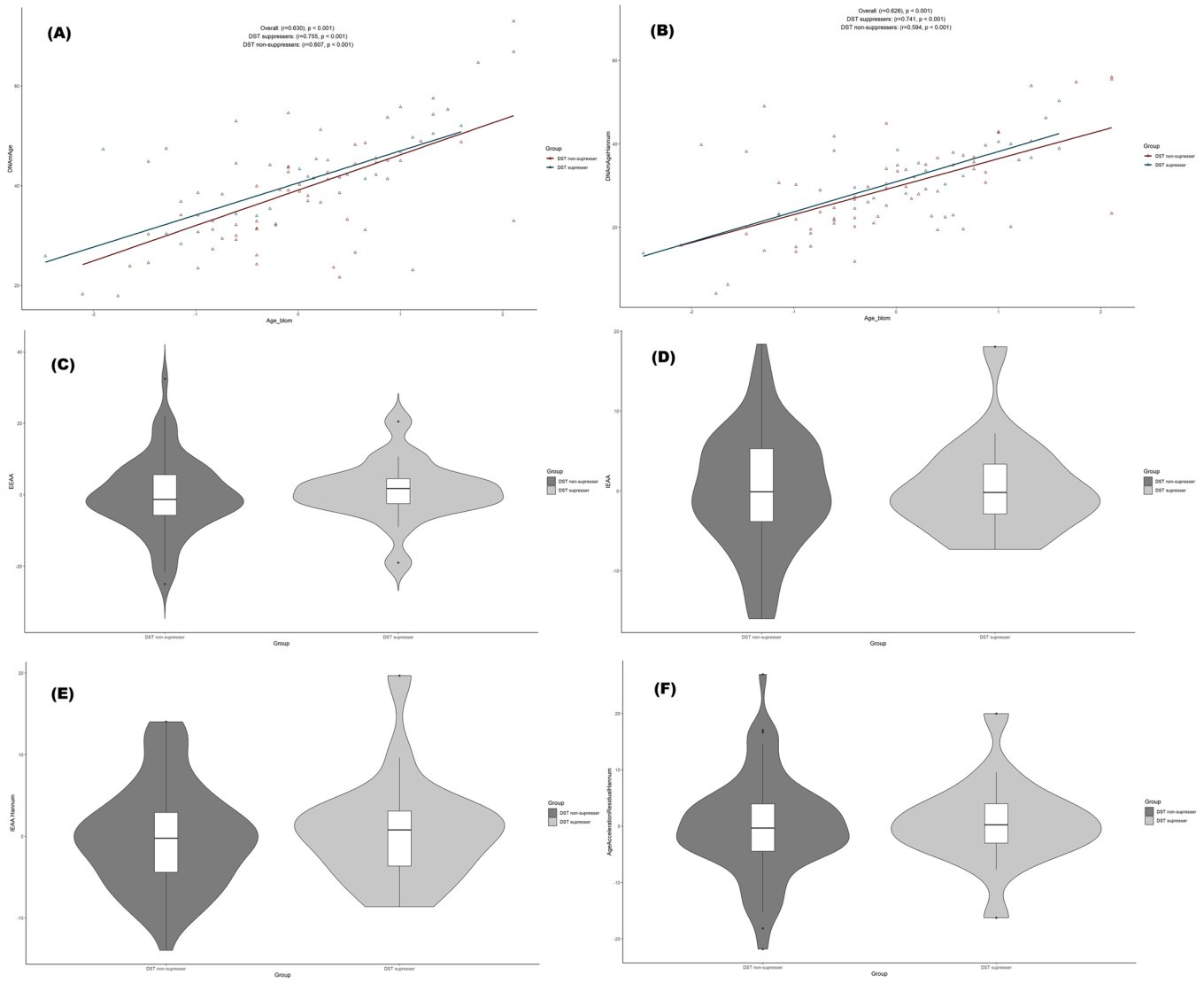

Fig. 4. Horvath and Hannum epigenetic age acceleration – DST suppressers vs non-suppresser controls, a, b Scatterplots show Horvath or Hannum Age vs chronological age. Pearson's correlation analysis indicated a moderate to strong correlation between DNA methylation age and chronological age in both groups. c-f Violin plot with boxplots show Horvath EAA, IEAA, Hannum EAA, or EEAA. Between-group comparisons were conducted using a Student's *t*-test. No significant between group differences were revealed ($p > 0.1$). EAA, epigenetic age acceleration; IEAA, intrinsic epigenetic age acceleration; EEAA, extrinsic epigenetic age acceleration.

mediated by its influence on the oxytocinergic system. A counterargument to this putative interpretation is however that it presumes that individuals with HD to a substantial degree engage in health promoting forms of sexual behavior, which can be considered doubtful. Perhaps even more surprising was the absence of relationship between HPA-axis dysregulation, indicated by the DST, and accelerated aging in the entire sample. Considering that a large body of previous research have evinced relationships between HPA-axis activity and somatic health (Adam et al., 2017) the non-result could be interpreted as casting doubt on the biological relevance of the DST and its ability to accurately identify clinically relevant hypercortisolism, when conducted according to the methodology used in this study. Reported dosages in psychiatric studies purporting to identify HPA-axis dysregulations vary and are many times considerably lower than the dexamethasone dosages recommended in the diagnosis of established endocrinological disease conditions with pronounced increases in HPA-axis activity with a clearly elucidated pathophysiological mechanism (Lindholm, 2014). For example, in a study investigating hypocortisolism in borderline personality disorder, 0.25 mg dexamethasone was administered in an overnight DST test (Carrasco et al., 2007), whereas examples exist of administering 1.5 mg (Schubert et al., 2019), 1 mg (Maes et al., 2006) and 0.5 mg (Carrasco et al., 2007) in overnight tests with psychiatric cohorts claiming to demonstrate hypercortisolism. Indeed, the use of the DST to identify the subclinical hypercortisolism purported to be associated with various psychiatric diseases have been previously criticized (Lindholm, 2014) and in several previous studies most healthy controls have been shown to exhibit non-suppressor status in the DST by the same dexamethasone dosage used in this study (Carrasco et al., 2007).

The capacity of this study to provide causal evidence is first handedly limited by the cross-sectional study design. Longitudinal analyzes of HD patients and DST non-suppressors would allow to take the dynamic quality of EAA into account. Second, the study sample was well-characterized and HD subjects exhibiting ongoing substance abuse or providing evidence of any serious comorbid somatic or psychiatric disorder were excluded. As EAA has previously been associated with substance abuse and somatic disease, it cannot be completely excluded that earlier findings of accelerated EA in relation to PTSD, depression or schizophrenia could have been conferred by such unaccounted-for

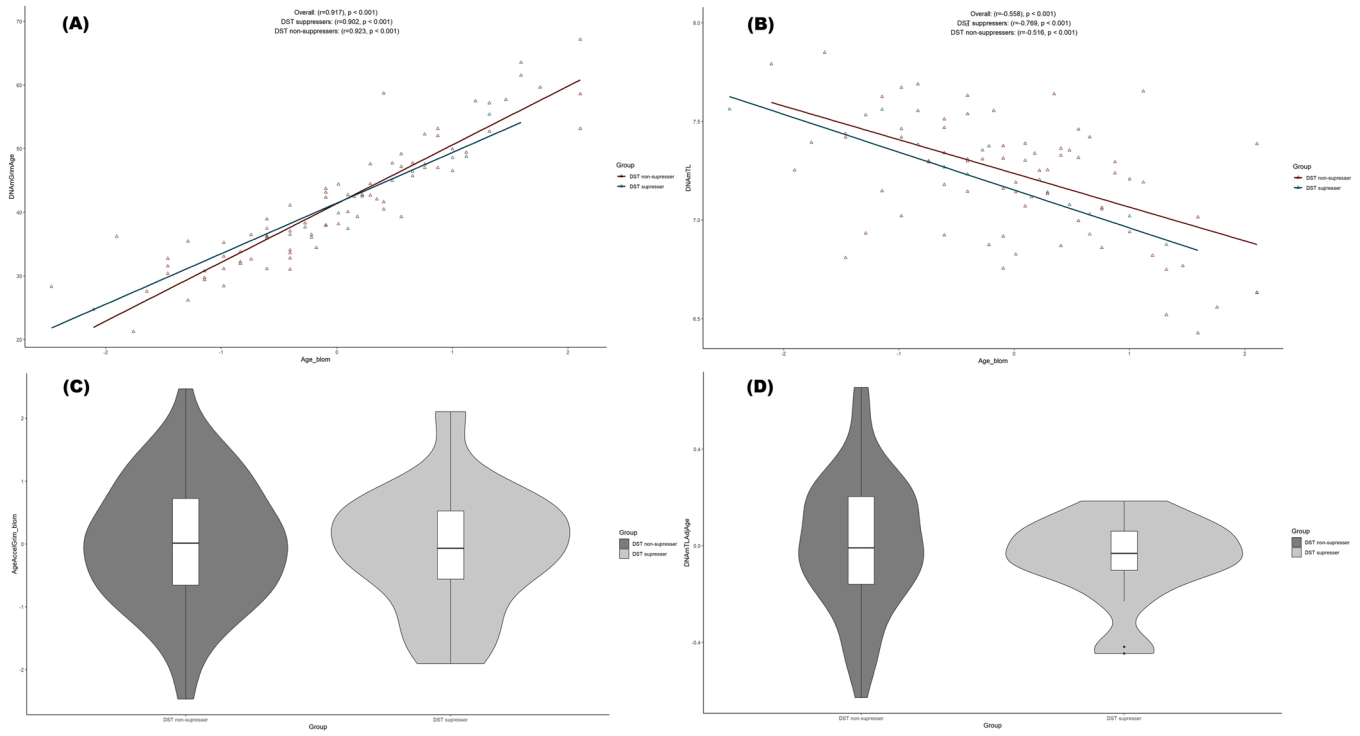

Fig. 5. Grim epigenetic age acceleration and DNA methylation-based telomere length – DST suppressers vs non-suppresser controls, a, b Scatterplots show GrimAge or DNAmTL vs. chronological age. Pearson's correlation analysis indicated a moderate to strong correlation between GrimAge/DNAmTL and chronological age in both groups. c, d Violin plot with boxplots shows Grim EAA or DNAmTLAdjAge. Student's *t*-test showed no significant between-group differences. EAA, epigenetic age acceleration; DNAmTL, DNA methylation-based telomere length; DNAmTLAdjAge, age-adjusted DNAmTL.

factors. Third, power-calculations implicated the study was not sufficiently powered to assuredly exclude small cumulative increases/decreases in excess mortality conferred by HD or DST non-suppression. Yet, this study is demonstrably sufficiently powered to detect clinically significant EA differences with large effect size. Assessing EA disparities of small magnitude would require a sizeable cohort of HD patients or DST non-suppressors and would, arguably, be unlikely to uphold any relevance to clinicians. Fourth, we administered a relatively low dose of dexamethasone (0.5 mg). Thus, it cannot be ruled out that DST non-suppressors identified through higher doses would exhibit differences in EA in comparison to suppressors and EAA in relation to their chronological age. However, the clinical relevance of this in relation to HD could arguably be seen as doubtful, since most of the HD sample were suppressors even by the low dose paradigm applied in this study. Fifth, the present study did not allow for comprehensive exclusion of potential confound from inflammatory markers in relation to EA in these populations. Including additional inflammatory markers, i.e., leukocyte counts or CRP, would have been of value. Lastly, while several non-beneficial life-style factors have been associated with HD (e.g. smoking, substance use, contraction of sexually transmittable disease and decreased subjective satisfaction with health (Långström and Karl Hanson, 2006), no previous study explored other important aspects that may influence health and EA (i.e., for example, exercise, social contexts or nutrition). Study strengths include the representative patient population of HD patients with thorough diagnostics of the psychiatric disorders and a careful assessment of severity of suicidal behavior as well as the consideration of possible confounders such as gender, childhood adversity, hormonal and inflammatory variables and comorbidity patterns. Such factors minimize risks of biased results due to hidden confound, albeit this cannot be completely excluded.

In conclusion, our results show that epigenetic age acceleration markers previously demonstrated to strongly predict physiological dysregulation and mortality are not related to HD or DST non-suppression status. Taken together, the findings presented herein could not contribute to provide objective evidence of accelerated cellular senescence in relation to HD, which separates HD from depression (Han et al., 2018), PTSD (Yang et al., 2020), and schizophrenia (Teeuw et al., 2021). Importantly, the unexpected independency of EA acceleration to HPA-axis dysregulation as measured by a low-dose regimen of dexamethasone (0.5 mg) implicates higher dosage-regimens may be needed to infer biologically relevant results when applied to psychiatric patients. Studies stratifying DST non-suppressors according to established dosage-regimens in somatic settings are needed to fully elucidate the putative contribution of HPA-axis dysregulation to EA. In a wider sense, these seemingly unintuitive findings re-update the question of the relationship of HD to neurobiological factors. As briefly outlined above, a plausible perspective is that multiple biological factors interact and to some extent could counteract each other in HD. Epigenetic aging would thus represent a sum of these complex processes. Additional research on HD is needed and it is possible that more careful delineation of potential sub-groups could provide important input for further biologically orientated research.

Author contributions

Design of study (A.D.B., J.J., A.C., S.A., K.G.Ö., M-R.A.). Collection of data (J.J., S.A., K.G.Ö., A.C., J.S.). Data analysis (A.D.B., J.J., P.A., M-R.A.). Drafting of manuscript (A.D.B., J.J., P.A.). (A.C., S.A., K.G.Ö., J.S., M-R.A.) contributed to extensive discussions and critical manuscript reading. All authors contributed to and have approved of the final manuscript. All authors are accountable for all aspects of the work.

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Declaration of Competing Interests

Dr. Rask-Andersen served as consultant for Olink Proteomics. There are no other commercial or otherwise potentially competing interests to report.

Data Availability

The data underlying the findings presented in this study are available upon reasonable request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.psyneuen.2022.105765.

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