

LETTER

Nasal epigenetic age and systemic steroid response in pediatric emergency department asthma patients

To the Editor,

Asthma is a leading cause of emergency department visits and hospitalizations in children with health-related costs amounting to over \$80 billion annually.¹ Hence, continued efforts to improve the risk stratification and management of pediatric asthma patients remain important. To better predict asthma severity, Zhu et al. performed an epigenome-wide association study in nasal epithelial cells of 55 African American children with asthma.² They identified 816 differentially methylated sites and 10 differentially methylated regions. An earlier study of 20 children by this same group similarly identified over 200 sites that were differentially methylated when comparing children who were good versus poor responders to acute systemic steroid treatment.³ Beyond single methylation sites or regions, a few studies have demonstrated relationships of pediatric asthma with blood and nasal cell epigenetic age acceleration—a novel marker of biological age.^{4,5} Despite this evidence, even fewer studies have evaluated relationships of epigenetic age with treatment responses in pediatric asthma. Given the robust associations of epigenetic age with healthspan and lifespan and its potential clinical utility, improving our understanding of its relationship with asthma could have a profound role in informing patient management and risk stratification moving forward.

To further our understanding of epigenetic age and asthma therapeutic relationships, we utilized the publicly available 20 participant systemic steroid response (baseline [T0] and ~24 h after steroid therapy [T1]) nasal DNA methylation data from Zhang et al.³ Per the Cincinnati Children's Hospital Medical Center evidence-based treatment protocol for in-patient asthma exacerbation, all study participants received 2 mg/kg/day of prednisone during their hospital stay.³ The Zhang et al. dataset included information on chronological age, sex, race, and hospital length of stay (LOS) [short (≤ 24 h) and long (> 24 h)]. Participants had a median (IQR) chronological age of 10 (6–15) years. Other demographic information has been previously published (Table S3).³ We calculated the T1-T0 change in epigenetic age acceleration (EAA Horvath) using an online calculator (<http://dnamage.genetics.ucla.edu>). We found no significant differences in median EAA by LOS, sex, or race (Figure 1A–C). However, females with short LOS were significantly younger than males with long LOS ($p = .049$, Figure 1D). In repeated measures mixed effects models examining relationships of LOS with EAA, we observed no direct associations of LOS, sex, or race with EAA. Nor did we observe

any significant effect modification of the LOS and epigenetic age relationship by race. However, we did observe a significant interaction between LOS and sex after adjusting for timepoint and race. Specifically, compared to males with long LOS, females with short LOS were on average 2.32 years younger in nasal EAA (95% CI: -4.06 , -0.57 , $p = .01$). EAA was not statistically different between males with short LOS ($\beta = .99$ years, 95% CI: -0.38 , 2.36 , $p = .15$) and males with long LOS. No significant differences were observed in chronological age among the sex and LOS categories (Table 1).

Anti-inflammatory processes are often associated with anti-aging. Conversely, inflammatory diseases and biomarkers are frequently associated with aging processes, including EAA.⁶ In the context of pediatric asthma, previous work in children from the Project Viva cohort (mean chronological age of 12.9 years) reported a greater EAA of 0.74 years in children with current asthma compared to their peers.⁴ Given this background literature and our study design of nasal methylation samples taken within 24 h of each other, we hypothesized that biologically older individuals may have differences in disease severity and may require different durations of therapeutics like steroids to temporize their disease. When tested directly in our study sample, albeit not statistically significant, the median EAA of individuals with long LOS (a proxy of more severe asthma) was greater than that of individuals with short LOS (a proxy of less severe asthma). Furthermore, statistically significant differences in EAA were observed when we examined the interaction between sex and LOS. Our results of significantly lower EAA in females with short LOS compared to males with long LOS may suggest less severe disease and/or better inflammatory regulation (including effectiveness of systemic steroids in mitigating the inflammatory response) in the former group.

Highlighting the sex-specific nature of our statistically significant observations is important because general sex differences in EAA have been previously reported with men oftentimes demonstrating greater EAA when compared to women.⁷ Although not statistically significant, we observe the same trend in our data (Figure 1B). There are also notable sex differences in asthma throughout life. Prior to puberty, asthma prevalence is greater in males. However, after puberty, asthma prevalence is greater in females.⁸ For these reasons, consistently evaluating sex interactions will be critical for building a more comprehensive understanding of sex-specific asthma therapy and EAA relationships in children and adults. Revisiting the

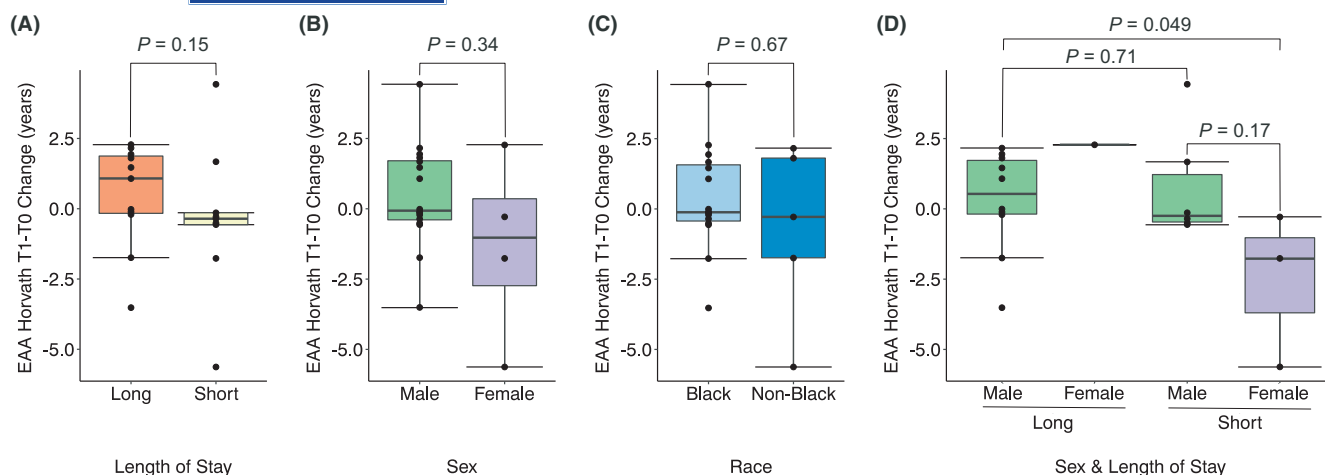


FIGURE 1 Change in T1-T0 Epigenetic Age by Demographic Variables. Figure 1 presents boxplots of the change (T1-T0) in epigenetic age acceleration (EAA) by length of stay {LOS} (A), sex (B), race (C), and sex-LOS categories (D). Each large dot represents a study participant. *p* values are from two-sided Wilcoxon rank sum tests. T1 = Timepoint 1 (after 24 h). T0 = Timepoint 0 (baseline)

Aging Models			
	Difference in Aging (95% CI)	<i>p</i>	N (Obs)
EAA Horvath units: years			
Males with long LOS	Reference	—	10 (20)
Females with long LOS	Not reported given observations from only one study subject	—	1 (2)
Males with short LOS	0.99 (−0.38, 2.36)	.15	6 (12)
Females with short LOS	−2.32 (−4.06, −0.57)	.01	3 (6)
Chronological Age units: years			
Males with long LOS	reference	—	10 (20)
Females with long LOS	Not reported given observations from only one study subject	—	1 (2)
Males with short LOS	−0.87 (−5.42, 3.67)	.69	6 (12)
Females with short LOS	1.82 (−3.95, 7.59)	.51	3 (6)

TABLE 1 Relationships of sex and length of stay (LOS) with nasal epigenetic age acceleration (EAA) and chronological age (Obs = 40)

Models adjusted for race. EAA model additionally adjusted for timepoint of each observation. Abbreviations: EAA, Epigenetic Age Acceleration; Obs, Observations.

aforementioned Project Viva study, the authors found that bronchodilator response at least 15 min after receiving two puffs (90 µg per puff) of albuterol was not significantly associated with EAA, but they did not include interactions with sex.⁴ Even though albuterol is primarily viewed as a bronchodilator, it also has some anti-inflammatory properties and it is possible that a sex-specific EAA relationship may also exist with this therapy. Additionally, although not best examined in non-randomized short-term (hours to days) therapeutic studies like our own, the potential role of asthma therapeutics in slowing or reversing EAA is also understudied and merits further evaluation.

Our analysis is from a small sample, with limited data on other important variables such as overall steroid use, and more extensive studies are needed to confirm our findings. Still, our preliminary data suggest that epigenetic age is associated with systemic steroid

asthma treatment responses in a sex-specific manner. As such, epigenetic age may be a useful adjunct in guiding emergent asthma treatment planning. Specifically, epigenetic aging may be helpful in risk stratifying patients as emergency providers consider in-patient admission versus other intermediate care options such as observation units. Presently, tools like the Pediatric Asthma Score (PAS) are used by some providers to quantify asthma severity following steroid initiation and have been associated with improved treatment outcomes and lower healthcare costs for asthmatics.⁹ Nevertheless, portions of the PAS algorithm are based on chronological age rather than biological age or EAA. It is our hypothesis that even if EAA proves not to be clinically useful in informing care on its own, substituting epigenetic age for chronological age perhaps may improve the usefulness of existing asthma care algorithms. Nonetheless, more

comprehensive research is necessary to evaluate this conjecture, especially in the context of sex-specific differences.

KEYWORDS

asthma exacerbation, child, DNA methylation, steroids

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CONFLICTS OF INTEREST

The authors do not endorse any conflicts of interest.

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