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ORIGINAL RESEARCH

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Immigrant status and citizenship relationships with epigenetic aging in a representative sample of United States adults

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ABSTRACT

Background: Immigrant status and citizenship influence health and well-being, yet their associations with DNA methylation (DNAm)-based biomarkers of aging – key predictors of healthspan and lifespan, also known as epigenetic aging – remain underexplored.

Methods: Using a representative sample of 2,336 United States (U.S.) adults from the 1999–2000 and 2001–2002 cycles of the National Health and Nutrition Examination Survey (NHANES), we analyzed cross-sectional associations of immigrant status and U.S. citizenship with seven epigenetic aging biomarkers: HannumAge, HorvathAge, SkinBloodAge, PhenoAge, GrimAge2, DNAm Telomere Length, and DunedinPoAm.

Results: After adjusting for demographic factors, immigrants had 2.53-year lower GrimAge2 measures (95%CI: −3.44, −1.63, $p < 0.001$) compared to non-immigrants. U.S. citizens had 1.98-year higher GrimAge2 measures (95%CI: 0.66, 3.30, $p = 0.005$) compared to non-citizens. The GrimAge2 associations with immigrant status (β = -1.04-years, 95%CI: −1.87, −0.21, $p = 0.02$) and citizenship (β = 1.35-years, 95% CI: 0.38, 2.32, $p = 0.02$) were attenuated after adjusting for other lifestyle/health variables. Immigrant status and citizenship were associated with estimated levels of several GrimAge2 DNAm component proteins, including adrenomedullin and C-reactive protein.

Conclusion: Our results support the paradigm of the immigrant mortality advantage and highlight the potential value of epigenetic age measures in studying socioeconomic and broader factors influencing citizen and immigrant health.

PLAIN LANGUAGE SUMMARY

This study explored whether being an immigrant or a U.S. citizen is linked to aging, based on changes in DNA methylation that can predict health and lifespan. Using data from 2,336 U.S. adults, we compared seven different DNA methylation-based aging measures between immigrants and non-immigrants, as well as citizens and non-citizens. Immigrants had younger DNA methylation ages than non-immigrants. For example, one marker (GrimAge2) showed that immigrants were about 2.5 years younger. U.S. citizens had older DNA methylation ages than non-citizens, about 2 years older in GrimAge2. These differences became smaller when considering lifestyle and health factors, showing that behaviors and environment matter. Immigrants and citizens also had different estimated levels of certain blood proteins linked to aging, like C-reactive protein (inflammation) and adrenomedullin (vascular health). Our findings support the idea that immigrants may have better health (the "immigrant mortality advantage"). Overall, this study shows how DNA methylation-based aging markers can help us understand how social and environmental factors affect health differences between groups.

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KEYWORDS

DNA methylation age; immigrant health; national health and nutrition examination survey (NHANES); ADM; CRP

1. Introduction

In 2023, 47.8 million immigrants lived in the United States (U.S.), accounting for 14.3% of the total U.S. population [1]. Many of these individuals immigrated to the U.S. for better opportunities for themselves and their families. Despite often experiencing improvements in their educational and financial situations, immigrants still reported facing challenges including communication barriers, discrimination, and difficulty meeting their basic needs of housing, nutrition, and healthcare [2]. Given the complex nature of immigrants' social experiences and their significant representation within the U.S. population, studying their health and well-being is an important focus for public health.

Many studies have examined the relationships of immigrant status with morbidity and mortality in the U.S. Although some

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Article highlights

- Immigrant status and citizenship are acknowledged as key factors affecting health.
- We studied immigrant status, citizenship, and epigenetic aging in U.S. adults.
- We observed that immigrants were epigenetically younger than non-immigrants.
- U.S. citizens were found to be epigenetically older than non-citizens.
- Epigenetic aging may be useful for studying citizen and immigrant health.

of these studies report worse health among immigrants – like one reporting a greater impact of COVID-19 on foreign-born populations [3] or another study of over 120,000 Latino adults reporting greater cardiometabolic and accidental mortality in non-citizens compared to U.S.-born citizens [4] – a substantial portion of the literature describes better health and lower mortality rates in immigrants compared to their native-born counterparts (an immigrant mortality advantage) [5–7]. Some studies have even identified mortality advantages for specific ethnic groups most notably a "Hispanic paradox" or mortality advantage [8,9]. The etiology of the Hispanic paradox and overall immigrant mortality advantage is believed to be multifactorial with contributions from lifestyle behaviors (e.g., smoking alcohol intake), cultural factors (e.g., social support, family cohesion, acculturation), and healthy selection [5].

Amid this evidence there is a need to test the relationships of immigrant status with epigenetic age biomarkers, which are DNA methylation (DNAm)-based measures that strongly predict morbidity and mortality [10–15]. Epigenetic age is impacted by lifestyle and environmental factors and existing literature suggests that it may have utility in predicting and/or monitoring clinical illness and mortality risk [16]. Still, there is limited data exploring epigenetic aging relationships with immigrant status. We identified one analysis of 4,237 individuals from the U.S. National Longitudinal Study of Adolescent to Adult Health that reported slower epigenetic aging, in the GrimAge measure, among first-generation immigrants when compared to their US-born peers [17]. More specifically, in multivariate models also including adjustments for leukocyte proportions, second and third or more generation Americans on average had higher GrimAges of 1.42 and 1.33 years respectively when compared to first-generation immigrants. Yet, this study was limited in that it used a cohort of patients who were adolescents in 1994 and now had a mean chronological age of 38.4 years. Furthermore, the study did not examine relationships with citizenship, which in addition to immigration status, has been conceptualized as an important factor, often determining access to key resources (e.g., healthcare) that can influence health and mortality. Mixed findings have been reported when examining citizenship relationships in Latino cohorts [18]; however, these studies have not examined DNAm-based measures and/or included nationally representative samples [19].

Considering these findings, the present study uses data from the National Health and Nutrition Examination Survey (NHANES), a nationally representative sample of individuals in the U.S., to evaluate the cross-sectional relationship of immigrant status with epigenetic age in adults aged 50 years and older. As a corollary, we also examine the cross-sectional relationship of U.S. citizenship with epigenetic age. With the context of the immigrant mortality advantage, we hypothesize that we will observe lower epigenetic age values in immigrants and U.S. non-citizens – suggesting reduced morbidity and mortality risk in these populations.

2. Methods

2.1. Study population

Through NHANES' interviews, physical examinations, and laboratory tests in a representative sample of the U.S. population, the National Center for Health Statistics (NCHS) assesses the health of the noninstitutionalized U.S. population [20]. In our study, we examined the relationships of U.S. citizenship and immigrant status with epigenetic age measures that are publicly available from the 1999–2000 and 2001–2002 NHANES cycles. The subsample consisted of 2,532 adults aged 50 years and older. To safeguard participant privacy, NHANES top-coded the ages of individuals 85 years and older as 85 years ($n = 130$), making their exact ages unknown. We excluded these participants to avoid misclassification error in the epigenetic age measures. Additionally, we removed participants whose DNAm-predicted sex did not match their self-reported sex ($n = 56$), leaving 2,346 participants. Of these, 2,336 had data on U.S. citizenship and immigration status ($n = 10$ missing), and 1,827 provided complete information on all covariates. All participants signed written informed consent forms, and the NCHS Research Ethics Review Board approved the study protocols (protocol #98–12).

2.2. U.S. Citizenship and immigrant status

Participants' U.S. citizenship and immigrant status was determined from self-report. For citizenship, participants answered the question, "Are you a citizen of the United States? [Information about citizenship is being collected by the U.S. Public Health Service to perform health related research. Providing this information is voluntary and is collected under the authority of the Public Health Service Act. There will be no effect on pending immigration or citizenship petitions.]" Participants who responded, "Citizen by birth or naturalization" were coded as "yes." Participants who answered, "Not a citizen of the U.S." were coded as "no." Participants who refused to answer, did not know their citizenship status, or for whom a response was missing were not included in the analyses.

For immigrant status, participants answered the question, "In what country were you born?" Participants who answered, "Born in Mexico" or "Born Elsewhere" were coded as "yes." Participants who responded, "Born in 50 U.S. States or Washington, DC" were coded as "no." Again, participants who did not answer, did not know where they were born, or for whom a response was missing were not included in the analyses. Data on the length of time participants were in the U.S. was available for a small subset of

participants and subsequently was only utilized in secondary analyses.

2.3. DNA methylation and epigenetic age

We accessed epigenetic age measures and DNA methylation-based leukocyte proportion estimates from the NHANES website (https://wwwn.cdc.gov/nchs/nhanes/dnam/), which also provides detailed information about DNA methylation analysis and processing. In summary, DNA was extracted from whole blood samples collected from NHANES participants aged 50 years or older during the 1999–2000 and 2001–2002 cycles. Genome-wide DNA methylation was then analyzed using the Illumina EPIC BeadChip array.

Our study incorporated several epigenetic age measures, including HannumAge, HorvathAge, SkinBloodAge, PhenoAge, GrimAge2, DunedinPoAm, and DNA methylation-based Telomere Length (DNAmTL). These measures were selected *a priori* as they have the most established relationships with health outcomes in the literature [10–15,21,22]. The HannumAge, HorvathAge, and SkinBloodAge measures primarily predict chronological age based on DNA methylation patterns, although research has linked them to broader health indicators [11,12,22]. PhenoAge, a leading biomarker of healthspan, was developed using nine clinical variables: albumin, creatinine, glucose, C-reactive protein, lymphocyte percentage, mean cell volume, red cell distribution width, alkaline phosphatase, and white blood cell count [15]. GrimAge2, a lifespan biomarker, integrates chronological age, gender, and 10 DNA methylation surrogates for cigarette pack-years and plasma protein markers, including adrenomedullin (ADM), beta-2-microglobulin (B2M), C-reactive protein (CRP), cystatin C, growth differentiation factor-15 (GDF15), hemoglobin A1c (A1c), leptin, plasminogen activator inhibitor-1 (PAI1), and tissue inhibitor metalloproteinase-1 (TIMP1) [13]. DNAmTL estimates telomere length based on DNA methylation patterns [14]. DunedinPoAm measures the pace of biological aging by assessing morbidity-related biomarkers. This metric was developed by analyzing longitudinal changes in 18 organ function biomarkers among individuals of the same chronological age, offering a robust indicator of aging pace [10]. The updated version of DunedinPoAm, DunedinPACE [23], was not available for download and analysis in NHANES.

2.4. Statistical analysis

We applied the R 'Survey' package to conduct generalized linear regression models, leveraging NHANES-provided participant sample weights designed for the epigenetic clock subsample [24]. To evaluate the associations of citizenship and immigration status with each epigenetic age measure, we used the svyglm function in R, which accounts for the survey's complex design. Our main model covariates were determined *a priori* and included chronological age (continuous, in years), chronological age^2 (continuous), sex (dichotomous: female vs. male), and self-identified ethnicity/race (categorical; Non-Hispanic White, Mexican American, Other Hispanic, Non-Hispanic Black, Other Race). Building on our primary epigenetic age findings, we then applied the same covariate adjustments to examine associations of citizenship and immigrant status with the DNAm-predicted blood biomarkers included in GrimAge2.

We performed three sensitivity analyses. The first stratified the study sample by Hispanic ethnicity to explore any influence of the Hispanic paradox on our findings. The second sensitivity analysis included additional lifestyle factor and health-related covariates: education (categorical; less than high school, high school diploma or GED, more than high school education, $n = 1$ missing), occupation (categorical; white-collar/professional, white-collar/semi-routine, blue-collar/high-skill, blue-collar /semi-routine, or no work, $n = 134$ missing), poverty-to-income ratio (PIR) (continuous, $n = 263$ missing), alcohol intake (categorical; abstainer, moderate drinker, heavy drinker, $n = 115$ missing), body mass index (BMI [kg/m^2]) (continuous, $n = 83$ missing), general health condition (categorical; good, fair, poor, $n = 2$ missing), smoking status (categorical; never, former, current, $n = 4$ missing), and physical activity (dichotomous; moderate/vigorous activity in the last 30 days: yes vs. no, $n = 0$ missing). The third sensitivity analysis adjusted for lifestyle and health covariates in addition to estimated leukocyte proportions (B cells, CD4 cells, CD8 cells, NK cells, monocytes, and neutrophils). To address missing covariate data for some participants, we used imputed values in the second and third sensitivity analyses. We performed multiple imputations using the *MICE* function in R, generating 10 imputed datasets. The estimates from these datasets were pooled using the *pool* function in R [25].

Lastly, previous research has raised questions about whether the immigrant mortality advantage persists with prolonged residence in the U.S. or diminishes, potentially due to the adoption of unhealthy behaviors and exposure to adverse environmental and social conditions [7,26]. To indirectly explore this, we conducted a secondary analysis focusing exclusively on immigrants to assess the relationship between citizenship status and epigenetic aging. This analysis used main model demographic covariates. We again hypothesized that citizens would exhibit greater epigenetic aging compared to non-citizens, but that this association might be attenuated among immigrants as compared to models using the general population because immigrants are epigenetically younger at baseline. Furthermore, we evaluated the role of time spent in the U.S., positing that longer residency – a potential proxy for assimilating detrimental health behaviors and exposures – may explain some differences when comparing the citizenship and epigenetic aging relationship in immigrant-specific analyses versus the general overall analysis. All statistical analyses were carried out using R Version 4.4.1 (R Core Team, Vienna, Austria). Statistical significance was set at a Bonferroni-adjusted p-value $<0.05/7$ ($p < 0.007$), accounting for seven independent epigenetic clocks. We discussed p-values <0.05 as marginal.

3. Results

3.1. Study sample characteristics

Table S1 presents the non-weighted study sample characteristics. Among the participants, 1827 (78%) had complete

Table 1. Complete case study sample characteristics by U.S. Citizenship and immigrant status ($n = 1827$).

	U.S. Citizens n = 1667	Non-Citizens n = 160	*P*-value	Immigrants n = 421	Non-Immigrants n = 1406	*P*-value
Aging Variables						
Age (years), mean (sd)	65.2 (9.3)	62.2 (8.2)	<0.001	63.3 (8.4)	65.4 (9.5)	<0.001
Epigenetic Age/Clocks, mean (sd)						
HannumAge (years)	66.3 (9.2)	65.0 (8.0)	0.07	65.4 (8.5)	66.4 (9.3)	0.04
HorvathAge (years)	66.4 (8.6)	63.4 (7.3)	<0.001	64.2 (8.0)	66.7 (8.7)	<0.001
SkinBloodAge (years)	63.7 (9.0)	61.2 (8.2)	<0.001	61.9 (8.4)	64.0 (9.0)	<0.001
PhenoAge (years)	54.9 (10.2)	52.6 (9.0)	0.003	52.7 (9.6)	55.3 (10.2)	<0.001
GrimAge2 (years)	71.5 (8.4)	68.8 (7.5)	<0.001	69.0 (7.8)	72.0 (8.5)	<0.001
DNAm Telomere Length (TL) (kb)	6.6 (0.3)	6.6 (0.3)	0.46	6.6 (0.3)	6.6 (0.3)	0.14
DunedinPoAm	1.11 (0.09)	1.11 (0.09)	0.71	1.09 (0.09)	1.11 (0.09)	<0.001
Demographic Variables						
Education, n (%)			<0.001			<0.001
Less Than High School	638 (38%)	122 (76%)		269 (64%)	491 (35%)	
High School Diploma (including GED)	385 (23%)	13 (8%)		46 (11%)	352 (25%)	
More Than High School	644 (39%)	25 (16%)		106 (25%)	563 (40%)	
Occupation, n (%)			<0.001			<0.001
Blue-collar (high skill)	233 (14%)	21 (13%)		66 (16%)	188 (13%)	
Blue-collar (semi-routine)	630 (38%)	107 (67%)		229 (54%)	508 (36%)	
White-collar (high skill)	437 (26%)	22 (14%)		76 (18%)	383 (27%)	
White-collar (semi-routine)	324 (19%)	7 (4%)		40 (10%)	291 (21%)	
Never worked	43 (3%)	3 (2%)		10 (2%)	36 (3%)	
Poverty to Income Ratio, mean (sd)	2.8 (1.6)	1.7 (1.2)	<0.001	2.1 (1.4)	2.9 (1.6)	<0.001
Race/Ethnicity Category, n (%)			<0.001			<0.001
Mexican American	394 (24%)	115 (72%)		237 (56%)	272 (19%)	
Other Hispanic	85 (5%)	19 (12%)		72 (17%)	32 (2%)	
Non-Hispanic White	760 (45%)	7 (4%)		40 (10%)	727 (52%)	
Non-Hispanic Black	377 (23%)	10 (6%)		34 (8%)	353 (25%)	
Other Race	51 (3%)	9 (6%)		38 (9%)	22 (2%)	
Sex, n (%)			0.002			0.004
Male	875 (52%)	105 (66%)		252 (60%)	728 (52%)	
Female	792 (48%)	55 (44%)		169 (40%)	678 (48%)	
Health Variables						
Alcohol Intake, n (%)			0.79			0.06
Abstainer	732 (44%)	71 (44%)		195 (46%)	608 (43%)	
Moderate Drinker	876 (53%)	85 (53%)		219 (52%)	742 (53%)	
Heavy Drinker	59 (3%)	4 (3%)		7 (2%)	56 (4%)	
Body Mass Index (kg/m^2), mean (sd)	28.9 (6.0)	28.4 (5.4)	0.24	28.2 (5.1)	29.1 (6.1)	0.004
General Health Condition, n (%)			<0.001			<0.001
Good	1172 (70%)	88 (55%)		249 (59%)	1011 (72%)	
Fair	386 (23%)	55 (34%)		133 (32%)	308 (22%)	
Poor	109 (7%)	17 (11%)		39 (9%)	87 (6%)	
Smoking, n (%)			0.36			<0.001
Current	259 (15%)	26 (16%)		51 (12%)	234 (17%)	
Former	678 (41%)	56 (35%)		138 (33%)	596 (42%)	
Never	730 (44%)	78 (49%)		232 (55%)	576 (41%)	
Physically Active, n (%)			<0.001			<0.001
Yes	903 (54%)	50 (31%)		187 (44%)	766 (54%)	
No	764 (46%)	110 (69%)		234 (56%)	640 (46%)	

P-values from T-tests or chi square tests comparing U.S. citizens to non-citizens or immigrants to non-immigrants.

covariate data. When compared to participants missing demographic data, complete cases were generally chronologically and epigenetically younger. Table 1 describes the complete case study sample characteristics by U.S. citizenship and immigrant status before survey weights were applied. In general, U.S. non-citizens and immigrants were significantly chronologically and epigenetically younger than their citizen and non-immigrant counterparts. Based on citizenship, the greatest difference was observed in HorvathAge with U.S. citizens being 3 years older on average ($p < 0.001$). Based on immigrant status, the greatest difference was observed in GrimAge2 with immigrants being on average 3 years younger ($p < 0.001$). The largest percentages of U.S. citizens were Non-Hispanic White (45%), had more than a high school education (39%), and reported being physically active (54%). In contrast, the largest percentages of immigrants were Mexican American (56%), had less than a high school education (64%), and reported not being physically active (56%). Other key citizen versus non-citizen and immigrant versus non-immigrant covariate differences are highlighted in Table 1. Figure S1 illustrates the correlations between chronological age and epigenetic age biomarkers in the full study sample ($n =$ 2,336). Epigenetic age showed strong correlations with chronological age, with SkinBloodAge exhibiting the strongest relationship ($r = 0.87$, Median Absolute Error [MAE] = 3.44 years). DNAmTL demonstrated a negative correlation with chronological age ($r = -0.58$).

3.2. Epigenetic age relationships

Table 2 presents the results from our analyses on immigrant status and U.S. citizenship relationships with epigenetic aging. In the models, which controlled for chronological age, chronological age^2, sex, and race/ethnicity, immigrants

Table 2. Relationships of immigrant status and U.S. Citizenship with epigenetic aging ($n = 2336$).

Model/Measure	Estimate (95% CI)	*P*-value
Immigrant Status		
HannumAge (years)	−0.06 (−1.10, 0.97)	0.90
HorvathAge (years)	−0.47 (−1.27, 0.33)	0.24
SkinBloodAge (years)	−0.02 (−0.75, 0.70)	0.95
PhenoAge (years)	−1.07 (−2.16, 0.02)	0.05
GrimAge2 (years)	−2.53 (−3.44, −1.63)	<0.001
DNAmTL (kb)	0.02 (−0.03, 0.06)	0.43
DunedinPoAm	−0.04 (−0.05, −0.02)	<0.001
U.S. Citizenship		
HannumAge (years)	−0.08 (−1.29, 1.12)	0.89
HorvathAge (years)	0.62 (−0.65, 1.89)	0.32
SkinBloodAge (years)	0.21 (−0.8, 1.23)	0.67
PhenoAge (years)	−0.28 (−1.88, 1.33)	0.72
GrimAge2 (years)	1.98 (0.66, 3.30)	0.005
DNAmTL (kb)	0.01 (−0.04, 0.06)	0.69
DunedinPoAm	0.01 (−0.01, 0.03)	0.18

Model estimates are for Immigrants and U.S. citizens (non-immigrants and non-citizens are the reference groups respectively). Model Adjustments: chronological age, chronological age2, sex, and race/ethnicity. $p < 0.007$: statistically significant $p < 0.05$: marginally significant.

Table 3. Relationships of Immigrant Status and U.S. Citizenship with Estimated GrimAge2 Components (n = 2336).

Model/Measure	Estimate (95% CI)	*P*-value
Immigrant Status		
A1c	-0.01 (-0.01, -0.002)	0.01
ADM	-4.59 (-7.63, -1.55)	0.005
B2M	-20249.78 (-35818.73, -4680.84)	0.01
CRP	-0.18 (-0.26, -0.10)	<0.001
Cystatin C	-8786.06 (-15773.41, -1798.72)	0.02
GDF15	-46.34 (-66.49, -26.18)	<0.001
Leptin	-412.83 (-805.19, -20.47)	0.04
Packyears	-3.85 (-5.84, -1.86)	<0.001
PAI1	-650.77 (-1272.73, -28.81)	0.04
TIMP1	-171.13 (-294.77, -47.48)	0.009
U.S. Citizenship		
A1c	0.01 (0.003, 0.01)	<0.007
ADM	6.10 (2.05, 10.15)	0.005
B2M	6102.40 (-10469.66, 22674.46)	0.45
CRP	0.17 (0.08, 0.26)	<0.001
Cystatin C	7801.39 (1511.69, 14091.10)	0.02
GDF15	23.71 (2.44, 44.98)	0.03
Leptin	620.10 (218.35, 1021.85)	0.004
Packyears	2.15 (-1.53, 5.84)	0.24
PAI1	1212.68 (633.27, 1792.10)	<0.001
TIMP1	148.08 (27.48, 268.67)	0.02

Model estimates are for Immigrants and U.S. citizens (non-immigrants and non-citizens are the reference groups respectively). Model Adjustments: chronological age, chronological age2, sex, and race/ethnicity. p <0.007: statistically significant p <0.05: marginally significant

had a statistically significant lower GrimAge2 (β = −2.53-years, 95%CI: −3.44, −1.63, $p < 0.001$) and DunedinPoAm (β = −0.04, 95%CI: −0.05, −0.02, $p < 0.001$) when compared to non-immigrants. Moreover, compared to non-citizens, citizens had a significantly higher GrimAge2 (β = 1.98-years, 95%CI: 0.66, 3.30, $p = 0.005$). Although the model estimates were slightly attenuated, associations remained marginally significant for relationships of immigrant status with GrimAge2 (β = −1.04-years, 95%CI: −1.87, −0.21, $p = 0.02$) and DunedinPoAm (β = −0.02, 95%CI: −0.03, −0.001, $p = 0.04$) in models adjusted for lifestyle and health factors (Table S2). Associations were attenuated and included the null when further adjusting for estimated leukocyte proportions (Table S3). U.S. citizenship remained associated GrimAge2 after health/lifestyle (β = 1.35-years, 95%CI: 0.38, 2.32, $p = 0.02$, Table S2) and leukocyte (β = 1.62-years, 95% CI: 0.62, 2.62, $p = 0.01$, Table S3) adjustments. We observed stronger immigrant status associations in non-Hispanics when compared to Hispanics (Table S4). We did not observe any marginal or statistically significant relationships of citizenship or immigrant status with HannumAge, HorvathAge, SkinBloodAge, PhenoAge, or DNAmTL in our primary models.

3.3. GrimAge2 component relationships

Given the significant associations of immigrant status and U.S. citizenship with GrimAge2, we evaluated relationships with GrimAge2 predicted biomarker components. Table 3 presents these results. Compared to non-immigrants, immigrants had significantly lower predicted levels of ADM (β = −4.59, 95%CI: −7.63, −1.55, $p = 0.005$), CRP (β = −0.18, 95%CI: −0.26, −0.10, $p < 0.001$), GDF15 (β = −46.34, 95%CI: −66.49, −26.18, $p < 0.001$), and pack-years (β = −3.85, 95%CI: −5.84, −1.86, $p < 0.001$). Compared to non-citizens, U.S. citizens had significantly higher predicted levels of A1c (β = 0.01, 95%CI: 0.003, 0.01, $p < 0.007$), ADM (β = 6.10, 95% CI: 2.05, 10.15, $p = 0.005$), CRP (β = 0.17, 95%CI: 0.08, 0.26, $p <$ 0.001), leptin (β = 620.10, 95%CI: 218.35, 1021.85, $p = 0.004$), and PAI1 (β = 1212.68, 95%CI: 633.27, 1792.10, $p < 0.001$).

3.4. U.S. Citizenship relationships in immigrants

Figure S2 depicts the distribution of time in the U.S. for naturalized citizens and non-citizens with this available data. When compared to non-citizens, naturalized citizens had been in the U.S. for greater periods of time ($p < 0.001$). Although the effect estimates with GrimAge2 remained positive, we observed no statistically significant relationships of epigenetic age with citizenship in immigrants both in main and time in U.S.-adjusted models (dichotomized as <20 years vs. ≥20 years based on the distribution in this study sample, Table 4).

4. Discussion

In this analysis of a representative cross-sectional sample of U.S. adults aged 50–84 years, we evaluated the relationships of immigrant status and U.S. citizenship with epigenetic aging biomarkers. By reporting similar observations in a study sample of older adults, our work expands and builds on a previous analysis with findings that suggest that epigenetic aging biomarkers are sensitive to the immigrant mortality advantage [17]. Furthermore, to the best of our knowledge, we describe the first associations of U.S. citizenship with epigenetic aging. From our models, we observed less epigenetic aging in non-citizens and immigrants when compared to U.S. citizens and non-immigrants respectively. These relationships were most robust for associations with the GrimAge2 measure. Additionally, we observed associations of several GrimAge2

Table 4. Relationships of U.S. Citizenship with Epigenetic Aging in Immigrants.

Model/Measure	Estimate (95% CI)	*P*-value	n
Main Model			
HannumAge (years)	-0.20 (-1.91, 1.50)	0.80	588
HorvathAge (years)	0.25 (-1.27, 1.76)	0.74	588
SkinBloodAge (years)	0.03 (-1.40, 1.47)	0.96	588
PhenoAge (years)	-1.36 (-3.52, 0.80)	0.20	588
GrimAge2 (years)	0.45 (-1.15, 2.05)	0.56	588
DNAmTL (kb)	0.03 (-0.03, 0.09)	0.36	588
DunedinPoAm	-0.01 (-0.04, 0.02)	0.40	588
Time in U.S. -Adjusted			
HannumAge (years)	0.02 (-2.31, 2.34)	0.99	581
HorvathAge (years)	0.28 (-1.69, 2.24)	0.77	581
SkinBloodAge (years)	0.08 (-2.06, 2.22)	0.94	581
PhenoAge (years)	-0.94 (-3.41, 1.54)	0.43	581
GrimAge2 (years)	0.57 (-1.16, 2.30)	0.49	581
DNAmTL (kb)	0.01 (-0.06, 0.09)	0.71	581
DunedinPoAm	-0.003 (-0.04, 0.03)	0.84	581

Model estimates are for U.S. citizens (non-citizens are the reference group). Model Adjustments: chronological age, chronological age2, sex, and race/ethnicity. Time in U.S. models are additionally adjusted for time in the U.S. (dichotomized as <20 years vs. ≥20 years) p <0.007: statistically significant p <0.05: marginally significant

estimated DNAm components with immigrant status (ADM, CRP, GDF15, and packyears) and citizenship (A1c, ADM, CRP, leptin, and PAI1).

Of all epigenetic age measures examined, our most consistent findings across groups were with GrimAge2, the epigenetic aging measure that best predicts mortality risk [13]. This close relationship with mortality may explain why the GrimAge2 findings were most robust. As such, our findings of higher GrimAge2 in citizens when compared to non-citizens and non-immigrants when compared to immigrants, supports the notion of an immigrant mortality advantage. Moreover, although a large proportion of our study sample was made up of ethnically Hispanic individuals, our sensitivity analysis demonstrating more negative model estimates for the relationship of immigrant status with epigenetic age in non-Hispanics compared to Hispanics suggests that our findings are not due to the Hispanic paradox. Additionally, we observed attenuation of the citizenship and, to a greater extent, the immigrant status relationships after adjusting for health/lifestyle variables and leukocyte proportions. We believe that this can be partially explained by noting that many of these variables may be important mediators in these relationships. For instance, prior work has noted that immigrants have healthier behavioral habits like lower levels of smoking [27]. We observe the same in our study sample and identify immigrants as having significantly lower levels of estimated DNAm packyears (one of the GrimAge2 components).

Although citizenship and immigration status are related and our results agree with the expected directionality under the immigrant mortality advantage framework, our findings highlight some nuances that should be considered in future research in this area. Both immigrant status and citizenship were associated with several GrimAge2 components, suggesting that their impact on health involves multiple physiological processes. Although there was overlap in physiological processes like vascular health and inflammation as evidenced by shared relationships with ADM and CRP respectively, the different associations of GrimAge2 components with immigrant status (cellular repair [GDF15] and cigarette packyears) and citizenship (blood glucose regulation [A1c], fat storage [leptin], and coagulation [PAI1]) may highlight physiological processes that differ between the two categorizations [28–33]. These differences may again be partially explained by socioeconomic differences in citizenship status versus immigration status. For example, poverty can impact health through a variety of mechanisms (e.g., access to healthcare, nutrition) [34]. In our study sample, the PIR difference when comparing citizens to non-citizens is 1.1, but only 0.8 when comparing non-immigrants to immigrants. Differences in socioeconomic variables and differences in sensitivity to physiological processes captured may also explain why only immigrant status was associated with a slower pace of aging in DunedinPoAm.

We also explored the relationship of citizenship status – an imperfect proxy for U.S. assimilation – with epigenetic aging only in immigrants. We hypothesized that the positive association between citizenship and accelerated aging, observed in the broader study sample, would be weaker among immigrants themselves, given their mortality advantage. Focusing on GrimAge2, which yielded statistically significant results in our primary analyses, we identified a positive but attenuated association between citizenship and aging in immigrants. However, this relationship did not reach statistical significance, likely due to the reduced statistical power of the smaller immigrant subgroup. Furthermore, adjusting for time spent in the U.S. had minimal impact on these results. Notably, naturalized citizens and non-citizens differed significantly in their average time spent in the U.S., yet this disparity did not translate to meaningful differences in our models. These null findings may reflect insufficient statistical power rather than a true absence of a relationship, underscoring the need for replication in larger cohorts.

Our study's strengths include the use of DNA methylation-based biomarkers to directly examine biological aging as a function of citizenship and immigrant status. However, we have some limitations. Our cross-sectional design limits our ability to assess longitudinal relationships essential for understanding aging over time. Moreover, we conducted sensitivity analyses using imputation models to address missing variables for some NHANES participants. Still, these analyses yielded consistent effect estimates when compared to our main models. Additionally, we observed a positive relationship of U.S. citizenship with GrimAge2 in analyses restricted to immigrants, but these results were not statistically significant. The lack of statistically significant results may be attributed to the analysis being limited to immigrants, who constituted approximately 25% of the total sample, thereby reducing statistical power and our ability to detect meaningful differences. Future work with a larger sample of immigrants and the ability to examine other nuances related to immigration such as time with or without documentation status, or circumstances of the immigration experience will be helpful for better characterizing epigenetic age relationships. Lastly, while our study utilizes the most recent methylation data available in NHANES at the time of analysis, these data are approximately two decades old. Given shifts in U.S. immigration patterns and demographic composition over the past 20 years, the generalizability of our findings to contemporary populations may be limited. Despite

this constraint, our research addresses novel questions and leverages a nationally representative sample, offering foundational insights that can guide future investigations in this area.

5. Conclusion

In summary, this study of U.S. adults aged 50–84 years reports relationships of citizenship and immigrant status with epigenetic aging-related measures (GrimAge2 and DNAm-predicted GrimAge2 proteins). If confirmed, these findings suggest that epigenetic aging measures may be valuable for studying socioeconomic and broader factors important to citizen and immigrant health.

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Author contributions

Jamaji Nwanaji-Enwerem conceived of the analyses, performed data analysis, visualization, original writing. Patricia Rodriguez Espinosa, David Rehkopf, Hanyang Shen, Nicole Gladish, Anne Bozack, Saher Daredia, and Belinda Needham contributed to the analysis. Andres Cardenas and David Rehkopf supervised the work. All authors contributed to writing/editing of the manuscript.

Disclosure statement

The authors have no relevant affiliations orfinancial involvement with any organization or entity with a financial interestin or financial conflict with the subject matter or materials discussed in themanuscript. This includes employment, consultancies, honoraria, stock ownershipor options, expert testimony, grants or patents received or pending, orroyalties.

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Ethical declaration

All NHANES participants gave written informed consent, and the study protocols received approval from the NCHS Research Ethics Review Board (protocol #98–12).

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Data availability statement

The datasets analyzed in the current study are available from the NHANES website.

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