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Running title: DNA methylation-predicted GDF-15 and mortality in cancer

DNA methylation-predicted GDF-15 and mortality in cancer survivors: a cohort study

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Developing non-invasive prognostic biomarkers remains critical to improving personalized cancer care. Growth differentiation factor-15 (GDF-15), a TGF-β family cytokine, plays a key role in tumorigenesis and immune evasion. Circulating GDF-15 serves as a biomarker for cancer prognosis, and DNA methylation (DNAm)-predicted GDF-15 has been linked to mortality risk in the general population. However, the association between DNAm-predicted GDF-15 and mortality risk in cancer survivors remains unexplored. We analyzed the association between DNAm-predicted GDF-15 and all-cause, long-term all-cause, and cancer mortality risks using a cohort of 343 cancer survivors from the National Health and Nutrition Examination Survey (NHANES) 1999-2002 with a median follow-up of 138 months. Multivariable Cox regression reporting hazard ratios (HRs) and 95% confidence intervals (CIs) demonstrated that each 1-standard deviation (SD) increment in DNAm-predicted GDF-15 was associated with a 60% higher all-cause mortality risk adjusted with model 1 of age and sex, and a 54% greater all-cause mortality risk in model 2 adjusted additionally for ethnicity, education, smoking, and coronary heart disease. Participants in the high GDF-15 tertile showed a 201% and 166% higher mortality risk in model 1 and model 2, respectively (both p for trend < 0.0001) compared to the low tertile. Its association with long-term mortality risk remains unchanged. Stratified analyses indicated consistent relationships across multiple subgroups. Kaplan-Meier and competing risk analyses revealed a graded increase in cancer mortality risk across ascending GDF-15 tertiles; Cox models confirmed a significant positive association per 1-SD increment in the unadjusted model and model 1, which remained consistent in direction and magnitude in model 2, with a marginally significant (p = 0.052). The current study provided evidence that DNAm-predicted GDF-15, an alternative and precise estimate of GDF-15 based on DNA methylation, is positively associated with all-cause and long-term all-cause mortality risks and showed a trend of positive association with cancer mortality among cancer survivors. Future larger longitudinal studies with serial DNAm-predicted GDF-15 assessments are needed to verify potential causal links.

Key words: GDF-15; mortality; cancer; methylation; survival

Cancer is the second leading cause of death in the United States and poses a significant public health challenge globally [1]. The aging and expanding population is expected to result in a nearly 50% rise in new cancer cases by 2050 [2]. Although there have been advancements in multidisciplinary treatment methods in recent years, the outlook for many patients with cancer continues to be unfavorable. Identifying more accurate, straightforward, and non-invasive screening markers related to cancer prognosis holds considerable clinical importance and has the potential to enhance prognostic predictions and facilitate personalized treatment strategies.

Growth differentiation factor-15 (GDF-15, also referred to as macrophage inhibitory cytokine-1, MIC-1) is a cytokine that belongs to the transforming growth factor-β (TGF-β) protein family [3]. The expression of GDF-15 is low under normal conditions except in the placenta. Its expression can be induced in response to stress conditions [4] and is reported to be abundantly produced in various cancers. It has been reported that GDF-15 plays an essential role in tumorigenesis [5-7]. In recent years, it has attracted growing interest as it has been found to interfere with antitumoral immune checkpoint blockade; neutralizing GDF-15 has shown potential for overcoming resistance and improving immunotherapy outcomes [8, 9]. Increasing evidence has demonstrated the circulating GDF-15 protein level to be an effective biomarker for early detection and prognosis in a spectrum of malignancies [10-15]. Epigenetic-related measures enable the quantification of DNA sequence-independent genomic alterations, providing a stable, long-term surrogate to circulating biomarkers [16-19]. DNA methylation (DNAm)-predicted GDF-15 was developed using DNAm levels of 137 CpGs as a surrogate of plasma level GDF-15 protein with a high correlation coefficient [20]. It has been reported to be an effective predictor for mortality risk in a general population [17]. However, the association between DNAm-predicted GDF-15 and mortality risk in cancer survivors remains unclear; in addition, the associations between DNAm-predicted GDF-15 and long-term mortality and cancer mortality are lacking.

We hypothesize that DNAm-predicted GDF-15 is positively associated with mortality risk in cancer individuals and investigate the relationship using a cohort of 343 cancer survivors from the NHANES dataset 1999-2002.

Patients and methods

Study population. This cross-sectional study utilized data from NHANES 1999-2000 and 2001-2002 as the DNA methylation epigenetic biomarker data, released on July 31, 2024, were exclusively available in these cycles in a selection of participants aged 50 years or older, and were not collected or released in any other NHANES cycles. The NHANES protocols, including experimental procedures, were approved by the National Centre for Health Statistics Research Ethics Review Board. All participants provided written informed consent without compensation, and the requirement for consent to use public data was waived. NHANES employed a complex, stratified, clustered probability design to recruit a nationally representative sample of non-institutionalized US civilians. The survey consists of two parts: interviews conducted at participants' homes and physical examinations carried out at mobile examination centers. Additional details about NHANES procedures are available at (https://www.cdc.gov/nchs/nhanes/index.htm). The study adhered to the Declaration of Helsinki principles, and its reporting was guided by the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guideline. From the initial pool of 21,004 participants, exclusions were made for the following reasons: self-reported denial of a cancer diagnosis (N=20,494); missing DNA epigenetic marker data (N=563). No participants were excluded due to missing mortality follow-up data. The final analysis included 343 cancer survivors.

Ascertainment of cancer. Cancer diagnoses were self-reported and determined [21] by participants in response to the question: "Has a doctor or health professional ever diagnosed you with cancer or any malignancy?". Trained interviewers administered this assessment using the Computer-Assisted Personal Interview (CAPI) system [22], which includes built-in consistency checks to reduce data entry errors.

DNAm-predicted GDF-15 measurement. DNAm-predicted GDF-15 was developed using methylation levels at 137 CpG sites, which showed high correlation with plasma GDF-15 protein levels [20]. DNA was extracted from whole blood samples collected from a randomly selected subset of NHANES participants aged 50 years or older, with storage at -80 °C. DNA methylation analysis was performed using the Illumina Infinium MethylationEPIC BeadChip v1.0 (Illumina, San Diego, CA, USA). The raw methylation data underwent preprocessing, normalization, and biomarker calculation in R (version 4.3). Detailed laboratory protocols and bioinformatics workflows are documented (https://wwwn.cdc.gov/nchs/data/nhanes/dnam/NHANES%20DNAm%20Epigenetic%20Biomarkers%20Data%20Documentation.pdf).

Mortality. Mortality status and follow-up data were sourced from the publicly available National Death Index-linked mortality file. All-cause mortality was defined as death due to any reason. Cancer mortality was defined as deaths attributed to malignant neoplasms (ICD-10 codes C00-C97). Person-months of follow-up were calculated from the NHANES mobile examination center visit date until either the date of death or the end of the mortality follow-up period (December 31, 2019).

Covariates. Participants were classified as smokers if they had smoked a cumulative total of 100 or more cigarettes during their lifetime; otherwise, they were categorized as non-smokers. The diagnoses of coronary heart disease (CHD), hypertension, diabetes, and chronic kidney disease were self-reported and determined according to the responses to the NHANES interview questionnaire [21, 23, 24]. Biological aging [25] was evaluated using the HorvathAge epigenetic clock [26], which was categorized into 3 tertiles (low, middle, and high). Frailty assessment used a modified version following the principle of the Modified Fried Frailty Phenotype [27]. As the NHANES 1999–2002 did not contain variables corresponding to the criterion of exhaustion, to be consistent with the conceptual framework of the original model, we included four of the five components (weakness, low physical activity, slow walking speed, and unintentional weight loss). Participants were subsequently categorized into two frailty strata for analysis: little or no (demonstrating zero to two criteria) and pronounced frailty (demonstrating three or more criteria).

Statistical analysis. Baseline characteristics were reported as means and standard deviations (SD) for continuous variables and counts and percentages for categorical variables. Group differences were assessed using Student's t-tests for continuous variables and Chi-square tests for categorical variables. DNAm-predicted GDF-15 was analyzed as a continuous measure (per 1-SD increase) or categorized into tertiles, with the low tertile as the reference. Rescrtic cubic spline and Kaplan-Meier curves were employed to demonstrate the association between DNAm-predicted GDF-15 and mortality. To mitigate potential reverse causality, the association between DNAm-predicted GDF-15 and long-term all-cause mortality was assessed by excluding participants who died within the first two years of follow-up. For the association between DNAm-predicted GDF-15 and cancer mortality, competing risk analysis using the Fine-Grey hazard model was conducted, treating deaths from non-cancer causes as competing events. To analyze the association between DNAm-predicted GDF-15 and mortality risk, we performed multivariable Cox regression, reporting hazard ratios (HRs) and 95% confidence intervals (CIs). Multivariable adjustments were made in two models. Model 1 was adjusted for age and sex. Model 2 was further adjusted for race/ethnicity (non-Hispanic White, non-Hispanic Black, Mexican American, others), education level (< high school, high school equivalent, ≥ college), smoking status (smoker, non-smoker), and CHD. Stratified analyses were conducted by sex (male/female), age (50-65 vs. > 65 years), race/ethnicity, education level, smoking status, and CHD. Each subgroup analysis was adjusted for all covariates except the stratification variable. Potential effect modification was tested using log-likelihood ratio tests. As sensitivity analyses, stratified analyses were further employed by adjusting for hypertension, diabetes, chronic kidney disease, biological aging evaluated by HorvathAge epigenetic clock tertiles, and frailty based on model 2 to assess whether DNAm-predicted GDF-15 offers prognostic value in cancer survivors beyond its general association with aging-related mortality. All statistical analyses were performed in R (version 4.4), with statistical significance set at p-value < 0.05 (two-tailed).

Results

Baseline characteristics. The baseline characteristics of 343 cancer survivors are presented in Table 1. Participants in the high tertile of DNAm-predicted GDF-15 were older, less likely to be well-educated, and more likely to be male (p < 0.05).

DNAm-predicted GDF-15 and mortality. A median follow-up of 138 months (range 7-248 months) documented 239 all-cause deaths. Cancer survivors exhibited a positive linear association between DNAm-predicted GDF-15 levels and increased all-cause mortality risk (Figure 1A), Kaplan-Meier analysis revealed those in the low GDF-15 tertile maintained the highest survival probability throughout follow-up (log-rank p < 0.001, Figure 1B). Multivariable Cox regression demonstrated each 1-SD increment in DNAm-predicted GDF-15 conferred a 60% greater mortality risk after age and sex adjustment, which remained a 54% higher after further adjustment for race/ethnicity, education level, smoking, and CHD. Participants in the high GDF-15 tertile showed a 201% and 166% increased mortality risk in model 1 and fully adjusted model, respectively (both p-values for trend < 0.0001), with the low tertile serving as reference (Table 2).

After excluding 23 subjects who died within two years of follow-up, the association between DNAm-predicted GDF-15 and long-term all-cause mortality remained consistent with the primary outcome (Table 3). Multivariable Cox regression indicated that each 1-SD increase in

DNAm-predicted GDF-15 was associated with a 64% higher long-term mortality risk in model 1, with this association persisting at a 54% higher risk in model 2. For tertile comparisons, the high GDF-15 tertile had a 211% and 151% higher long-term mortality risk compared to the low tertile in models 1 and 2, respectively (both p-values for trend < 0.0001).

Sixty-nine cancer deaths were documented. Kaplan-Meier analysis demonstrated significantly higher cancer mortality risk for participants in the high tertile of DNAm-predicted GDF-15 compared to those in the low tertile (log-rank p < 0.001; Figure 2A). The cumulative incidence curves from the competing risk analysis showed a graded increase in the probability of cancer death across ascending GDF-15 tertiles (Figure 2B). Cox regression analyses revealed that each 1-SD increase in DNAm-predicted GDF-15 was associated significantly with a 72% and a 39% increase higher risk of cancer mortality in the unadjusted model and model 1 respectively. In model 2, the magnitude and direction of the association were consistent with those observed in model 1, with marginal significance (p=0.052). A positive but non-significant trend was observed across increasing GDF-15 levels in model 2 (p-value for trend = 0.057) when analyzed by tertiles (Table 4).

Stratified analyses. Stratified analyses (Figure 3) indicated that the observed association between DNAm-predicted GDF-15 and all-cause mortality was consistent across various subgroups, including age, sex, ethnicity, education level, and CHD. Smoking status showed effect modification when unadjusted for multiple comparisons. After Benjamini–Hochberg false discovery rate (FDR) correction (q=0.05), none of the variables show statistically significant effect modification.

Sensitivity analyses. After further adjusting for hypertension, diabetes, chronic kidney disease, HorvathAge, and frailty based on model 2, stratified analysis showed that the association between DNAm-predicted GDF-15 and all-cause mortality remained consistent across the majority of subgroups, including cancer survivors without hypertension (1.89, 1.37-2.61), without diabetes (1.58, 1.30-1.92), without chronic kidney disease (1.62, 1.36-1.93), those with slower epigenetic aging (HorvathAge low tertile, 1.46, 1.04-2.06; middle tertile, 1.71, 1.16-2.52), and those with little or no frailty (1.61, 1.35-1.93), with most interaction terms being statistically nonsignificant except for education level and chronic kidney disease. The subgroup of chronic kidney disease should be considered as exploratory due to the small sample size (n=21) (Supplementary Table S1).

Discussion

This prospective cohort study of 343 cancer survivors with a median follow-up of 138 months demonstrated that the baseline DNAm-predicted GDF-15 was significantly and positively associated with the risk of all-cause and long-term all-cause mortality. The association remained consistent in both unadjusted and fully adjusted models. Stratified analyses indicated robust relationships across multiple subgroups. A positive association was consistently observed between DNAm-predicted GDF-15 and cancer mortality. These results suggest that DNAm-predicted GDF-15 may serve as a potential prognostic biomarker for the risk of mortality in cancer individuals.

Our study showed a positive association between DNAm-predicted GDF-15 and all-cause and long-term mortality in cancer individuals. Similarly, Luo and Shen reported that DNAm-predicted GDF-15 was significantly associated with all-cause mortality risks among a population of NHANES 1999-2002 without disease-based selection (N=1,912, with 267 cancer participants included) [17]. However, Luo et al. did not assess the association between DNAm-predicted GDF-15 and the risks of long-term and cancer-specific mortalities; in addition, our study was restricted to cancer survivors (N=343), which extends previous findings by validating DNAm-GDF-15 as an effective prognostic marker in a distinct high-risk group and enhances generalizability. DNAm-predicted GDF-15 was constructed using a subset of 137 CpGs that linear combination best predicted plasma GDF-15 protein level, and has been proven to be an effective surrogate measure, with a reported correlation of 0.74 and 0.53 of plasma GDF-15 protein in the training and test data, respectively [20]. The circulating DNAm-predicted GDF-15 and GDF-15 protein are both related, yet distinct. Methylation patterns can vary in response to disease conditions, aging, and environmental stressors, with DNA methylation at specific CpG sites influencing gene expression. Consequently, DNAm-predicted GDF-15 may offer a more consistent and long-term assessment of physiological stress compared to plasma GDF-15 protein to some degree. Although few studies have explored the association between DNAm-predicted GDF15 and mortality, the associations between elevated circulating GDF-15 and mortality risks [28-30], metabolic dysfunctions, frailty, and biological aging [31-33] have been established across diverse patient populations and clinical settings. Our findings could partly be explained by that, as a stress-responsive cytokine, GDF-15 expression was induced by oxidative stress, inflammation, and mitochondrial dysfunction, leading to an increase in circulating GDF-15 concentration among cancer survivors with higher mortality risk. Considering that GDF-15 is strongly associated with frailty, a state of reduced stress tolerance to external stressors [34] that may result from disease progression, and to mitigate reverse causality, where elevated DNAm-predicted GDF-15 could merely reflect frailty-induced outcomes as a consequence of the late stage of disease, we excluded participants who died within two years of follow-up. DNAm-predicted GDF-15 remained significantly associated with an increased risk of long-term mortality. Notably, stratified analyses showed consistent findings of the positive association between DNAm-predicted GDF-15 and all-cause mortality in cancer survivors without hypertension, diabetes, chronic kidney disease, with slower epigenetic aging, and those with little or no frailty, these support that the relationship between DNAm-predicted GDF-15 and mortality risk in the cancer population may involve broader mechanisms beyond merely reflecting frailty status or aging-related decline.

We also found a trend of positive association between DNAm-predicted GDF-15 and cancer mortality risk, which has not been reported before. In the realm of cancer, a body of clinical literature has proven plasma GDF-15 to be an effective prognostic marker of survival in various malignancies. In a cohort of locally advanced NSCLC patients undergoing chemoradiotherapy, elevated baseline plasma GDF-15 levels demonstrated significant positive correlations with larger gross tumor volumes and independently predicted inferior relapse-free survival and overall survival in multivariate analyses [14]. A meta study showed that lung cancer patients with high GDF-15 levels were strongly associated with poorer 3-year overall survival (OR 4.05, 95% CI 1.92-8.51) compared to those with low levels, supporting its role as a robust prognostic biomarker in cancer outcomes [13]. In lower-grade glioma patients from the TCGA cohort, elevated GDF-15 expression was found to be correlated with aggressive clinical features and served as an independent predictor of poor overall survival [11]. In both pancreatic ductal adenocarcinoma patients and preclinical mouse models, GDF-15 was reported to be a robust independent prognostic biomarker, with rising levels correlating to tumor burden, cachexia development, and poorer survival outcomes, suggesting its utility for early detection and risk stratification [12]. A possible explanation for the association, although the precise mechanism needs further elucidation, is that GDF-15 has protumorigenic properties. On one hand, it could mediate multiple downstream signaling cascades involved in cancer progression. These include the oncogenic pathways of PI3K/AKT and MAPK signaling, as evidenced by phosphorylation of AKT1 and MAPK in prostate, cervical, and colorectal cancers, enhancing cell survival and therapy resistance [35, 36], epithelial-mesenchymal transition (EMT) and metastatic invasion through IGF1R and MAPK phosphorylation, facilitating tumor dissemination in breast cancer [37], and SMAD signaling in head and neck cancer and glioblastoma, sustaining cancer stem cell populations and conferring resistance to radiation therapy [38, 39]. On the other hand, GDF-15 plays a critical role in suppressing T cell migration, facilitating tumor immune evasion, creating an immunosuppressive tumor microenvironment, and leading to resistance to cancer immunotherapy [9]. Neutralizing GDF-15 has shown promising potential in sensitizing resistant tumors to the immune checkpoint inhibitors [8]. Additionally, GDF-15 has been reported to activate the hypoxia-inducible factor-1α (HIF-1α) and facilitate tumor angiogenesis [40]. However, a dual role of GDF-15 in tumorigenesis has been proposed [41, 42]. In contrast to its oncogenic property, GDF-15 could also exhibit a tumoral suppressor property, although the latter is reported much less often than the former. For example, in preclinical animal studies, the overexpression of GDF-15 in cancer cell lines, including HCT116, MCF-7, PC-3, and glioblastoma, could inhibit tumor growth [43, 44]. Moreover, the expression of GDF-15 has been shown to induce apoptosis in various cancer cells *in vitro* [45]. Its antitumor and protumor effects may vary depending on the type and stage of cancer [42]. In addition to its context-dependent anti- and pro-tumorigenic functions, GDF15 has been implicated in cachexia [46], a condition often viewed as a consequence of either direct tumor progression or the host's aberrant homeostatic response to cancer-induced systemic physiological alterations spanning the processes of tumor initiation and progression [47]. As our study focused on a pan-cancer population and lacked distinct cancer staging, and given the diversity of cancer types, it was not feasible to analyze the relationship for each specific cancer. In addition, it should be noted that although the observed hazard ratios for DNAm-predicted GDF-15 consistently suggested a positive trend of elevated cancer mortality risk, the 95% confidence intervals in the adjusted models were wide and included the null value at the lower bound (e.g., model 2, per 1-SD: 1.00–1.90), resulting in borderline P values ($P = 0.052$). The lack of definitive statistical significance is likely attributable in large part to a low event-per-variable ratio, thereby limiting the statistical power of the multivariate models. Therefore, the association between DNAm-predicted GDF-15 and cancer-specific mortality remains inconclusive and should be validated through larger cohort studies focusing on specific cancer types.

The current study has several strengths. We provide the first evidence of a positive association between DNAm-predicted GDF-15 and mortality risks among the cancer population. The use of a prospective cohort based on a non-institutional U.S cancer sample enhances generalizability. Detailed covariate data were considered as possible confounders. Our study reported a positive association between DNAm-predicted GDF-15 levels and long-term mortality, which supports the potential role of GDF-15 in predicting survival beyond its established link to frailty and mitigates concerns about reverse causality. Several limitations of the current study should be acknowledged. First, DNAm-predicted GDF-15 was measured only once, limiting insight into temporal fluctuations compared to repeated assessments. Second, although cancer diagnoses followed standardized protocols, self-reported data inherently carry risks of recall bias and misclassification without clinical verification. Third, cause-of-death information from death certificates may not always be precise. Fourth, the dataset lacked granular details on cancer types, stages, and treatment records, which could potentially influence the observed associations. Fifth, the independent association between GDF-15 and cancer mortality should be considered tentative due to the low number of cancer-specific deaths. In addition, despite our efforts to adjust for confounders, residual or unmeasured factors may still have affected the results. Finally, the current findings cannot establish causality among cancer survivors. Future larger longitudinal studies involving serial DNAm-predicted GDF-15 assessments are required.

In conclusion, the current study provided preliminary evidence that DNAm-predicted GDF-15, an alternative and precise estimate of GDF-15 based on DNA methylation, is an effective predictor positively associated with all-cause and long-term all-cause mortality risks and showed a trend of positive association with cancer mortality among cancer survivors. Future larger longitudinal studies with serial DNAm-predicted GDF-15 assessments are needed to verify potential causal links.

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Supplementary data are available in the online version of the paper.

References

[1] SIEGEL RL, MILLER KD, WAGLE NS, JEMAL A. Cancer statistics, 2023. CA Cancer J Clin 2023; 73: 17-48. https://doi.org/10.3322/caac.21763

[2] SUNG H, FERLAY J, SIEGEL RL, LAVERSANNE M, SOERJOMATARAM I et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin 2021; 71: 209-249. https://doi.org/10.3322/caac.21660

[3] BOOTCOV MR, BAUSKIN AR, VALENZUELA SM, MOORE AG, BANSAL M et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-beta superfamily. Proc Natl Acad Sci U S A. 1997; 94: 11514-11519. https://doi.org/10.1073/pnas.94.21.11514

[4] TSAI VWW, HUSAINI Y, SAINSBURY A, BROWN DA, BREIT SN. The MIC-1/GDF15-GFRAL Pathway in Energy Homeostasis: Implications for Obesity, Cachexia, and Other Associated Diseases. Cell Metab 2018; 28: 353-368. https://doi.org/10.1016/j.cmet.2018.07.018

[5] KOTSONI A, KOZAKI LV, STYLIANOU A, GKRETSI V. Interdependent roles for growth differentiation factor-15 (GDF15) and LIMS1 in regulating cell migration: Implications for colorectal cancer metastasis. Biochim Biophys Acta Mol Cell Res 2025; 1872: 119904. https://doi.org/10.1016/j.bbamcr.2025.119904

[6] JOO M, KIM D, LEE MW, LEE HJ, KIM JM. GDF15 Promotes Cell Growth, Migration, and Invasion in Gastric Cancer by Inducing STAT3 Activation. Int J Mol Sci 2023; 24. https://doi.org/10.3390/ijms24032925

[7] RAI AB, CODI JAK, SUCHITHA GP, HEMAVATHI KN, DAGAMAJALU S et al. Mapping growth differentiation factor-15 (GDF15)-mediated signaling pathways in cancer: insights into its role across different cancer types. Discov Oncol 2025; 16: 386. https://doi.org/10.1007/s12672-025-02121-1

[8] MELERO I, DE MIGUEL LUKEN M, DE VELASCO G, GARRALDA E, MARTIN-LIBERAL J et al. Neutralizing GDF-15 can overcome anti-PD-1 and anti-PD-L1 resistance in solid tumours. Nature 2025; 637: 1218-1227. https://doi.org/10.1038/s41586-024-08305-z

[9] MELERO I, KLAR K, LEO E. GDF-15 blockade: A multi-directional approach to potentiate cancer immunotherapy and alleviate cancer cachexia. Clin Transl Med 2025; 15: e70280. https://doi.org/10.1002/ctm2.70280

[10] DEPOTTE L, NAY P, BORG C, MEURISSE A, HENRIQUES J et al. Interplay between sarcopenia, GDF-15, and the efficacy of nivolumab plus ipilimumab in patients with mismatch repair deficient metastatic colorectal cancer: final survival analysis of the phase II GERCOR NIPICOL study. J Immunother Cancer 2025; 13. https://doi.org/10.1136/jitc-2024-011220

[11] WANG Y, CHEN J, CHEN C, PENG H, LIN X et al. Growth differentiation factor-15 overexpression promotes cell proliferation and predicts poor prognosis in cerebral lower-grade gliomas correlated with hypoxia and glycolysis signature. Life Sci 2022; 302: 120645. https://doi.org/10.1016/j.lfs.2022.120645

[12] ZHU X, OLSON B, KEITH D, NORGARD MA, LEVASSEUR PR et al. GDF15 and LCN2 for early detection and prognosis of pancreatic cancer. Transl Oncol 2024; 50: 102129. https://doi.org/10.1016/j.tranon.2024.102129

[13] PAN T, DUAN R, XU Z, HE X, LUO X et al. GDF-15 as a biomarker for diagnosis and prognosis of lung cancer: a meta-analysis. Front Oncol 2025; 15: 1447990. https://doi.org/10.3389/fonc.2025.1447990

[14] DI PASTENA F, POND G, TSAKIRIDIS EE, GOUVEIA A, AHMADI E et al. Growth differentiation factor 15 (GDF15) predicts relapse free and overall survival in unresected locally advanced non-small cell lung cancer treated with chemoradiotherapy. Radiat Oncol 2024; 19: 155. https://doi.org/10.1186/s13014-024-02546-y

[15] NOPP S, MOIK F, KRALER S, ENGLISCH C, PREUSSER M et al. Growth differentiation factor-15 and prediction of cancer-associated thrombosis and mortality: a prospective cohort study. J Thromb Haemost 2023; 21: 2461-2472. https://doi.org/10.1016/j.jtha.2023.04.043

[16] VERSCHOOR CP, VLASSCHAERT C, RAUH MJ, PARE G. A DNA methylation based measure outperforms circulating CRP as a marker of chronic inflammation and partly reflects the monocytic response to long-term inflammatory exposure: A Canadian Longitudinal Study on Aging analysis. Aging Cell 2023; 22: e13863. https://doi.org/10.1111/acel.13863

[17] LUO H, SHEN Y. Association between DNA methylation predicted growth differentiation factor 15 and mortality: results from NHANES 1999-2002. Aging Clin Exp Res 2024; 36: 234. https://doi.org/10.1007/s40520-024-02896-3

[18] BANNISTER S, KIM B, DOMINGUEZ-ANDRES J, KILIC G, ANSELL BRE et al. Neonatal BCG vaccination is associated with a long-term DNA methylation signature in circulating monocytes. Sci Adv 2022; 8: eabn4002. https://doi.org/10.1126/sciadv.abn4002

[19] EISENBERG DTA, RYAN CP, LEE NR, CARBA DB, MACISAAC JL et al. DNA methylation-based estimators of telomere length show low correspondence with paternal age at conception and other measures of external validity of telomere length. Geroscience 2024; 46: 3957-3969. https://doi.org/10.1007/s11357-024-01114-2

[20] LU AT, QUACH A, WILSON JG, REINER AP, AVIV A et al. DNA methylation GrimAge strongly predicts lifespan and healthspan. Aging (Albany NY) 2019; 11: 303-327. https://doi.org/10.18632/aging.101684

[21] NONG J, TONG J, WANG R, SHI K, ZHANG Y. Associations of sleep disorders with all-cause and cause-specific mortality in cancer survivors: a cross-sectional analysis of the NHANES 2005-2016. BMC Psychiatry 2024; 24: 118. https://doi.org/10.1186/s12888-024-05589-3

[22] LI C, FORD ES, ZHAO G, TSAI J, BALLUZ LS. A comparison of depression prevalence estimates measured by the Patient Health Questionnaire with two administration modes: computer-assisted telephone interviewing versus computer-assisted personal interviewing. Int J Public Health 2012; 57: 225-233. https://doi.org/10.1007/s00038-011-0253-9

[23] NONG J, WANG R, ZHANG Y. Association of lymphocyte-to-C-reactive protein ratio with all-cause and cause-specific mortality among US cancer survivors. Eur J Med Res 2025; 30: 312. https://doi.org/10.1186/s40001-025-02527-1

[24] NONG J, ZHANG Y. Circulating Klotho and mortality patterns among US cancer survivors: A cohort study. Medicine 2025; 104. https://doi.org/10.1097/md.0000000000043471

[25] PERRI G, FRENCH C, AGOSTINIS-SOBRINHO C, ANAND A, ANTARIANTO RD et al. An expert consensus statement on biomarkers of ageing for use in intervention studies. J Gerontol A Biol Sci Med Sci 2024. https://doi.org/10.1093/gerona/glae297

[26] HORVATH S. DNA methylation age of human tissues and cell types. Genome Biol. 2013; 14: R115. https://doi.org/10.1186/gb-2013-14-10-r115

[27] KURNAT-THOMA EL, MURRAY MT, JUNEAU P. Frailty and Determinants of Health Among Older Adults in the United States 2011-2016. J Aging Health 2022; 34: 233-244. https://doi.org/10.1177/08982643211040706

[28] XIE S, LI Q, LUK AOY, LAN HY, CHAN PKS et al. Major Adverse Cardiovascular Events and Mortality Prediction by Circulating GDF-15 in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis. Biomolecules 2022; 12. https://doi.org/10.3390/biom12070934

[29] CICERI P, BONO V, MAGAGNOLI L, SALA M, ARTIOLI L et al. MO409: GDF-15 is a Predictor of Mortality in Chronic Kidney Disease Patients With Covid-19 Infection. Nephrol Dial Transplant 2022; 37: 2295 https://doi.org/10.1093/ndt/gfac070.023

[30] KOKKORAKIS M, FOLKERTSMA P, FORTE JC, WOLFFENBUTTEL BHR, VAN DAM S et al. GDF-15 improves the predictive capacity of steatotic liver disease non-invasive tests for incident morbidity and mortality risk for cardio-renal-metabolic diseases and malignancies. Metabolism 2025; 163: 156047. https://doi.org/10.1016/j.metabol.2024.156047

[31] TIAN T, LIU M, LITTLE PJ, STRIJDOM H, WENG J et al. Emerging Roles of GDF15 in Metabolic and Cardiovascular Diseases. Research (Wash D C) 2025; 8: 0832. https://doi.org/10.34133/research.0832

[32] GONCALVES R, MACIEL ACC, ROLLAND Y, VELLAS B, DE SOUTO BARRETO P. Frailty biomarkers under the perspective of geroscience: A narrative review. Ageing Res Rev 2022; 81: 101737. https://doi.org/10.1016/j.arr.2022.101737

[33] CONTE M, GIULIANI C, CHIARIELLO A, IANNUZZI V, FRANCESCHI C et al. GDF15, an emerging key player in human aging. Ageing Res Rev 2022; 75: 101569. https://doi.org/10.1016/j.arr.2022.101569

[34] KAMPER RS, NYGAARD H, PRAEGER-JAHNSEN L, EKMANN A, DITLEV SB et al. GDF-15 is associated with sarcopenia and frailty in acutely admitted older medical patients. J Cachexia Sarcopenia Muscle 2024; 15: 1549-1557. https://doi.org/10.1002/jcsm.13513

[35] CHEN SJ, KARAN D, JOHANSSON SL, LIN FF, ZECKSER J et al. Prostate-derived factor as a paracrine and autocrine factor for the proliferation of androgen receptor-positive human prostate cancer cells. Prostate 2007; 67: 557-571. https://doi.org/10.1002/pros.20551

[36] NAZAROVA N, QIAO S, GOLOVKO O, LOU YR, TUOHIMAA P. Calcitriol-induced prostate-derived factor: autocrine control of prostate cancer cell growth. Int J Cancer 2004; 112: 951-958. https://doi.org/10.1002/ijc.20510

[37] PEAKE BF, EZE SM, YANG L, CASTELLINO RC, NAHTA R. Growth differentiation factor 15 mediates epithelial mesenchymal transition and invasion of breast cancers through IGF-1R-FoxM1 signaling. Oncotarget 2017; 8: 94393-94406. https://doi.org/10.18632/oncotarget.21765

[38] PENG H, LI Z, FU J, ZHOU R. Growth and differentiation factor 15 regulates PD-L1 expression in glioblastoma. Cancer Manag Res 2019; 11: 2653-2661. https://doi.org/10.2147/CMAR.S192095

[39] LI YL, CHANG JT, LEE LY, FAN KH, LU YC et al. GDF15 contributes to radioresistance and cancer stemness of head and neck cancer by regulating cellular reactive oxygen species via a SMAD-associated signaling pathway. Oncotarget 2017; 8: 1508-1528. https://doi.org/10.18632/oncotarget.13649

[40] MIELCARSKA S, STOPINSKA K, DAWIDOWICZ M, KULA A, KICZMER P et al. GDF-15 Level Correlates with CMKLR1 and VEGF-A in Tumor-free Margin in Colorectal Cancer. Curr Med Sci 2021; 41: 522-528. https://doi.org/10.1007/s11596-021-2335-0

[41] WANG X, BAEK SJ, ELING TE. The diverse roles of nonsteroidal anti-inflammatory drug activated gene (NAG-1/GDF15) in cancer. Biochem Pharmacol 2013; 85: 597-606. https://doi.org/10.1016/j.bcp.2012.11.025

[42] HASANPOUR SEGHERLOU Z, NOURI-VASKEH M, NOROOZI GUILANDEHI S, BAGHBANZADEH A, ZAND R et al. GDF-15: Diagnostic, prognostic, and therapeutic significance in glioblastoma multiforme. J Cell Physiol 2021; 236: 5564-5581. https://doi.org/10.1002/jcp.30289

[43] MARTINEZ JM, SALI T, OKAZAKI R, ANNA C, HOLLINGSHEAD M et al. Drug-induced expression of nonsteroidal anti-inflammatory drug-activated gene/macrophage inhibitory cytokine-1/prostate-derived factor, a putative tumor suppressor, inhibits tumor growth. J Pharmacol Exp Ther 2006; 318: 899-906. https://doi.org/10.1124/jpet.105.100081

[44] LAMBERT JR, KELLY JA, SHIM M, HUFFER WE, NORDEEN SK et al. Prostate derived factor in human prostate cancer cells: gene induction by vitamin D via a p53-dependent mechanism and inhibition of prostate cancer cell growth. J Cell Physiol 2006; 208: 566-574. https://doi.org/10.1002/jcp.20692

[45] ALBERTONI M, SHAW PH, NOZAKI M, GODARD S, TENAN M et al. Anoxia induces macrophage inhibitory cytokine-1 (MIC-1) in glioblastoma cells independently of p53 and HIF-1. Oncogene 2002; 21: 4212-4219. https://doi.org/10.1038/sj.onc.1205610

[46] HULLWEGEN M, KLEINERT M, VON HAEHLING S, FISCHER A. GDF15: from biomarker to target in cancer cachexia. Trends Cancer 2025; 11: 1093-1105. https://doi.org/10.1016/j.trecan.2025.06.007

[47] SLOMINSKI RM, RAMAN C, CHEN JY, SLOMINSKI AT. How cancer hijacks the body's homeostasis through the neuroendocrine system. Trends Neurosci 2023; 46: 263-275. https://doi.org/10.1016/j.tins.2023.01.003

Figure Legends

Figure 1. The association between DNAm-predicted GDF-15 and all-cause mortality risk in cancer survivors A) The restricted cubic spline model shows a linear association between DNAm-predicted GDF-15 level and all-cause mortality risk. The hazard ratio (solid line) was adjusted for age, sex, race, education level, smoking status and CHD. Shaded areas represent 95% CIs. The model was conducted with 3 knots. B) Kaplan-Meier survival curves for the mortality outcome by tertiles of DNAm-predicted GDF-15. Abbreviations: DNAm-DNA methylation; CHD-coronary heart disease.

Figure 2. The association between DNAm-predicted GDF-15 and cancer mortality risk in cancer survivors. A) Kaplan-Meier survival curves for the cancer mortality by DNAm-predicted GDF-15 tertiles. B) Cumulative incidence curves from the competing risk analysis of cancer mortality stratified by DNA-predicted GDF-15 tertiles, with non-cancer death considered as a competing risk. Abbreviation: DNAm-DNA methylation

Figure 3. Stratified analyses of the association between DNAm-predicted GDF-15 and all-cause mortality risk. The forest plot illustrates the Cox regression analysis of the DNAm-predicted GDF-15-mortality association stratified by subgroups, adjusting for sex, age (50-65, > 65), race, education level, smoking, and CHD, except for each stratification variable itself. Abbreviations: DNAm-DNA methylation; CHD-coronary heart disease.

Table 1. Characteristics of the cancer survivors classified by the DNAm-predicted GDF-15 tertiles.

Characteristics	**Total (n = 330)**	**Low (n=114)**	**Middle (n=114)**	**High (n=115)**	**p-value**
		< 958	959-1109	> 1110	
Age, mean (SD)	70.5 (9.7)	61.5 (6.5)	71.6 (7.1)	78.0 (6.9)	< 0.001
Age					< 0.001
50-65	103 (30.0)	80 (70.2)	15 (13.2)	8 (7.0)	
> 65	240 (70.0)	34 (29.8)	99 (86.8)	107 (93.0)	
Sex					0.048
male	186 (54.2)	52 (45.6)	63 (55.3)	71 (61.7)	
Female	157 (45.8)	62 (54.4)	51 (44.7)	44 (38.3)	
Race and ethnicity					0.132
White	226 (65.9)	67 (58.8)	73 (64.0)	86 (74.8)	
Black	47 (13.7)	19 (16.7)	16 (14.0)	12 (10.4)	
Mexican American	50 (14.6)	23 (20.2)	17 (14.9)	10 (8.7)	
Others	20 (5.8)	5 (4.4)	8 (7.0)	7 (6.1)	
Education level					0.032
< high school	107 (31.2)	29 (25.4)	38 (33.3)	40 (34.8)	
High school or equivalent	80 (23.3)	25 (21.9)	20 (17.5)	35 (30.4)	
College or above	156 (45.5)	60 (52.6)	56 (49.1)	40 (34.8)	
Smoking status					0.091
smoker	214 (62.4)	63 (55.3)	79 (69.3)	72 (62.6)	
Non-smoker	129 (37.6)	51 (44.7)	35 (30.7)	43 (37.4)	
Coronary heart disease					0.129
Yes	44 (13.0)	10 (8.9)	14 (12.4)	20 (17.9)	
No	294 (87.0)	103 (91.1)	99 (87.6)	92 (82.1)	

Notes: Continuous variables: mean (SD); Categorical variables: number (95% CI)
Abbreviations: SD-standard deviation; CI-confidence interval

Table 2. Cox regression for the associations between DNAm-predicted GDF-15 and all-cause mortality.

Models	HR (95% CI); p-value				
	Continuous	Low tertile	Middle tertile	High tertile	p-value trend
Crude	1.97 (1.75, 2.22); < 0.0001	reference	2.93 (2.02, 4.23); < 0.0001	6.07 (4.22, 8.74); < 0.0001	< 0.00001
Model 1	1.60 (1.36, 1.88); < 0.0001	reference	1.79 (1.18, 2.73); 0.006	3.01 (1.90, 4.79); < 0.0001	< 0.00001
Model 2	1.54 (1.30, 1.83); < 0.0001	reference	1.63 (1.06, 2.49); 0.025	2.66 (1.67, 4.24); < 0.0001	0.00002

Notes: Model 1: Adjusted for age (continuous) and sex (male or female); Model 2: Further adjusted for race, education level, smoking status and coronary heart disease.

Abbreviation: CI-confidence interval

Table 3. Cox regression for the associations between DNAm-predicted GDF-15 and long-term all-cause mortality.

Models	HR (95% CI); p-value				
	Continuous	Low tertile	Middle tertile	High tertile	p-value trend
Crude	1.99 (1.75, 2.26); < 0.0001	reference	3.25 (2.19, 4.82); < 0.0001	5.99 (4.07, 8.82); <0.0001	< 0.0001
Model 1	1.64 (1.39, 1.95); < 0.0001	reference	2.11 (1.36, 3.27); 0.0009	3.11 (1.92, 5.04); <0.0001	< 0.0001
Model 2	1.54 (1.29, 1.84); < 0.0001	reference	1.82 (1.17, 2.82); 0.0080	2.51 (1.55, 4.06); 0.0002	0.0002

Notes: Long-term mortality: excluded 23 subjects who died within the first two years of follow-up; Model 1: Adjusted for age (continuous) and sex (male or female); Model 2: Further adjusted for race, education level, smoking status and coronary heart disease

Abbreviation: CI-confidence interval

Table 4. Cox regression for the associations between DNAm-predicted GDF-15 and cancer mortality.

Models	HR (95% CI); p-value				
	Continuous	Low tertile	Middle tertile	High tertile	p-value trend
Crude	1.72 (1.38, 2.15); <0.0001	reference	2.99 (1.51, 5.91); 0.002	4.50 (2.27, 8.93); < 0.0001	< 0.0001
Model 1	1.39 (1.02, 1.90); 0.040	reference	1.96 (0.90, 4.25); 0.089	2.39 (0.99, 5.73); 0.052	0.060
Model 2	1.38 (1.00, 1.90); 0.052	reference	1.94 (0.87, 4.31); 0.103	2.43 (1.00, 5.93); 0.051	0.057

Notes: Model 1: Adjusted for age (continuous) and sex (male or female); Model 2: Further adjusted for race, education level, smoking status and coronary heart disease

Abbreviation: CI-confidence interval

Fig. 1 Download full resolution image

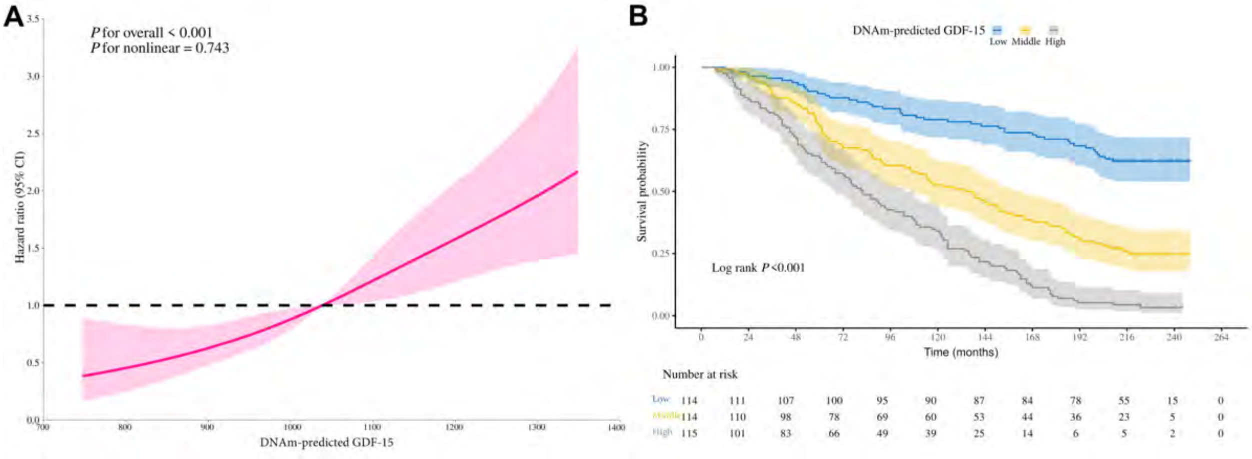


Fig. 2 Download full resolution image

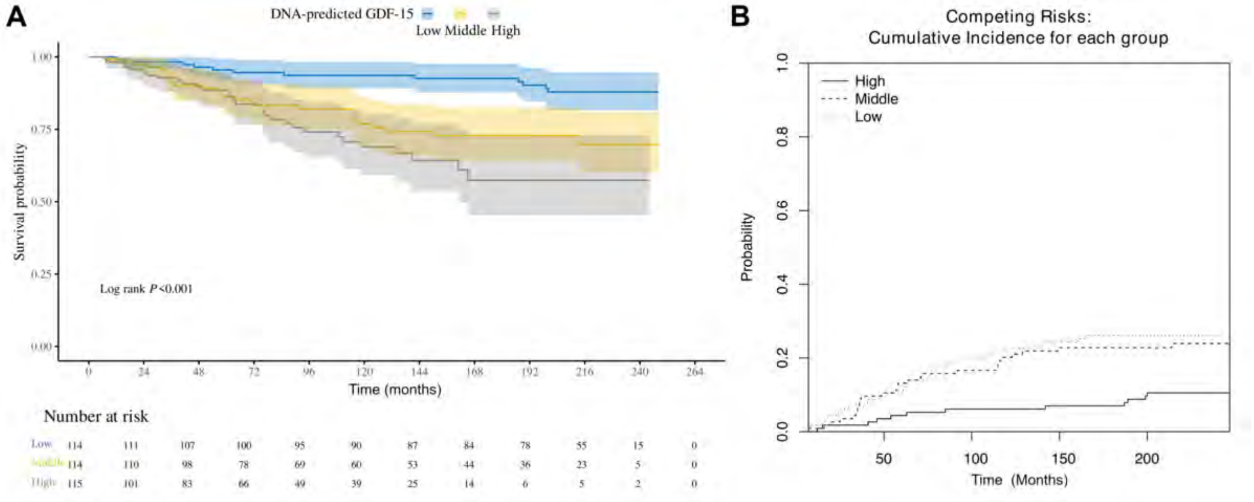
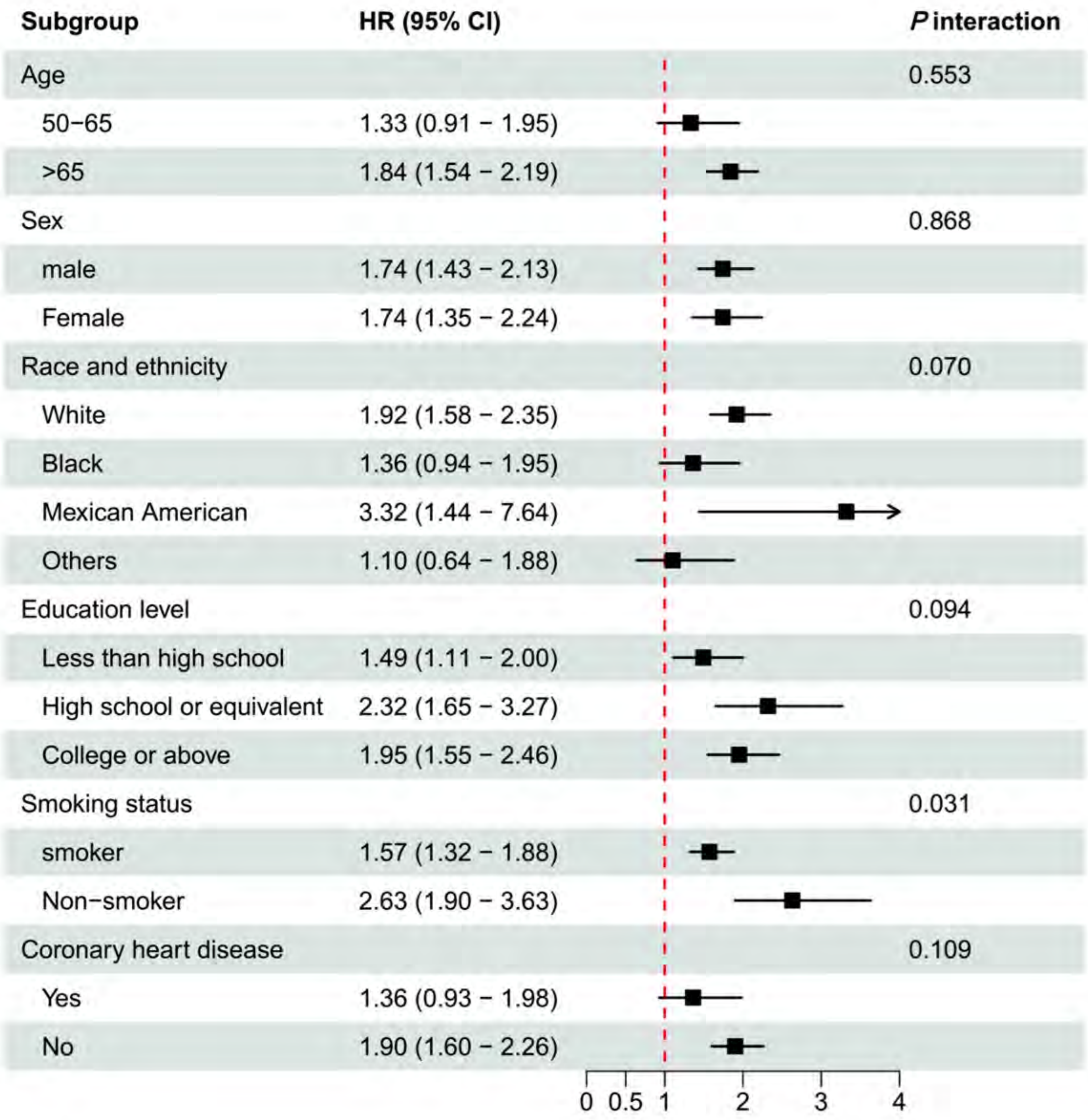


Fig. 3



Subgroup	HR (95% CI)	*P* interaction
Age		0.553
50−65	1.33 (0.91 − 1.95)	
>65	1.84 (1.54 − 2.19)	
Sex		0.868
male	1.74 (1.43 − 2.13)	
Female	1.74 (1.35 − 2.24)	
Race and ethnicity		0.070
White	1.92 (1.58 − 2.35)	
Black	1.36 (0.94 − 1.95)	
Mexican American	3.32 (1.44 − 7.64)	
Others	1.10 (0.64 − 1.88)	
Education level		0.094
Less than high school	1.49 (1.11 − 2.00)	
High school or equivalent	2.32 (1.65 − 3.27)	
College or above	1.95 (1.55 − 2.46)	
Smoking status		0.031
smoker	1.57 (1.32 − 1.88)	
Non−smoker	2.63 (1.90 − 3.63)	
Coronary heart disease		0.109
Yes	1.36 (0.93 − 1.98)	
No	1.90 (1.60 − 2.26)	