Gao et al. Clinical Epigenetics (2018) 10:84
https://doi.org/10.1186/s13148-018-0515-y

Clinical Epigenetics

RESEARCH Open Access

# Vitamin D status and epigenetic-based mortality risk score: strong independent and joint prediction of all-cause mortality in a population-based cohort study

Xu Gao[1,5], Yan Zhang[1], Ben Schöttker[1,2] and Hermann Brenner[1,2,3,4*]

## Abstract

**Background:** Vitamin D deficiency and insufficiency have been established to be strongly associated with increased overall mortality and deaths from specific aging-related diseases. Recently, an epigenetic "mortality risk score" (MS) based on whole blood DNA methylation at the 10 most prominent mortality-related cytosine-phosphate-guanine (CpG) sites has also been found to be highly related to all-cause mortality. This study aimed to explore whether vitamin D status, defined by serum 25-hydroxyvitamin D [25(OH)D] concentrations, is associated with the MS and to what extent both indicators are individually and jointly capable of predicting all-cause mortality in a general population sample of older adults.

**Results:** The MS was derived from the blood DNA methylation profiles measured by Illumina Human Methylation 450K Beadchip, and serum 25(OH)D concentration was measured among 1467 participants aged 50–75 of the German ESTHER cohort study. There was no association between vitamin D status and the MS at baseline, but both metrics were prominently and independently associated with all-cause mortality during a median follow-up of 15.2 years. The combination of both indicators showed the potential to be a particularly strong prognostic index for all-cause mortality. Participants with vitamin D deficiency (< 30 nmol/L) and high MS (> 5 CpG sites with aberrant methylation) had almost sixfold mortality (hazard ratio 5.79, 95% CI 3.06–10.94) compared with participants with sufficient vitamin D (≥ 50 nmol/L) and a low MS (0–1 CpG site with aberrant methylation).

**Conclusions:** This study suggests that vitamin D and the MS are strong independent predictors of all-cause mortality in older adults.

**Keywords:** DNA methylation, Epigenetic mortality risk score, Vitamin D, All-cause mortality, Epigenetic epidemiology, Precision medicine

## Background

Vitamin D is a critical nutrient that is, apart from some limited supply from diet and supplement use, mainly obtained from the biosynthesis within the human body in response to the exposure of solar ultraviolet B radiation [1]. Vitamin D status is commonly measured via assessing 25-hydroxyvitamin D [25(OH)D] concentrations in serum [1]. Vitamin D deficiency and insufficiency have been shown to be strongly associated with increased overall mortality, as well as deaths from specific aging-related diseases, such as cardiovascular disease (CVD) and various forms of cancer [2–4]. We previously performed a meta-analysis to summarize the results of eight prospective cohort studies from European countries and the USA to investigate the prognostic association of vitamin D status and mortality [3]. Comparing bottom vs. top quintiles of 25(OH)D concentrations resulted in a risk ratio of 1.57 (95% CI 1.36–1.81) for all-cause mortality.

* Correspondence: h.brenner@dkfz-heidelberg.de
[1]Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Im Neuenheimer Feld 581, 69120 Heidelberg, Germany
[2]Network Aging Research, University of Heidelberg, Bergheimer Straße 20, 69115 Heidelberg, Germany
Full list of author information is available at the end of the article

Recently, DNA methylation, one of the most studied and stable epigenetic modifications, has been shown to be associated with aging and aging-related health outcomes [5, 6] and recognized as the indicator for all-cause and disease-specific mortality [7]. In a recent epigenome-wide association study (EWAS) with approximately 1900 older adults followed up for 14 years and an external validation with 1727 participants, we identified 58 cytosine-phosphate-guanine (CpG) sites within 19 chromosomes that were associated with all-cause mortality [8]. We constructed a "mortality risk score" (MS) based on the 10 most robustly mortality-related loci, which was found to be a robust and informative predictor of all-cause, CVD, and cancer mortality. It is unclear, however, to what extent its association is independent of other well-established indicators of mortality risks. This study aimed to explore whether vitamin D status, defined by serum 25(OH)D concentrations, is associated with the MS and to what extent both indicators are individually and jointly capable of predicting all-cause mortality in a general population sample of older adults.

## Methods

### Study design and population

Study subjects were chosen from the ESTHER study, an ongoing statewide population-based cohort study conducted in Saarland, a state located in southwestern Germany. Details of the study design have been reported previously [8, 9]. As shown in Fig. 1, 9949 older adults (aged 50–75 years) were enrolled by their general practitioners during a routine health checkup between July 2000 and December 2002 and followed up thereafter. The cross-sectional analysis of this study is based on the data and biospecimen collected at baseline from 1467 participants (close to 100% Caucasian) who were randomly selected for the measurements of 25(OH)D concentrations and DNA methylation profiles among participants recruited consecutively at the start of the ESTHER study between July 2000 and March 2001 [10]. Participants were then regularly followed up with respect to the incidence of major chronic diseases and mortality. The ESTHER study was approved by the ethics committees of the University of Heidelberg and the state medical board of Saarland, Germany. Written informed consent was obtained from all participants.

### Vitamin D measurements

Blood samples were taken during the health checkup and stored at − 80 °C until further processing. As previously described [11], the automated Diasorin–Liaison analyzer (Diasorin, Inc.) was used to measure total serum 25(OH)D concentrations in women from baseline serum samples in the central laboratory of the University Clinic of Heidelberg in 2006 within the framework of a project on women's health. Additional funding was obtained in 2009 to measure total serum 25(OH)D concentrations in men as well. As the Diasorin–Liaison method used for women was no longer available at that time, the automated IDS-iSYS analyzer (Immunodiagnostic Systems, GmbH) was used instead. Both assays with their within- and between-assay coefficients and lower limits of detection have been comprehensively described elsewhere [12, 13]. Both immunoassays were

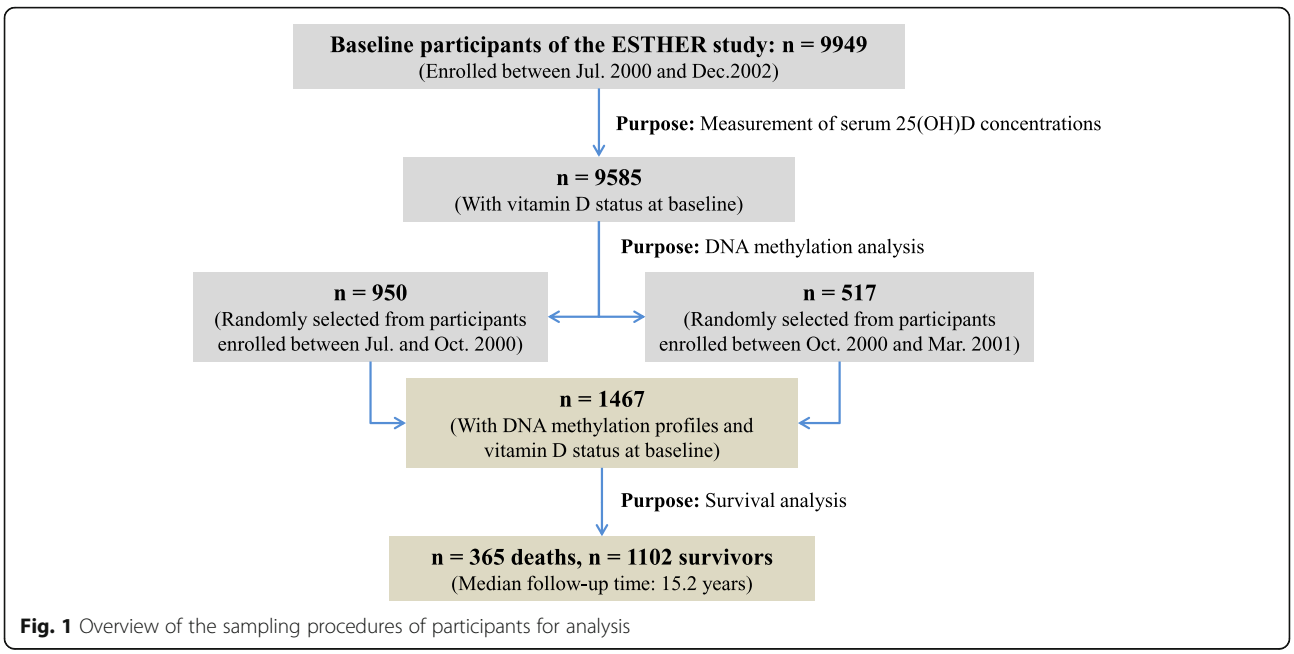


**Fig. 1** Overview of the sampling procedures of participants for analysis