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Reduced peripheral blood telomere length in inflammatory bowel disease has limited predictive value for treatment response

Evangelia Legaki[a], Nikolas Dovrolis[b], Georgios Stravodimos[a], Ioannis Papaconstantinou[c], Nikos Viazis[d], Giorgos Bamias[e], Maria Gazouli[a]

School of Medicine, National and Kapodistrian University of Athens, Greece; School of Medicine, Democritus University of Thrace, Alexandroupolis, Greece; Aretaieion General Hospital, National and Kapodistrian University of Athens, Greece; Evangelismos Hospital, Athens, Greece; University of Athens-Sotiria Hospital-GI Unit, Athens, Greece

Abstract

Background Telomere shortening is associated with cellular aging and chronic inflammation, but evidence in inflammatory bowel disease (IBD), particularly in relation to peripheral blood, remains limited. We investigated peripheral blood telomere length in Crohn's disease (CD) and ulcerative colitis (UC) and explored its potential value in predicting treatment response.

Methods Peripheral blood telomere length was analyzed in 70 individuals retrospectively selected from our institutional biobank, including 14 healthy controls, 28 patients with CD and 28 with UC. Group differences were evaluated using multivariate regression, adjusted for age and sex. Treatment-response discrimination was assessed using repeated stratified cross-validation.

Results Both CD and UC were associated with shorter telomere length than healthy controls, after adjustment for age and sex, with adjusted differences of −1.55 kb and −2.24 kb, respectively. Telomere length did not differ significantly between CD and UC. Among 56 patients with treatment-response data, predictive discrimination was limited, with an area under the receiver operating characteristic curve of 0.53 for telomere length alone, and 0.55 when combined with clinical covariates. Wide confidence intervals indicated substantial uncertainty.

Conclusions Peripheral blood telomere length was significantly shorter in patients with CD and UC than in healthy controls, after adjustment for age and sex. These findings support an association between IBD and altered peripheral blood telomere biology. Baseline telomere length provided little additional discrimination of subsequent treatment response in this cohort, suggesting that its potential relevance may lie beyond short-term therapeutic prediction.

Keywords Inflammatory bowel disease, telomere length, biological aging, inflammaging, treatment response

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Conflict of Interest: None

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Correspondence to: Maria Gazouli, PhD, Professor Department of Basic Medical Sciences, School of Medicine, National and Kapodistrian University of Athens, Michalakopoulou 176, 11527 Athens, Greece, e-mail: mgazouli@med.uoa.gr

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Introduction

Telomeres are nucleoprotein structures composed of repetitive TTAGGG sequences that protect chromosome ends and maintain genomic stability [1]. They progressively shorten during cell division, contributing to cellular senescence and apoptosis [2-5]. Although telomerase maintains telomere length in certain cell populations, telomere dysfunction is associated with aging and chronic inflammation, while inherited defects in telomere maintenance underlie specific telomere biology disorders [6-8].

Telomere erosion has been extensively documented in immune-mediated inflammatory diseases. In rheumatoid arthritis (RA), telomeres of peripheral blood mononuclear cells, T-cell subsets, and hematopoietic progenitors are significantly shorter compared with healthy controls, often independently of disease activity or duration [9-12]. In systemic lupus

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erythematosus, leukocytes also display telomere shortening, although no consistent associations with disease severity have been found. Comparative studies have reported pronounced telomere erosion in chronic inflammatory diseases, such as RA, ankylosing spondylitis and psoriatic arthritis, whereas osteoarthritis, lacking a persistent inflammatory component, does not show similar alterations [13].

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic multifactorial disorders involving genetic susceptibility, environmental factors, gut microbiota alterations, and dysregulated immune responses. Despite their classification as immune-mediated diseases, the mechanisms initiating intestinal inflammation remain incompletely understood [14].

Chronic intestinal inflammation and telomere attrition are increasingly recognized as complementary processes in IBD pathogenesis. Persistent mucosal inflammation may promote telomere shortening through oxidative stress, increased replicative demand and reduced telomerase activity [2]. Conversely, critically shortened or dysfunctional telomeres can activate DNA damage and inflammatory pathways, including ataxia-telangiectasia mutated (ATM), nuclear factor kappa B (NF-κB), and cyclic GMP-AMP synthase (cGAS)-stimulator of interferon genes (STING) signaling [2,15,16]. In intestinal epithelial cells, telomere dysfunction may also enhance pro-inflammatory signaling through Yes-associated protein 1 (YAP1) activation, and increased expression of mediators such as interleukin (IL)-18 [17]. These findings support a bidirectional relationship in which inflammation accelerates telomere attrition, while telomere dysfunction may further sustain inflammatory responses and impaired tissue repair.

Telomere attrition has been particularly well documented in UC. Telomere shortening in colonic epithelial cells was first reported more than 2 decades ago [18] and subsequently confirmed [19]. O'Sullivan *et al* demonstrated significantly shorter telomeres in non-dysplastic colonic mucosa from patients with UC who had dysplasia or cancer elsewhere in the colon (UC progressors), compared with UC patients without dysplasia or cancer (UC non-progressors) and non-UC controls [20]. Telomere shortening has also been associated with disease duration and with clinical phenotypes relevant to UC-associated neoplastic progression [21]. Reduced telomere length has also been associated with more severe and early-onset UC. Although shortening has been observed in both colonocytes and peripheral leukocytes, it appears more pronounced in the colonic epithelium, potentially contributing to accelerated colonic aging and the increased risk of colorectal cancer in UC [19,21]. Nevertheless, telomere shortening in IBD does not appear to represent a primary inherited telomeropathy. A multigenerational study found no association between telomere length and genetic anticipation, suggesting that telomere dysfunction in IBD is more likely to be acquired and inflammation-related, rather than caused by a monogenic defect in telomere maintenance [22].

Evidence from peripheral blood remains limited and inconsistent. Getliffe *et al* reported similar age-related lymphocyte telomere loss in UC, CD and controls, and identified effects of smoking and azathioprine on telomerase-related measures [23], while Risques *et al* observed only modest leukocyte telomere shortening in UC [24]. Laish *et al* reported greater telomere dysfunction in peripheral blood lymphocytes from patients with primary sclerosing cholangitis than in those from patients with IBD alone and healthy controls [25]. Moreover, a Mendelian randomization study found no evidence supporting a causal association between leukocyte telomere length and IBD, UC or CD [26] Overall, the available literature indicates heterogeneous findings and an uncertain causal relationship between peripheral blood telomere length and IBD [27].

Current advanced therapies for IBD target inflammatory pathways and include proinflammatory cytokine inhibitors (e.g., anti-tumor necrosis factor [TNF]) agents, integrin antagonists, and small-molecule inhibitors. Anti-TNF antibodies, such as infliximab and adalimumab, are effective in both UC and CD, although primary non-response and secondary loss of response remain common. Anti-integrin therapies and Janus kinase (JAK) inhibitors provide additional therapeutic options, particularly in patients with refractory disease [28]. Despite these advances, the high treatment costs, variable therapeutic response, and safety considerations underscore the need for improved prognostic and predictive biomarkers in IBD.

The primary objective of this study was to determine whether average peripheral blood telomere length differs between healthy controls and patients with CD or UC, after adjustment for age and sex. A secondary objective was to explore whether telomere length was associated with treatment response, and whether it contributed discriminatory information beyond basic clinical covariates.

Materials and methods

The study was approved by the Institutional Review Board of the Medical School, National and Kapodistrian University of Athens (approval No. 461/24, 9 February 2024) and was conducted in accordance with the Declaration of Helsinki.

Participants

A total of 70 individuals were retrospectively selected from the biobank of the Laboratory of Biology, School of Medicine, National and Kapodistrian University of Athens. Samples

[a]Department of Basic Medical Sciences, Laboratory of Biology, School of Medicine, National and Kapodistrian University of Athens, Greece (Evangelia Legaki, Georgios Stravodimos, Maria Gazouli); [b]Laboratory of Biology, School of Medicine, Democritus University of Thrace, Alexandroupolis, Greece (Nikolas Dovrolis); [c]Second Department of Surgery, Aretaieion General Hospital, National and Kapodistrian University of Athens, Greece (Ioannis Papaconstantinou); [d]Department of Gastroenterology, Evangelismos Hospital, Athens, Greece (Nikos Viazis); [e]3rd Academic Department of Internal Medicine, University of Athens-Sotiria Hospital-GI Unit, Athens, Greece (Giorgos Bamias)

collected over the preceding 10 years were included in the primary analysis. The study population comprised 14 healthy controls (Ctrl), 28 patients with CD, and 28 patients with UC. Participants were not selected consecutively for the present study. Patients with IBD had an established diagnosis of CD or UC, based on standard clinical, endoscopic, radiological and histopathological criteria, and had an available peripheral blood sample suitable for telomere-length analysis. Healthy controls were selected from biobanked individuals without a diagnosis of IBD and were not individually matched to patients for age or sex; these variables were accounted for in the multivariate analyses. No individuals included in the study were excluded from the primary analysis because of missing data on age, sex, disease status or telomere length.

Following baseline blood sampling, patients were started on biological or targeted therapies, and were stratified according to treatment regimen. Peripheral blood samples used for telomere-length measurement were collected before the initiation of treatment. All patients were biologic-naïve at the time of blood sampling. Thus, telomere length represented a pretreatment baseline measurement, with no prior exposure to biological or targeted agents. Among patients with CD, 22 received anti-TNF therapy and 6 received anti-integrin therapy. Among patients with UC, 18 received JAK inhibitor therapy, 7 received anti-integrin therapy and 3 received anti-TNF therapy. Because subsequent treatment selection was strongly associated with IBD diagnosis, diagnosis-related differences could not be reliably distinguished from treatment-class-related patterns in the treatment-response analyses. Therefore, treatment-specific analyses were considered exploratory and were not used to infer independent treatment effects. Treatment response was determined retrospectively from clinical records, based on the treating physician's assessment, incorporating clinical symptoms and available biochemical and/or endoscopic findings during routine clinical follow up. Response assessment was performed during routine clinical follow up rather than at a uniform predefined time point. Patients were classified as responders or non-responders according to the documented treatment outcome. Primary non-response and secondary loss of response were not systematically distinguished, and were therefore analyzed together as non-response.

Quantification of average telomere length by qPCR

Genomic DNA was isolated from peripheral blood using the NucleoSpin Blood Kit (Macherey-Nagel, Germany), according to the manufacturer's instructions. DNA quality and input requirements were assessed according to the specifications of the assay manufacturer. Average telomere length was determined by quantitative real-time polymerase chain reaction (qPCR) using the Absolute Human Telomere Length Quantification qPCR Assay Kit (ScienCell Research Laboratories, Carlsbad, CA, USA), following the manufacturer's protocol. The assay is based on amplification of telomeric repeats (TEL) and a single-copy reference sequence (SCR) located on chromosome 17. Amplification was performed on a SaCycler Real-Time PCR system (Sacace Biotechnologies). Samples and assay controls were analyzed according to the technical replication and quality-control procedures specified by the manufacturer. Cycle threshold values for TEL and SCR were used to calculate average absolute telomere length using the calculation method provided by the manufacturer, and results were expressed in kilobases (kb).

Primary statistical model

Group differences in average peripheral blood telomere length were assessed using a multivariate ordinary least squares (OLS) regression model, with Ctrl as the reference group and chronological age and sex included as covariates. The model was specified as: $TL_i = \beta_0 + \beta_1 I(CD_i) + \beta_2 I(UC_i) + \beta_3 Age_i + \beta_4 Male_i + \varepsilon_i$ where TL_i denotes the average telomere length (kb) of individual i; β_0 is the intercept; β_1 and β_2 represent the adjusted differences in telomere length for CD and UC, respectively, relative to healthy controls; β_3 represents the change in telomere length per year of age; β_4 represents the difference between males and females; and ε_i is the residual error term. $I(CD_i)$ and $I(UC_i)$ are binary indicator variables identifying the respective disease groups, while $Male_i$ is coded as 1 for males and 0 for females. Heteroskedasticity-consistent type 3 (HC3) standard errors were used, following the covariance estimation approach described by MacKinnon and White [29]. The 2 primary disease-group comparisons, CD vs. Ctrl and UC vs. Ctrl, were corrected for multiple testing using the Benjamini-Hochberg false discovery rate (FDR) procedure, with adjusted P-values reported as q-values. In a secondary analysis restricted to patients with IBD, UC was compared directly with CD using an age- and sex-adjusted OLS model with HC3-robust standard errors. To assess whether the association between chronological age and telomere length differed among disease groups, an exploratory model incorporating age-by-disease-group interaction terms was fitted, adjusting for sex. The interaction terms were evaluated jointly using an HC3-robust Wald F test. No *a priori* sample size calculation was performed because the study was based on retrospectively selected biobank samples. To characterize the statistical sensitivity of the study, we estimated the approximate minimum detectable adjusted difference using the observed group sizes (14 healthy controls and 28 patients in each IBD group) and the residual standard deviation of the age- and sex-adjusted linear model (1.842 kb). Assuming 80% power and a conservative 2-sided significance threshold of 0.025 for the 2 primary disease–control comparisons, the minimum detectable differences were approximately 1.92 kb for CD vs. healthy controls and 1.92 kb for UC vs. healthy controls. These estimates were based on conventional linear-model assumptions.

Influence and sensitivity analysis

Influential observations were assessed using Cook's distance from the primary OLS regression model. Observations

exceeding the conventional threshold of 4/n were flagged as potentially influential, but were retained in the primary analysis. To assess the robustness of the estimated disease-group effects, the primary model was repeated as a sensitivity analysis after excluding observations above this threshold. The 2 disease–control comparisons in the sensitivity analysis were adjusted for multiple testing using the same Benjamini-Hochberg FDR procedure as in the primary analysis. To evaluate the influence of limited age overlap among study groups, an additional sensitivity analysis was performed after restricting the sample to the common observed age range across healthy controls, CD and UC. The original age- and sex-adjusted OLS model with HC3-robust standard errors and Benjamini-Hochberg FDR correction was applied to this restricted sample.

Predictive modelling of treatment response

Treatment-response discrimination was evaluated in the 56 patients with IBD using repeated stratified cross-validation. Three logistic regression models were assessed: telomere length alone; clinical covariates comprising age, sex and diagnosis; and a combined model incorporating telomere length and these clinical covariates. Logistic regression with L2 regularization was implemented using the liblinear solver and a fixed inverse regularization strength of C=1.0, corresponding to the scikit-learn default, without additional hyperparameter tuning. Numerical predictors were standardized and categorical predictors were encoded within each training fold. Fivefold stratified cross-validation was repeated 20 times using a fixed random seed of 20260814. For each participant, predicted probabilities were averaged across the iterations in which that participant was held out. Predictive performance was summarized using the area under the receiver operating characteristic curve (AUC-ROC) calculated from these out-of-fold predictions. Ninety-five percent confidence intervals (CIs) were estimated using 2000 bootstrap resamples of the out-of-fold predictions.

The pooled analysis evaluated whether telomere length contributed to discrimination of documented treatment response across the IBD cohort, with diagnosis included in the clinical and combined models. Given the small treatment strata and strong association between diagnosis and treatment class, treatment-specific comparisons were limited to descriptive summaries of telomere length among responders and non-responders, without fitting separate predictive models. The pooled predictive analysis was considered exploratory, because treatment-response assessment was not standardized across participants.

Software and reproducibility

All statistical analyses were performed in Python v3.10.6 using pandas v1.5.3, NumPy v1.24.2, statsmodels v0.14.6, SciPy v1.15.3, scikit-learn v1.7.2, matplotlib v3.10.8, and seaborn v0.13.2. HC3 heteroskedasticity-robust inference was implemented using statsmodels, while repeated stratified cross-validation and L2-regularized logistic regression were implemented using scikit-learn. Figures were generated using matplotlib and seaborn. A fixed random seed was used throughout the analysis to ensure reproducibility.

Results

Cohort characteristics and analytic sample

The study included 70 individuals retrospectively selected from the biobank, comprising 14 Ctrl, 28 patients with CD and 28 with UC. Baseline demographic and clinical characteristics are summarized in Table 1. All 70 individuals had complete data regarding age, sex, disease status and telomere length, and were included in the primary disease-group analysis. Treatment-response information was available for all 56 patients with IBD, including 31 responders and 25 non-responders. Following baseline blood sampling, 22 patients with CD received anti-TNF therapy and 6 received anti-integrin therapy, whereas 18 patients with UC received JAK inhibitor therapy, 7 received anti-integrin therapy and 3 received anti-TNF therapy. Potentially influential observations identified by Cook's distance in the primary disease-group analysis were evaluated separately in sensitivity analyses.

Telomere length is significantly reduced in both CD and UC relative to Ctrl

Average peripheral blood telomere length differed across the 3 study groups, with the highest values observed in Ctrl and progressively lower values in CD and UC (Fig. 1). Mean telomere length was 8.16±1.71 kb in Ctrl, 6.70±2.35 kb in CD, and 5.74±1.35 kb in UC. These differences remained significant after adjustment for chronological age and sex in the primary multivariate OLS model (Table 2; Supplementary Fig. 1). Compared with Ctrl, CD was associated with an adjusted reduction in average telomere length of 1.55 kb

Table 1 Demographic and telomere length characteristics of the primary analytic cohort (n=70). Values are mean±standard deviation unless otherwise stated

Characteristic	Ctrl (n=14)	CD (n=28)	UC (n=28)
Age, years (mean±SD)	38.9±11.2	33.9±17.2	43.3±16.3
Sex, male/female	8/6	19/9	20/8
Average telomere length, kb, mean±SD	8.16±1.71	6.70±2.35	5.74±1.35
Average telomere length, kb, median	8.55	6.37	5.80
IQR, kb	6.85-9.01	4.83-7.97	5.00-6.65

Ctrl, healthy controls; CD, Crohn's disease; UC, ulcerative colitis; SD, standard deviation; IQR, interquartile range

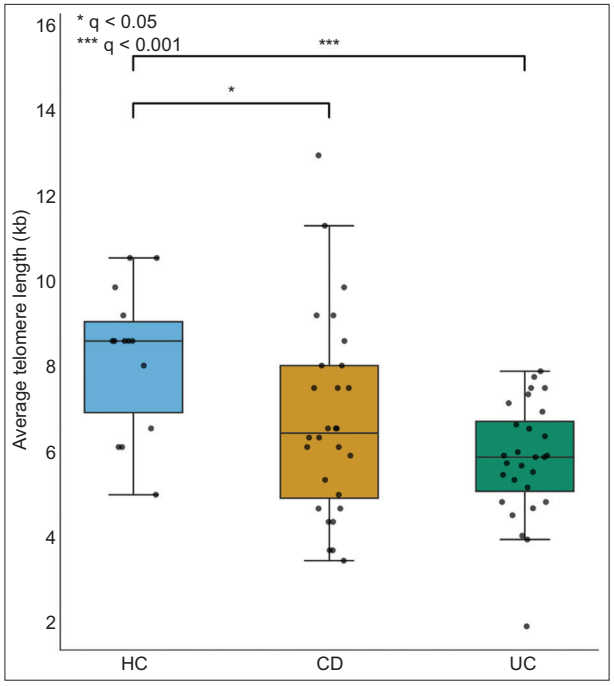


Figure 1 Average peripheral blood telomere length across study groups. Box plots with overlaid individual observations show average telomere length in healthy controls (HC), Crohn's disease (CD), and ulcerative colitis (UC). Boxes represent the interquartile range, with the median indicated by the central line, and individual observations are shown as jittered points. Group differences were assessed using multivariate ordinary least squares regression adjusting for chronological age and sex, with HC3 heteroskedasticity-robust standard errors. Benjamini-Hochberg false discovery rate (FDR) correction was applied to the CD vs. HC and UC vs. HC comparisons. The FDR-adjusted P-values are denoted by q: *q<0.05; ***q<0.001

(β=−1.545, 95%CI −2.838 to −0.252, FDR q=0.019), while UC was associated with a reduction of 2.24 kb (β=−2.237, 95%CI −3.312 to −1.161, FDR q<0.001). Age showed a negative but non-significant association with telomere length in the full cohort (β=−0.026 kb per year, 95%CI −0.055 to 0.002, P=0.068) (Fig. 2), while sex was not significantly associated with telomere length (β=−0.478, 95%CI −1.495 to 0.540, P=0.358). An exploratory analysis incorporating age-by-disease-group interaction terms did not identify statistically significant differences in age-related telomere length slopes among Ctrl, CD and UC (HC3-robust Wald F(2, 63)=1.351, P=0.266).

In a secondary analysis restricted to patients with IBD, UC patients had numerically shorter telomeres than CD patients after adjustment for age and sex, but this difference was not statistically significant (β=−0.652 kb, 95%CI −1.649 to 0.346, P=0.201). Within this IBD-only model, increasing age was associated with shorter telomere length (β=−0.0310 kb per year, 95%CI −0.0620 to −0.0001, P=0.049).

Five observations exceeded the Cook's-distance threshold of 4/n, comprising 1 Ctrl, 3 patients with CD and 1 patient with UC. These observations were retained in the primary analysis and excluded only in the sensitivity analysis (n=65). The adjusted differences remained significant after FDR

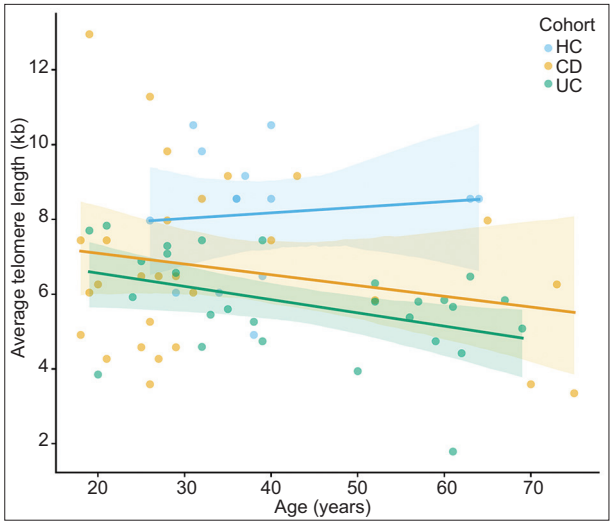


Figure 2 Relationship between chronological age and average peripheral blood telomere length. Scatterplots show individual observations for healthy controls (HC), Crohn's disease (CD), and ulcerative colitis (UC), with group-specific linear regression lines and 95% confidence bands. Average telomere length is expressed in kilobases (kb). The primary multivariate analysis evaluated age together with disease group and sex, using ordinary least squares regression with HC3 heteroskedasticity-robust standard errors. The group-specific regression lines and confidence bands represent descriptive relationships and should not be interpreted as evidence of statistically significant differences in age-related slopes

Table 2 Multivariate OLS regression model of average telomere length (kb). Disease group was entered with healthy controls as the reference category, with chronological age and sex included as covariates. HC3 heteroskedasticity-robust standard errors were used. Benjamini-Hochberg FDR correction was applied to the 2 primary disease-group comparisons; age and sex were treated as covariates and were not FDR-corrected

Predictor	β	95%CI	P-value	FDR q
CD vs. Ctrl	−1.545	−2.838 to−0.252	0.019	0.019
UC vs. Ctrl	−2.237	−3.312 to−1.161	<0.001	<0.001
Sex (male vs. female)	−0.478	−1.495 to 0.540	0.358	N/A
Age (per year)	−0.026	−0.055 to 0.002	0.068	N/A

OLS, ordinary least squares; FDR, false discovery rate; Ctrl, healthy controls; CD, Crohn's disease; UC, ulcerative colitis; CI, confidence interval; N/A, not applicable; β, regression coefficient; q, FDR-adjusted P-value

correction for both CD vs. Ctrl (β=−1.98 kb, 95%CI −3.04 to −0.91, q<0.001) and UC vs. Ctrl (β=−2.35 kb, 95%CI −3.23 to −1.46, q<0.001), supporting the robustness of the primary findings.

An additional sensitivity analysis restricted to the common age range of 26-64 years included 50 individuals (14 Ctrl, 15 CD and 21 UC). The adjusted differences remained negative for both CD vs. Ctrl (β=−1.22 kb, 95%CI −2.77 to 0.33, FDR q=0.123) and UC vs. Ctrl (β=−2.36 kb, 95%CI −3.49 to −1.23, FDR q<0.001). The UC association remained statistically

significant, whereas the CD comparison was less precise and no longer statistically significant in the restricted sample.

Telomere length shows limited cross-validated discrimination for treatment response

Given the substantial telomere shortening observed in IBD patients, we next examined whether baseline telomere length carried predictive information for treatment response. Predictive performance was evaluated using repeated stratified cross-validation and out-of-fold predictions (Table 3; Fig. 3). Across the full IBD cohort, telomere length alone showed limited discrimination for treatment response, with a cross-validated AUC of 0.532 (95%CI 0.375-0.685). A model including age, sex and diagnosis yielded a similar AUC of 0.534 (95%CI 0.385-0.692). Addition of telomere length to these clinical covariates resulted in only a small increase in discrimination, with an AUC of 0.548 (95%CI 0.388-0.704). However, the wide confidence intervals around all AUC estimates indicate substantial uncertainty, and preclude firm conclusions regarding predictive performance.

Telomere length distributions overlapped between responders and non-responders within each treatment class (Fig. 3A; Supplementary Table 1). Given the small subgroup sizes and strong association between diagnosis and treatment class, these descriptive comparisons were not used to infer independent treatment-specific predictive relationships.

Table 3 Cross-validated discrimination of treatment response in the overall IBD cohort. Predictive performance was estimated using repeated stratified cross-validation and subject-level out-of-fold predictions. AUC-ROC values are shown with 95% bootstrap confidence intervals. The 3 models evaluated telomere length alone, clinical covariates alone, and telomere length combined with clinical covariates.

Model	n	Cross-validated AUC	95%CI
Telomere length only	56	0.532	0.375-0.685
Age+sex+diagnosis	56	0.534	0.385-0.692
Telomere length+age+sex+diagnosis	56	0.548	0.388-0.704

IBD, inflammatory bowel disease; AUC-ROC, area under the receiver operating characteristic curve; AUC, area under the curve; CI, confidence interval

Discussion

In this study, we investigated the association between IBD, telomere length and therapeutic response in a retrospective cohort. Patients with both CD and UC had significantly shorter average peripheral blood telomere length than Ctrl, independently of age and sex. These associations

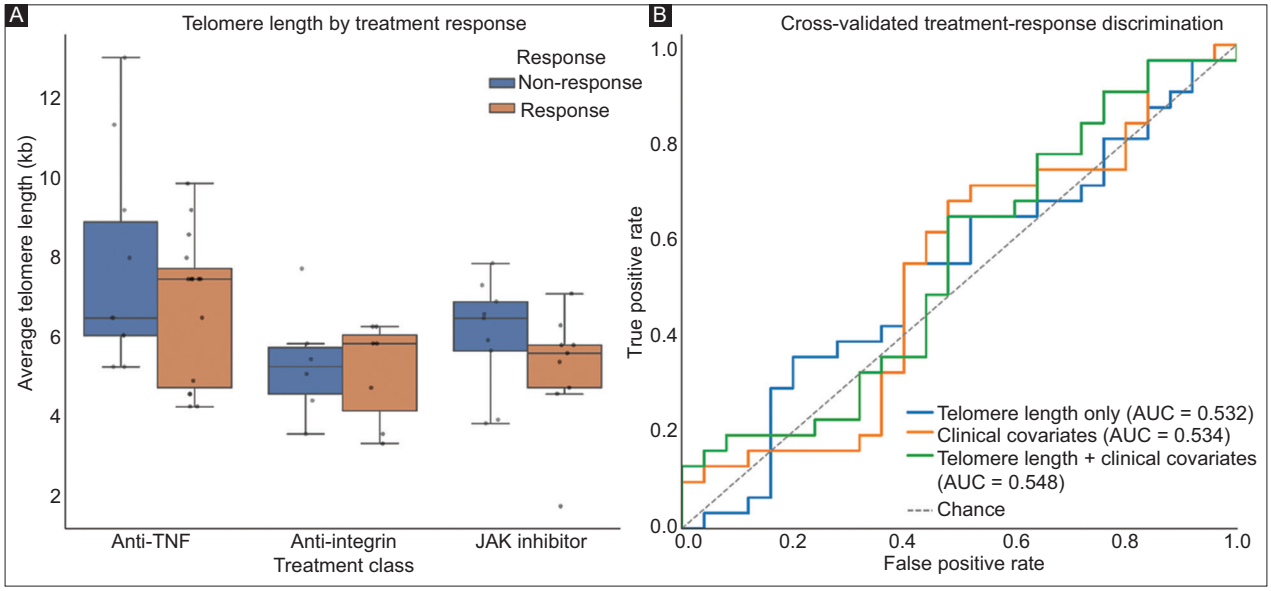


Figure 3 Association of average peripheral blood telomere length with treatment response and cross-validated predictive discrimination. (A) Box plots with overlaid individual observations show average telomere length in responders and non-responders within the anti-tumor necrosis factor (anti-TNF), anti-integrin, and Janus kinase (JAK) inhibitor treatment groups. Boxes represent the interquartile range, with the median indicated by the central line. (B) Receiver operating characteristic (ROC) curves generated from subject-level out-of-fold predictions obtained using repeated stratified cross-validation across the overall inflammatory bowel disease cohort. Three logistic regression models were evaluated: telomere length alone, clinical covariates including age, sex and diagnosis, and a combined model including telomere length together with these clinical covariates. The corresponding cross-validated areas under the ROC curve (AUCs) were 0.532, 0.534 and 0.548, respectively. The dashed diagonal line indicates chance-level discrimination

remained robust in sensitivity analyses excluding potentially influential observations identified by Cook's distance. In contrast, telomere length did not differ significantly between CD and UC after covariate adjustment, and cross-validated models showed limited ability of telomere length to discriminate treatment response. Our results are consistent with previous IBD studies reporting telomere shortening in colonic epithelial cells and mucosal tissue, particularly in UC [19-21]. Together with previous reports from intestinal tissue and peripheral blood, our findings support an association between IBD and shorter telomere length across different biological compartments; however, the present cross-sectional blood-based study cannot establish systemic telomere attrition or its temporal relationship with disease. In contrast to our results, Getliffe *et al* found that telomere loss in the peripheral lymphocytes of UC and CD patients presented no statistically significant difference ($P>0.05$) compared to Ctrl, though their study's small sample size (n=54 total) inherently limited its statistical power [23].

Similar findings have also been reported in other immune-mediated inflammatory diseases, including rheumatoid arthritis [9,30], systemic lupus erythematosus [31,32] and psoriasis [33], where persistent immune activation may increase cellular turnover and replicative stress, contributing to premature telomere erosion [2]. However, findings across these autoimmune diseases, as well as in ankylosing spondylitis and multiple sclerosis, are heterogeneous, with some studies reporting preserved or even elongated telomeres, depending on disease stage, cell population and methodology [9,13,30-37].

Evidence regarding peripheral blood telomere length in IBD remains limited and heterogeneous. Our findings provide additional evidence of shorter peripheral blood telomere length in both CD and UC. Although the underlying mechanisms cannot be established by the present study, chronic inflammation, oxidative stress and increased cellular turnover represent potential mechanisms linking IBD with telomere shortening [2]. Telomeres are particularly susceptible to oxidative injury, while sustained immune-cell proliferation may further increase replicative stress. Proinflammatory cytokines, including TNF-α and IL-6, may additionally contribute to oxidative and metabolic stress [38-41].

Telomere dysfunction may not simply represent a consequence of chronic inflammation, but could also contribute to its maintenance. Critically shortened telomeres activate DNA damage responses and cellular senescence, promoting a senescence-associated secretory phenotype and the release of proinflammatory mediators [42,43]. Telomere dysfunction may also activate innate immune pathways, including cGAS-STING, Toll-like receptor 9, and NF-κB signaling [2,15,16]. In intestinal epithelial cells, telomere dysfunction has been shown to activate an ATM-YAP1-pro-IL-18 pathway, increasing IL-18 production in tissues from patients with both UC and CD [17]. Together, these observations support a bidirectional model, in which chronic inflammation may accelerate telomere shortening, while telomere dysfunction may further promote inflammatory signaling and cellular senescence [2].

Notably, the association between IBD and shorter telomere length remained significant after adjustment for chronological age, suggesting that the observed differences are not explained by age alone. This finding is consistent with the possibility that chronic inflammatory stress contributes to telomere attrition in IBD. Such an interpretation aligns with the broader concept of "inflammaging", in which persistent inflammatory activity and age-related cellular dysfunction may reinforce one another [17,42,44]. However, because the present study measured telomere length, rather than a validated composite biological-age metric, these findings should be interpreted as evidence of telomere shortening associated with IBD rather than as a direct quantification of accelerated biological aging.

Our findings are further supported by emerging epidemiological evidence linking biological aging to IBD susceptibility. In a large prospective UK Biobank study involving more than 400,000 participants, accelerated biological aging, assessed using the PhenoAge metric, was associated with a significantly increased risk of both UC and CD, independently of chronological age [45]. Additionally, Wang and colleagues demonstrated that accelerated biological aging not only increased IBD risk, but also interacted with genetic susceptibility, so that individuals with both elevated PhenoAge acceleration and high genetic risk exhibited the greatest likelihood of developing disease [46]. Interestingly, biological aging was also found to mediate part of the association between established environmental risk factors, including smoking and alcohol consumption, and IBD risk [46]. Taken together, these findings support the broader concept that biological aging processes may be relevant to IBD pathogenesis.

Beyond the association with disease status, we examined whether baseline telomere length could contribute to discrimination of therapeutic response. However, telomere length showed limited predictive performance in the overall IBD cohort. Importantly, subsequent treatment class was strongly associated with IBD diagnosis, limiting our ability to distinguish diagnosis-related differences from treatment-class-specific patterns in the prediction of therapeutic response. Accordingly, treatment-specific comparisons were restricted to descriptive summaries, and were not used to infer differential predictive performance across treatment classes. Across all 3 treatment classes, non-responders exhibited numerically longer mean telomeres than responders, although the differences varied in magnitude. However, these descriptive findings do not support a straightforward association between shorter baseline telomeres and poorer treatment response, and the small subgroup sizes preclude definitive conclusions. Larger cohorts, with sufficient representation of each treatment class within both CD and UC,

will be needed to determine whether pretreatment telomere length is associated with response to specific therapies, independently of diagnosis. Nevertheless, the biological rationale for investigating telomere length in this context remains plausible, as telomere shortening has been linked to immunosenescence, reduced lymphocyte proliferative capacity, altered cytokine production and impaired adaptive immune responses [47-49]. Chronic inflammation represents a possible mechanism underlying the observed association; however, this cannot be established from the present data.

The association between IBD and shorter peripheral blood telomeres raises the possibility that telomere length captures aspects of the cumulative biological burden associated with chronic intestinal inflammation. Persistent inflammatory signaling, oxidative stress, and repeated immune-cell proliferation may influence telomere dynamics through mechanisms linked to immunosenescence and inflammaging [42-44].

In this context, peripheral blood telomere length may provide information distinct from conventional inflammatory biomarkers, which primarily reflect current disease activity. Its potential value may therefore extend beyond predicting the response to individual therapies, particularly toward understanding longer-term immune-system alterations associated with IBD. Longitudinal studies incorporating serial telomere measurements, inflammatory activity and clinical outcomes could establish whether telomere length contributes additional information to the characterization of disease burden and progression.

Our cross-sectional design prevents generalized conclusions regarding causality or temporal relationships between telomere shortening and disease development. Complete records of disease duration, Montreal classification and disease activity were not available. In addition, smoking status and other potential confounders, including body mass index, alcohol consumption, physical activity and previous exposure to corticosteroids or thiopurines, were not systematically available across the cohort and therefore could not be included as covariates. As several of these factors may influence telomere dynamics, residual confounding cannot be excluded. The study's sample size was relatively small, particularly the healthy control group (n=14), resulting in limited statistical precision and relatively wide confidence intervals for some disease-group comparisons. The treatment-response analysis was limited by the small sample size and wide CIs around the cross-validated AUC estimates. Furthermore, response assessment was not standardized across participants, and primary nonresponse and secondary loss of response were analyzed together. This heterogeneity may have reduced the ability to identify associations between baseline telomere length and subsequent therapeutic response. An additional limitation is the strong association between IBD diagnosis and subsequent treatment selection, which limits the interpretation of treatment-class-specific patterns in therapeutic response. Age distributions were uneven, with 12 of 14 control subjects aged 26-40 years, limiting the ability of statistical adjustment to fully account for age-related differences. In the age-restricted sensitivity analysis, the UC association remained significant, whereas the CD estimate retained its direction but became less precise. Furthermore, telomere length was measured in peripheral blood rather than intestinal tissue, and variations in peripheral leukocyte composition may have contributed to between-group differences. Nevertheless, peripheral blood provides a minimally invasive and clinically practical source for telomere assessment.

In conclusion, our study demonstrated that peripheral blood telomere length is significantly shorter in patients with both CD and UC compared with control values, independently of chronological age and sex. These associations were further supported by sensitivity analyses addressing influential observations. The findings suggest that altered telomere biology may represent a systemic correlate of chronic inflammatory disease, potentially reflecting aspects of cumulative inflammatory exposure and immune-cell aging. Although baseline telomere length provided limited discrimination of subsequent treatment response, its association with IBD status highlights a distinct biological dimension worthy of further investigation. Longitudinal studies integrating telomere dynamics with inflammatory burden and clinical outcomes may help establish its relevance to disease progression and biological aging in IBD.

Footnotes

Institutional review board statement: The study was approved by the Institutional Review Board of the Medical School, National and Kapodistrian University of Athens, and was conducted in accordance with the Declaration of Helsinki (approval No. 461/24, 9 February 2024). The present study used archived peripheral blood samples from the laboratory biobank, collected over the preceding 10 years in the context of previously approved studies conducted by our group. The relevant ethics approvals and informed-consent procedures for the original sample collections have been reported in the corresponding publications (*World J Gastrointest Pharmacol Ther* 2017;**8**:193-200; *Inflamm Bowel Dis* 2022;**28**:87-95). Written informed consent had been obtained from all participants at the time of the original sample collection.

Declaration of generative AI use

Generative artificial intelligence tools were used solely for language editing, including improvements to grammar, syntax, clarity and readability. All scientific content, interpretation, analyses and conclusions were developed and verified by the authors, who take full responsibility for the final manuscript.

Summary Box

What is already known:

- Telomere shortening has been associated with aging, chronic inflammation, and immune-mediated inflammatory diseases
- In inflammatory bowel disease, telomere shortening has been mainly described in intestinal tissue, particularly in ulcerative colitis (UC)
- Evidence regarding peripheral blood telomere length in Crohn's disease (CD) and UC remains limited and heterogeneous

What the new findings are:

- Peripheral blood telomere length was significantly shorter in both CD and UC patients compared with healthy controls, after adjustment for age and sex
- Telomere length did not differ significantly between CD and UC after covariate adjustment
- Baseline telomere length showed limited cross-validated discrimination for treatment response, with substantial uncertainty due to the small sample size

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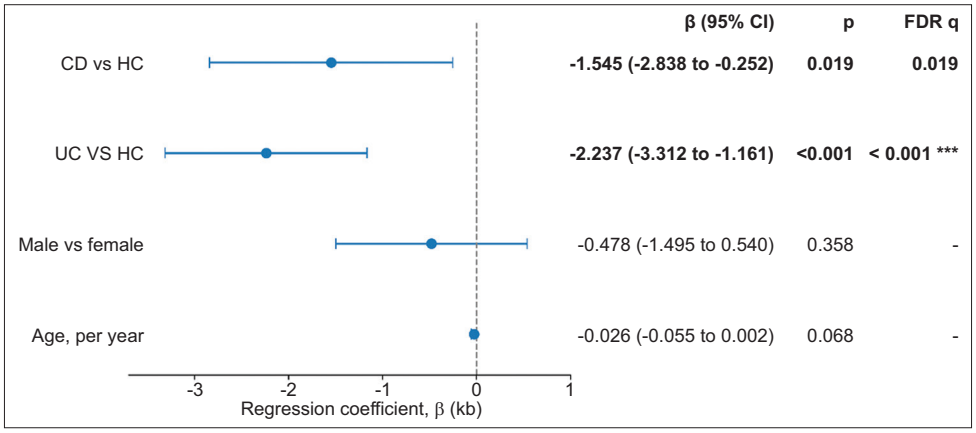
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Supplementary material

Supplementary Table 1 Average peripheral blood telomere length according to treatment class and response status

Treatment	Response status	n	Telomere length (kb), mean±SD
Anti-TNF	Responders	15	6.79±1.84
Anti-TNF	Non-responders	10	7.69±2.65
Anti-integrin	Responders	7	5.13±1.24
Anti-integrin	Non-responders	6	5.35±1.40
JAK inhibitor	Responders	9	5.23±1.49
JAK inhibitor	Non-responders	9	6.05±1.38

SD, standard deviation; TNF, tumor necrosis factor; JAK, Janus kinase



Supplementary Figure 1 Adjusted predictors of average peripheral blood telomere length. Forest plot showing regression coefficients (β) and 95% confidence intervals (CIs) from the primary multivariate ordinary least squares regression model. Healthy controls (HC) were the reference group for comparisons with Crohn's disease (CD) and ulcerative colitis (UC), and female sex was the reference category for sex. The model included chronological age and sex as covariates, and used heteroskedasticity-consistent type 3 (HC3) robust standard errors. Benjamini-Hochberg false discovery rate (FDR) correction was applied to the CD vs. HC and UC vs. HC comparisons. The FDR-adjusted P-values are denoted by q. The dashed vertical line indicates the null value (β=0). *q<0.05; ***q<0.001