THE EUROPEAN SOCIETY OF MEDICINE
Medical Research Archives, Volume 13 Issue 12

RESEARCH ARTICLE

Global deoxyribonucleic acid methylation and telomere length in patients with systemic metabolic disorders

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OPEN ACCESS

PUBLISHED
31 December 2025

CITATION
Kolesnikova, O., V., Radchenko, A., O., et al. Global deoxyribonucleic acid methylation and telomere length in patients with systemic metabolic disorders. [online] 13(12). https://doi.org/10.18103/mra.v13i12.7180

DOI
https://doi.org/10.18103/mra.v13i12.7180

ISSN
2375-1924

ABSTRACT

Background: Epigenetic mechanisms reflect biological ageing and cardiometabolic risk. In patients with systemic metabolic disorders (SMD), these processes are perturbed, yet joint dynamics of global 5-methylcytosine (5-mC) and telomere length remain insufficiently described.

Aims: To assess associations between 5-mC, telomere length and cardiometabolic profiles by SMD stage and over time.

Methods: We studied 78 SMD patients (median age 55.4 years; 56% men): stage 1 (n=59) and stage 2 (n=19). Global 5-mC was measured by enzyme-linked immunosorbent assay and telomere length by real-time quantitative polymerase chain reaction. 67 patients had a follow-up visit after a median of 63 weeks.

Results: Stage 2 patients showed higher body mass index, aspartate/alanine aminotransferases, alkaline phosphatase, glucose, triglycerides, very-low-density lipoprotein cholesterol, and lower high-density lipoprotein cholesterol (all $p<0.05$). Only trends toward hypermethylation and shorter telomere length were observed in stage 2. Within stage 1, 5-mC associated with hematocrit; telomere length associated with red blood cell count, platelet distribution width, and total cholesterol. Within stage 2, alkaline phosphatase inversely predicted 5-mC; telomere length was predicted by age, body weight, erythrocyte sedimentation rate, mean corpuscular hemoglobin concentration, alkaline phosphatase, creatinine, and uric acid (all $p<0.05$). Change in 5-mC related to baseline body mass index, mean corpuscular volume, glucose, red cell distribution width, mean platelet volume, creatinine, and low-density lipoprotein cholesterol; telomere length change related to baseline glucose, direct bilirubin, and albumin (all $p<0.05$).

Conclusions: Stage 2 SMD reflects greater metabolic burden with unfavorable 5-mC, telomere length trends. Cardiometabolic indices show independent links with 5-mC, telomere length, supporting composite biomarker strategies for ageing-risk monitoring in SMD.

Keywords: Systemic metabolic disorders, 5-methylcytosine, telomere length, cardiometabolic risk factors

Introduction

The shared pathophysiological basis of various metabolic disorders particularly their association with excess adiposity leading to insulin resistance and systemic inflammation is linked to a substantial risk of serious long-term health consequences worldwide. This recognition has prompted recent changes in current management strategies, aiming to enhance the impact of treatment on risk reduction. As a result, patients have been recently stratified into three actionable stages of systemic metabolic disorder (SMD) to reflect disease progression and enable an earlier approach to prevention from metabolic abnormalities without organ damage (stage 1) to early organ damage (stage 2) and to late-stage organ disease (stage 3).[1]

Each individual component of SMD including pre-diabetes (pre-DM) and type 2 diabetes (T2DM), metabolic dysfunction–associated steatotic liver disease (MASLD), hypertension (HPT), atherogenic dyslipidemia (DL), obesity (OB), and others has been associated with accelerated biological aging, which in turn reflects increasing cardiovascular risk and overall mortality.[2-6]

Among the molecular mechanisms underlying aging, alterations in epigenetic regulation such as deoxyribonucleic acid (DNA) methylation have emerged as powerful tools for identifying individuals with premature aging.[7] Lifestyle factors including diet, physical activity, smoking, other environmental exposures, and metabolic load can induce changes in gene expression without altering the DNA sequence, primarily through the attachment of methyl groups to nitrogenous bases of specific genes. Over time, the human DNA methylation landscape accumulates substantial changes that have been associated with a wide spectrum of age-related diseases, including metabolic disorders. Thus, detecting epigenetic marks holds promise for the early identification of individuals at risk of age-related health decline and the onset and progression of chronic diseases. Various age-related DNA methylation patterns have been described from differential and variable methylation at individual cytosine-phosphate-guanine dinucleotides (CpG) sites to whole-methylome alterations, including changes in entropy and correlation networks.[8] Among these, the most powerful biomarkers of aging are based on selected individual CpG sites strongly correlated with chronological age, forming the foundation of epigenetic clocks derived from DNA methylation profiles across multiple tissues with remarkable precision.[9, 10]

Although global (genome-wide) DNA methylation a measure of the overall methylation status across the genome may overlook critical promoter-specific CpG changes relevant to disease pathogenesis, it remains a standardized and widely applicable parameter that allows for population-level comparisons and provides an integrated view of the organism's epigenetic state.[11] Moreover, global methylation analysis is technically straightforward, does not require sequencing or library preparation, is cost-effective for large cohorts, and offers high reproducibility, which explains its continued widespread use.

In parallel, telomere length (TL) has been established as another hallmark of cellular and premature aging. Multiple studies consistently demonstrate that shorter telomeres are associated with metabolic syndrome, type 2 diabetes, and increased cardiometabolic risk.[12] TL depends on both inherited telomerase activity and progenitor telomere length. Although TL is not a classical epigenetic marker (like DNA methylation, histone modifications, or non-coding ribonucleic acids), it can be considered an epigenetic trait.[13] Furthermore, a significant relationship between differentially methylated CpG sites and shortened telomeres has been demonstrated.[14] Another study reported a significant association between global DNA methylation level (% 5- methylcytosine) and leukocyte TL in females among 542 healthy adolescents (44.8% African Americans; 55.2% females, aged 14–18 years).[15]

While it remains unclear whether these two markers independently reflect altered aging dynamics or interact synergistically to accelerate existing epigenetic changes, accumulating evidence supports the role of both methylation level alterations and TL shortening in shaping "epigenetic metabolic aging."

Despite numerous studies investigating aging biomarkers in patients with individual SMD components or comorbid conditions, integrated analyses evaluating both global DNA methylation and TL particularly simultaneously and across different SMD stages are currently lacking. Moreover, the clinical relevance of these epigenetic markers in SMD remains uncertain, specifically whether they can complement traditional biomarkers in predicting cardiovascular risk and mortality. The value of such biomarkers is further enhanced by their dynamic nature, as both can deteriorate under adverse influences and improve in response to therapy.[16]

Finally, hematological parameters are often overlooked as potential covariates or mediators linking DNA methylation, TL, and metabolic state, despite their routine availability in clinical practice. Recently, an increasing number of aging calculators and assessment algorithms have been developed that rely solely on circulating blood biomarkers to estimate an individual's biological or phenotypic age.[17,18,19] These markers could enhance the practical utility of composite models of aging within the SMD framework.

Therefore, the aim of this study was to assess the associations between 5-methylcytosine (5-mC), TL, and cardiometabolic profiles according to SMD stage and over time.

Material and Methods

Our study included 78 patients with systemic metabolic disorders (SMD), with a median age of 55.4 [49.0; 61.4] years; 56% were male. The study population comprised individuals diagnosed with HPT, OB, MASLD, T2DM, pre-DM, and DL.

Participants were stratified into two groups according to the disease stage:

- Group 1: stage 1 SMD (n = 59)
- Group 2: stage 2 SMD (n = 19).

The groups were well matched for age (54.0 [49.1; 60.7] vs. 56.1 [48.9; 62.9] years; p = 0.526) and sex distribution (53% vs. 68% males; p = 0.225).

All participants underwent anthropometric assessment, including measurement of height and body weight, followed by calculation of the body mass index (BMI).

A comprehensive clinical blood analysis and biochemical profiling were performed using standard laboratory methods. The biochemical panel included the following parameters: alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase (ALP), gamma-glutamyltransferase, total and fractionated bilirubin, glucose, total cholesterol, triglycerides, very-low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, uric acid, urea, creatinine, total protein, and protein fractions.

In addition, all participants were assessed for global DNA methylation, expressed as the percentage of 5-mC in leukocyte DNA extracted from peripheral blood, using a commercial 5-mC DNA ELISA Kit (Zymo Research Corp., USA). Relative telomere length (TL) was determined in peripheral blood leukocytes by quantitative real-time polymerase chain reaction (PCR).

Blood samples were collected in the morning after an overnight fast. Prior to amplification, DNA concentration was quantified fluorometrically using a Qubit 3.0 fluorometer (Life Technologies, USA) and adjusted to approximately 5 ng/μL. For global DNA methylation analysis, DNA concentration was re-measured with the Qubit dsDNA HS Assay Kit (Life Technologies, USA) and normalized to approximately 100 ng per ELISA well. Optical density for ELISA measurements was determined

using a semi-automated Immunochem-2100 microplate reader (USA).

Quantitative PCR was performed using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad Laboratories, USA) and gene-specific primers (Thermo Fisher Scientific, USA).

Telomere primers:

Tel-G:
ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT

Tel-C:
TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA

Reference gene (albumin) primers:

ALBU:
CGGCGGCGGGCGGCGCGGGCTGGGCGGAAATGCTGCACAGAATCCTTG

ALBD:
GCCCGGCCCGCCGCGCCCGTCCCGCCGGAAAAGCATGGTCGCCTGTT

Relative TL, expressed in arbitrary units, was calculated as the ratio of the number of telomeric repeat copies (T) to the number of copies of a single-copy reference gene (S), normalized to a reference DNA sample included in each run.

Follow-up and repeated measurements

A total of 67 patients participated in the follow-up assessment (median age, 53.9 [45.7; 59.8] years; 56% male), of whom 13% (n = 9) had stage 2 SMD. Throughout the study period, all participants continued to receive standard pharmacological therapy according to current Ukrainian and European clinical guidelines, depending on comorbid conditions.

The median interval between visits was 63 weeks (range: 26–160 weeks). Based on the duration of follow-up, participants were divided into two groups:

- Group 1: re-examination within less than 1 year (n = 19)
- Group 2: re-examination after more than 1 year (n = 48).

The groups did not differ significantly in age (54.6 [46.7; 63.1] vs. 56.9 [47.2; 62.9] years; p = 0.589) or sex distribution (60% vs. 54% males; p = 0.755). Among patients with stage 2 SMD, 4 belonged to Group 1 and 5 to Group 2.

At the follow-up visit, all participants underwent reassessment of body weight, BMI, complete blood count, and selected biochemical parameters, including aspartate aminotransferase, alanine aminotransferase, glucose, total cholesterol, triglycerides, very-low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, uric acid, urea, creatinine, and albumin.

In addition, global DNA methylation (5-mC level) and TL were re-evaluated in all patients according to the methods described above.

Statistical analysis

All statistical analyses were performed using STATISTICA software and Microsoft Excel for Windows. Given the non-normal distribution of the data, nonparametric statistical methods were applied. Continuous variables are presented as median [interquartile range] (Me [Q1; Q3]). Between-group comparisons for independent samples were performed using the Mann–Whitney U test, and within-group (pre- vs. post-treatment) comparisons were evaluated using the Wilcoxon signed-rank test for paired data. Differences in categorical variables were assessed using Pearson's chi-squared (χ^2) test. To further explore predictors of the outcome variables, multiple linear regression models with stepwise inclusion of predictors were employed, provided that the model residuals met the assumptions of normality. A p-value < 0.05 was considered statistically significant.

Results

Comparative characteristics of patients with systemic metabolic disorders according to disease stage

Depending on the stage of SMD, significant differences were observed in body weight and BMI. Patients with stage 2 SMD had higher body weight (89.0 [81.0; 107.0] kg vs. 82.0 [74.0; 92.8] kg, p = 0.042) and BMI (32.1 [28.9; 34.4] kg/m² vs. 27.4 [25.7; 30.6] kg/m², p = 0.001) compared with those at stage 1.

No significant intergroup differences were found in the complete blood count parameters.

In contrast, the biochemical profile revealed that patients with stage 2 SMD had significantly higher levels of aspartate aminotransferase (26 [20; 35] U/L vs. 22 [18; 26] U/L; p = 0.015), alanine aminotransferase (42 [20; 54] U/L vs. 24 [19; 32] U/L; p = 0.005), ALP (73 [63; 91] U/L vs. 61 [48; 77] U/L; p = 0.014), glucose (5.84 [4.92; 6.44] mmol/L vs. 5.07 [4.70; 5.32] mmol/L; p = 0.002), triglycerides (1.65 [1.31; 2.16] mmol/L vs. 1.20 [0.89; 1.61] mmol/L; p = 0.003), very-low-density lipoprotein cholesterol (0.83 [0.59; 0.97] mmol/L vs. 0.54 [0.40; 0.72] mmol/L; p = 0.004), and lower high-density lipoprotein cholesterol levels (1.19 [0.96; 1.34] mmol/L vs. 1.41 [1.20; 1.63] mmol/L; p = 0.007).

No statistically significant differences were observed in 5-mC (4.69 [3.94; 5.58] % vs. 4.27 [3.17; 4.95]%; p = 0.088) or TL (1.02 [0.75; 1.24] r.u. vs. 1.06 [0.82; 1.37] r.u.; p = 0.425) between stage 2 and stage 1 SMD, respectively, although a tendency toward increased global methylation and shorter telomeres was noted in patients with stage 2 disease.

A stepwise multiple linear regression model assessing predictors of 5-mC level among all SMD patients (R^2 = 0.277; adjusted R^2 = 0.226) identified erythrocyte-related parameters mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume, hematocrit, and plateletcrit as independent determinants. Higher MCHC was positively associated with increased 5-mC levels (B = 0.032; β = 0.297; p = 0.012), whereas higher mean corpuscular volume was inversely associated (B = –0.074; β = –0.297; p = 0.012). Hematocrit (B = 0.006; β = 0.272; p = 0.019) and plateletcrit (B = 0.467; β = 0.253; p = 0.029) also showed significant positive associations with 5-mC level.

Within the stage 1 SMD subgroup, a statistically significant positive association between hematocrit and 5-mC content was observed (R^2 = 0.086; adjusted R^2 = 0.066), where higher hematocrit correlated with greater global DNA methylation (B = 0.006; β = 0.293; p = 0.041).

Among patients with stage 2 SMD, ALP was the only independent predictor of 5-mC levels (R^2 = 0.418; adjusted R^2 = 0.365). Higher ALP activity was associated with lower global DNA methylation (B = –0.040; β = –0.646; p = 0.017).

In the stepwise multiple linear regression model assessing predictors of relative TL among all patients with SMD (R^2 = 0.186; adjusted R^2 = 0.159), the independent determinants were red blood cell count and platelet distribution width (PDW). Higher red blood cell count was associated with longer TL (B = 0.002; β = 0.369; p = 0.003), whereas increased PDW was linked to shorter TL (B = –0.073; β = –0.255; p = 0.034).

Within the stage 1 SMD subgroup, independent predictors of TL included red blood cell count, total cholesterol, and PDW (R^2 = 0.342; adjusted R^2 = 0.298). Elevated red blood cell count was positively associated with TL (B = 0.002; β = 0.469; p = 0.001), while higher total cholesterol (B = –0.132; β = –0.313; p = 0.019) and PDW (B = –0.090; β = –0.284; p = 0.028) were inversely associated.

Analysis of TL determinants among patients with stage 2 SMD demonstrated that the independent predictors were age category (<45 years, 45–60 years, >60 years), body weight, ALP, erythrocyte sedimentation rate, creatinine, MCHC, and uric acid (R^2 = 0.999; adjusted R^2 = 0.997). Elongation of TL was associated with higher erythrocyte sedimentation rate (B = 0.024; β = 0.943; p <

0.001), MCHC (B = 0.003; β = 0.137; p = 0.003), and uric acid (B = 0.0001; β = 0.092; p = 0.007), whereas older age category (B = –0.160; β = –0.558; p < 0.001), increased body weight (B = –0.004; β = –0.290; p < 0.001), higher ALP (B = –0.009; β = –0.720; p < 0.001), and elevated creatinine (B = –0.004; β = –0.257; p < 0.001) were associated with shorter TL.

Dynamics of parameters over time and according to the duration of follow-up

Significant differences between the first and follow-up visits among patients with SMD were observed only in serum albumin levels (43.5 [41.5; 47.5] g/L vs. 41.9 [38.1; 45.3] g/L; p = 0.042). Levels of 5-mC (5.25 [4.04; 6.51] % vs. 4.50 [3.74; 5.66] %; p = 0.168) and TL (1.06 [0.83; 1.16] r.u. vs. 1.04 [0.79; 1.33] r.u.; p = 0.759) did not change significantly. The median individual change in 5-mC was –0.66 [–1.66; 0.40] % and in TL was 0.02 [–0.22; 0.29] r.u., indicating a trend toward decreasing global DNA methylation over time without substantial telomere alterations.

When comparing subgroups by follow-up duration, no significant differences in studied parameters were observed among patients re-examined within one year. In those followed for more than one year, improvements were noted in aspartate aminotransferase (21 [17; 28] U/L vs. 26 [20; 36] U/L; p = 0.047), glucose (5.13 [4.70; 5.42] mmol/L vs. 5.44 [5.18; 5.67] mmol/L; p = 0.020), and albumin (41.5 [38.0; 44.4] g/L vs. 44.3 [41.8; 48.8] g/L; p = 0.004).

No significant differences between patients followed for <1 year and >1 year were found for median individual changes in 5-mC (–0.66 [–1.67; 0.21] % vs. –0.66 [–1.70; 0.41] %; p = 0.515) or TL (0.18 [–0.08; 0.28] r.u. vs. –0.04 [–0.25; 0.34] r.u.; p = 0.515).

Distribution analysis showed that 62% of patients exhibited a decrease in 5-mC, while 38% showed an increase after the follow-up period. These subgroups were comparable in age (53.9 [44.9; 61.7] vs. 59.3 [54.0; 62.8] years; p = 0.169) but differed by sex (71% vs. 31% males; p = 0.020), suggesting a potential sex-related influence on DNA methylation dynamics.

When grouped by TL change, 50% of patients demonstrated telomere shortening. The two subgroups did not differ significantly in age (59.3 [49.6; 63.3] vs. 55.0 [46.4; 60.4] years; p = 0.496) or sex (41% vs. 71% males; p = 0.084).

In the stepwise multiple linear regression model with 5-mC level at follow-up as the dependent variable and all second-visit parameters as independent variables, direct bilirubin and PDW emerged as independent predictors (R^2 = 0.307; adjusted R^2 = 0.259). Higher direct bilirubin (B = –0.485; β = –0.468; p = 0.006) and increased PDW (B = –0.351; β = –0.369; p = 0.025) were associated with lower 5-mC levels.

Among patients re-examined within one year, white blood cells (WBC) count, glucose, and triglycerides were identified as independent predictors of 5-mC (R^2 = 0.927; adjusted R^2 = 0.891). Higher triglycerides (B = –1.684; β = –0.663; p = 0.001) and glucose (B = –0.557; β = –0.321; p = 0.035) were associated with lower 5-mC, while higher WBC (B = 0.385; β = 0.612; p = 0.002) associated with increased 5-mC. No significant predictors of 5-mC were identified among patients with a follow-up interval longer than one year.

For TL, the only independent predictor at follow-up across all patients was MCHC (R^2 = 0.141; adjusted R^2 = 0.112), where higher MCHC was associated with longer TL (B = 0.011; β = 0.376; p = 0.034). Among patients with a follow-up of less than one year, WBC was the sole independent predictor of TL (R^2 = 0.520; adjusted R^2 = 0.461): higher WBC was associated with shorter TL (B = –0.103; β = –0.721; p = 0.019). No significant TL predictors were identified among patients with longer follow-up.

Correlation analysis revealed that changes in 5-mC were significantly associated with age (r = 0.356; p = 0.039), sex (r = 0.477; p = 0.004), and baseline 5-

mC level (r = –0.533; p = 0.001). Changes in TL showed a positive association with baseline 5-mC (r = 0.440; p = 0.009) and an inverse association with baseline TL (r = –0.449; p = 0.008).

In the subgroup of patients followed for less than one year, no such correlations were observed. However, in those with a follow-up exceeding one year, patterns were similar to those in the total cohort: changes in 5-mC correlated significantly with sex (r = 0.514; p = 0.010) and baseline 5-mC (r = –0.541; p = 0.006), while changes in TL were associated with baseline 5-mC (r = 0.488; p = 0.015) and baseline TL (r = –0.519; p = 0.009).

Table 1 summarizes baseline variables included in the stepwise multiple regression model with Δ5-mC as the dependent variable (R^2 = 0.786; adjusted R^2 = 0.753). Although the interval between visits was initially a significant predictor (p = 0.040), it was not retained in the final model due to a reduction in partial correlation and overlap with other predictors, suggesting that the effect of observation duration is mediated by other metabolic and cellular parameters.

Table 1. Predictors of changes in 5-methylcytosine levels among patients with systemic metabolic disorders

Predictors	B	β	p
5-mC	–0.859	–0.856	< 0.001
RDW	0.556	0.682	< 0.001
MPV	1.153	0.452	< 0.001
TL	–2.634	–0.447	< 0.001
Glucose	–0.445	–0.189	0.022
MCV	–0.091	–0.376	< 0.001
Creatinine	0.036	0.357	< 0.001
VLDL-C	0.417	0.299	< 0.001
BMI	–0.079	–0.214	0.033
5-mC, 5-methylcytosine; BMI, body mass index; creatinine, serum creatinine; glucose, fasting plasma glucose; MCV, mean corpuscular volume; MPV, mean platelet volume; RDW, red cell distribution width; TL, telomere length; VLDL-C, very low-density lipoprotein cholesterol.			

Independent predictors of changes in TL identified in the stepwise regression model (R^2 = 0.532; adjusted R^2 = 0.494) are presented in Table 2.

Table 2. Predictors of changes in telomere length among patients with systemic metabolic disorders

Predictors	B	β	p
TL	–0.706	–0.468	< 0.001
5-mC	0.087	0.337	0.001
Direct bilirubin	–0.021	–0.227	0.012
Albumin	0.024	0.221	0.019
Glucose	–0.110	–0.182	0.045
5-mC, 5-methylcytosine; TL, telomere length.			

Discussion

The comparison of patients at different stages of SMD revealed a deterioration in anthropometric profile and less favorable markers of metabolic status particularly those reflecting disturbances in carbohydrate and lipid metabolism as well as liver function tests. These differences may result from disease progression, including transition from

steatosis to steatohepatitis or hepatic fibrosis, from pre-DM to T2DM, and the development of subclinical atherosclerosis against the background of DL and increasing OB severity.

At the same time, the patterns of 5-mC and TL likely reflect long-term metabolic stress under the influence of the therapy carried out so far, which may account for the lack of significant differences.[20] These findings emphasize the need for more detailed categorization of patients, considering the duration and severity of each condition contributing to SMD stage, as well as incorporating follow-up duration to better estimate the timing of meaningful epigenetic alterations.

In the context of MASLD and liver fibrosis, the lack of significant group differences may be partly explained by the relatively young age of the study population. According to Rattan et al. (2022), the association between TL and liver disease particularly advanced fibrosis was observed only in individuals aged ≥ 65 years.[21]

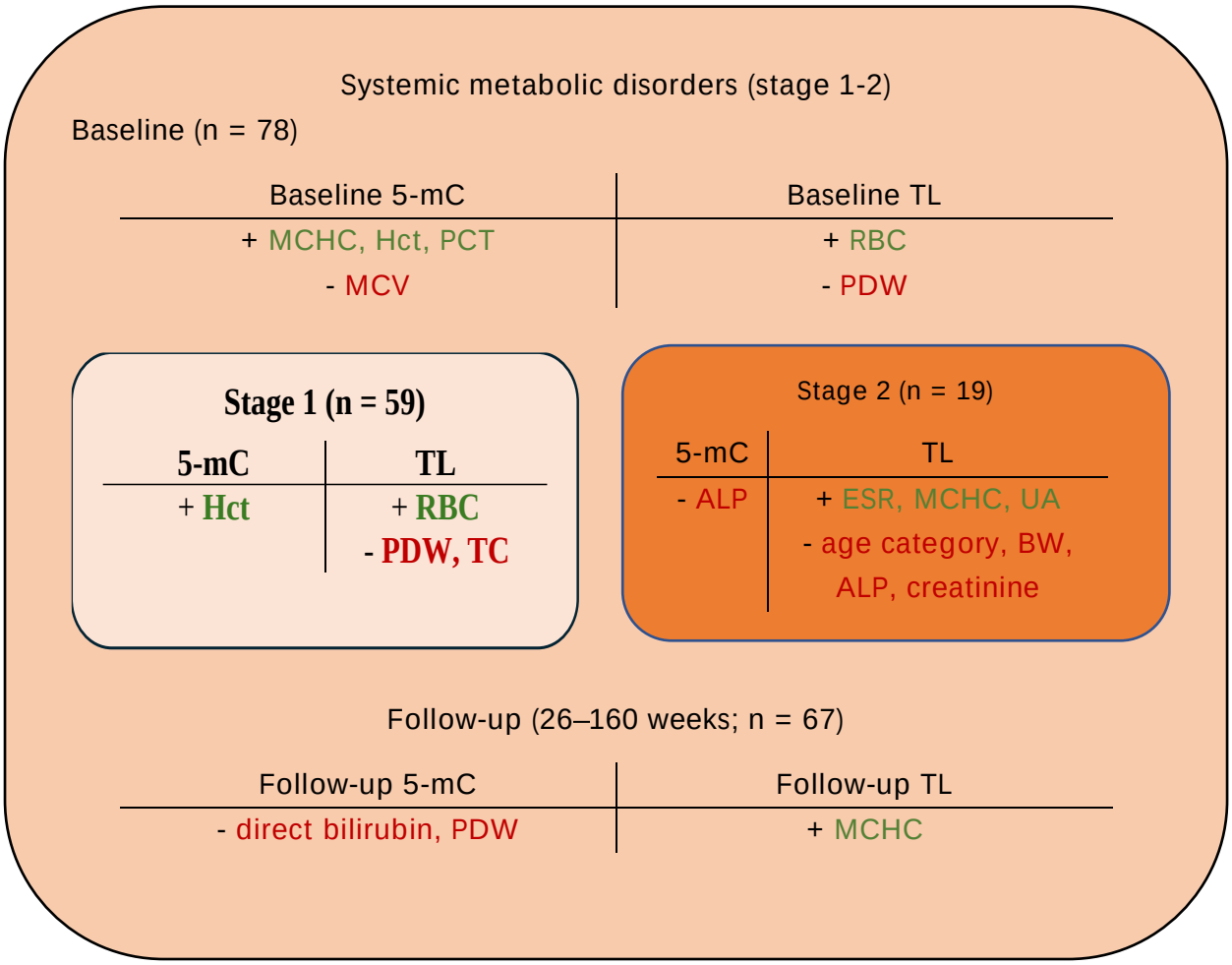
In the context of T2DM, the absence of major differences between patients with stage 2 and stage 1 SMD (including prediabetes), despite significant differences in glucose levels, could be due to well-controlled disease and maintenance of normoglycemia in most patients (as suggested by the median glucose levels presented above). Supporting this hypothesis, Lyu et al. (2020) reported that TL was inversely related to hemoglobin A1c, interleukin-6, tumor necrosis factor alpha, and that a link between telomere shortening and mitochondrial dysfunction was evident only under hyperglycemia, a key pathogenic mechanism underlying many age-related and cardiometabolic disorders.[22]

With respect to HTN, telomere shortening is typically observed and is concomitantly associated with the development of heart failure another component of stage 2 SMD. Among 646 hypertensive patients under 65 years with diabetes, coronary heart disease (CHD), or ≥ 3 cardiovascular risk factors (Zhang A. et al., 2025), heart failure prevalence increased with shorter TL (15.7%, 11.2%, and 7.9% across leukocyte TL tertiles; $p = 0.037$) at 5-year follow-up.[23] Thus, the absence of significant differences in our study may stem from the low prevalence of heart failure among patients with stage 2 SMD. However, this assumption requires further investigation, as we did not assess the specific contribution of each comorbidity to the observed outcomes, given that it was not the primary aim of this analysis. Moreover, Zhang et al. reported that participants in the short-TL group, compared with those with long telomeres, had a higher prevalence of male sex, hyperlipidemia, diabetes, and CHD, along with elevated blood pressure and fasting glucose factors that would be expected to be more common and pronounced in stage 2 than in stage 1 SMD. Therefore, further studies are warranted not only across SMD stages but also considering within-stage stratification by TL to better understand its role in cardiometabolic aging.

In addition, the high interindividual variability of 5-mC and TL levels driven by underlying influencing factors rather than the disease itself: genetic background, lifestyle factors (stress, diet, physical activity), and pharmacological impact (patient compliance, treatment modality, and therapy duration) not assessed in this study may also explain the absence of statistically significant between-group differences.[24, 25]

Overall, 5-mC levels and TL in patients with SMD were determined primarily by hematologic and metabolic parameters, but the pattern of associations varied substantially by SMD stage and over time (Fig. 1). ↑↓

Figure 1. Significant predictors of 5-methylcytosine levels and telomere length in patients with systemic metabolic disorders at different stages.



5-mC, 5-methylcytosine; ALP, alkaline phosphatase; BW, body weight; ESR, erythrocyte sedimentation rate; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PDW, platelet distribution width; PCT, plateletcrit; RBC, red blood cell count; TC, total cholesterol; TL, telomere length; UA, uric acid.

MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PDW, platelet distribution width; PCT, plateletcrit; RBC, red blood cell count; TC, total cholesterol; TL, telomere length; UA, uric acid.

Levels of 5-mC were mainly driven by erythropoietic and platelet-related markers (MCHC, mean corpuscular volume, hematocrit, plateletcrit) in the overall cohort, with hematocrit remaining the key determinant in stage 1 and ALP emerging as the single predictor in stage 2. At follow-up, the determinants shifted toward markers of hepatobiliary function and platelet activity (direct bilirubin, PDW). In patients re-examined within one year, metabolic and inflammatory indicators (WBC, glucose, triglycerides) became dominant predictors, suggesting that treatment- or disease-related metabolic changes modulate DNA methylation dynamics.

In the entire cohort, TL was influenced by erythropoietic and thrombopoietic activity (red blood cell count, PDW), with stage-specific profiles: red blood cell count, total cholesterol, and PDW predicted TL in stage 1, whereas in stage 2 TL reflected a broader set of age-, metabolic-, inflammatory, and erythropoietic-related determinants (age category, body weight, ALP, erythrocyte sedimentation rate, creatinine, MCHC, uric acid). At follow-up, TL was largely dependent on MCHC overall, while WBC predicted TL shortening in patients with <1-year intervals, indicating that inflammatory status and possible

therapy-related changes influence telomere dynamics over time.

Based on data from 11,775 individuals across six independent population-based cohorts within the BBMRI-NL consortium, Van der Spek et al. (2022) demonstrated that biomarkers involved in lipid metabolism were also associated with TL biology. However, among these metabolic biomarkers, this relationship did not include total cholesterol but rather two cholesterol-to-lipid ratios in small VLDLs.[26]

Among patients with stage 2 SMD, where OB is typically more prevalent and pronounced as it often forms part of the comorbid profile accompanying T2DM and hepatic fibrosis the observed association between body weight and TL is expected. According to Lejawa et al. (2021), telomeres shorten in individuals with metabolic dysregulation related to OB and oxidative stress.[27]

It is considered that uric acid acts as one of the body's non-enzymatic antioxidant defense mechanisms, which may explain the positive correlation observed between uric acid and TL in our study.[28] However, this protective effect appears to exist only within physiological ranges, and not under hyperuricemia. Moreover, the effects of uric acid lowering therapy among patients with hyperuricemia remain controversial, further suggesting that uric acid may possess context-dependent protective properties.[29]

A significant decline in albumin levels between baseline and follow-up visits among SMD patients may indicate chronic low-grade inflammation, reduced hepatic protein-synthetic capacity, or altered protein metabolism under sustained metabolic load.

In patients followed for more than one year, a moderate improvement in certain biochemical parameters (aspartate aminotransferase, glucose, albumin) was observed, possibly reflecting lifestyle modification or therapeutic intervention. At the same time, changes in 5-mC and TL showed divergent trends some patients exhibited an increase, while others a decrease highlighting substantial interindividual variability in epigenetic responses and underscoring the need for a more detailed analysis of the factors driving DNA methylation and TL dynamics in SMD. The observed sex-related difference in 5-mC changes (lower proportion of males among patients with increased 5-mC) may indicate the influence of sex hormones on epigenetic regulation, particularly greater female sensitivity to metabolic and inflammatory changes affecting DNA methyltransferase activity. The absence of significant age- or sex-related differences between these groups suggests that TL changes over the observation period were primarily driven by metabolic and cellular factors rather than demographic characteristics.

According to the analysis of two large patient populations from the Vanderbilt University and Marshfield Clinic biobanks both linked to genomic and phenomic medical record data only 23.7% of TL variance was explained by genome (12.8%), age (8.5%), phenome (1.5%), and sex (0.9%), supporting its potential as a clinically relevant biomarker.[30] Conversely, in a cohort of 317 randomized non-smoking participants with metabolic syndrome, Marti et al. (2023) reported that a 3-year lifestyle intervention resulting in a mean weight reduction of −3.7 ± 4 kg produced no significant TL change in the overall cohort.[31] However, a significant TL increase was observed only among women after 3 years (+0.25 ± 0.9 r.u.). In our study, TL dynamics were not analyzed separately by sex, and the proportion of women and men was comparable, which may have masked potential sex-specific effects of therapy.

The correlation analysis demonstrated that the dynamics of epigenetic markers namely, changes in 5-mC and TL were largely determined by baseline characteristics. Notably, patients with higher initial 5-mC levels exhibited TL elongation at follow-up despite an overall decline in 5-mC. This pattern likely reflects that these two parameters capture distinct yet interconnected

aspects of epigenetic regulation, which do not necessarily correlate directly in shaping a favorable aging phenotype. The correlations between 5-mC, sex, and age underscore the strong influence of demographic factors on the pace of epigenetic change. The absence of similar associations in the short-term follow-up group (<1 year) suggests that substantial epigenetic shifts emerge only under conditions of prolonged metabolic exposure whether through adverse metabolic load or because of pharmacological and therapeutic interventions highlighting the importance of long-term monitoring.

In the multiple linear regression model, a decrease in 5-mC (i.e., dynamic hypomethylation) was associated with higher baseline levels of 5-mC, glucose, mean corpuscular volume, BMI, and TL, whereas an increase in Δ5-mC (hypermethylation) was observed with higher RDW, MPV, creatinine, and very-low-density lipoprotein cholesterol. These findings indicate that the dynamics of global DNA methylation in patients with SMD are multifactorial and closely interconnected with both metabolic status and cellular characteristics of peripheral blood, as well as with other epigenetic markers. It is plausible that both hypomethylation and hypermethylation may represent a reactive response to disturbances in metabolic profile and cellular homeostasis in SMD. Considering our findings, assessing the individual longitudinal dynamics of 5-mC over time seems more appropriate than seeking a universal threshold applicable to all patients with SMD.

The analysis of TL change predictors demonstrated that higher glucose levels, a tendency toward hypermethylation, elevated direct bilirubin concentrations, and lower albumin levels at baseline were associated with telomere shortening over time. This biochemical profile reflects metabolic imbalance and oxidative stress, both of which accelerate telomere attrition. Conversely, the inverse relationship between TL dynamics and baseline TL may be explained by an age-related effect. It is plausible that these patients had milder metabolic disturbances and lower treatment adherence, which may have limited reactive TL elongation. To confirm these assumptions, prospective studies with careful monitoring of treatment compliance, therapeutic adjustments, and markers of oxidative stress and inflammation are warranted. Such investigations would help clarify whether TL elongation reflects the biological effect of successful correction of metabolic dysfunction.

Conclusions

Stage 2 systemic metabolic disorder (SMD) exhibited a less favorable anthropometric and biochemical profile, together with a shift toward deoxyribonucleic acid hypermethylation and telomere attrition, suggesting potential exhaustion of compensatory epigenetic mechanisms. Predictors of 5-methylcytosine (5-mC) levels differed by stage: hematocrit ($p = 0.041$) in stage 1 and alkaline phosphatase ($p = 0.017$) in stage 2. In stage 1 SMD, telomere length (TL) was positively associated with red blood cell count ($p = 0.001$) and negatively associated with platelet distribution width ($p = 0.028$) and total cholesterol ($p = 0.019$). In stage 2, TL elongation correlated with higher erythrocyte sedimentation rate ($p < 0.001$), mean corpuscular hemoglobin concentration ($p = 0.003$), and uric acid ($p = 0.007$), whereas TL shortening was associated with older age ($p < 0.001$), greater body weight ($p < 0.001$), elevated alkaline phosphatase ($p < 0.001$), and increased creatinine ($p < 0.001$).

Longitudinal analyses indicated that a decline in 5-mC was predicted by higher baseline 5-mC, TL, glucose, mean corpuscular volume, and body mass index. In contrast, increases in 5-mC were associated with higher red cell distribution width, mean platelet volume, creatinine, and low-density lipoprotein cholesterol ($p < 0.05$ for all pairs). Telomere shortening over follow-up was associated with elevated baseline glucose ($p = 0.045$) and direct bilirubin ($p = 0.012$), together with lower albumin ($p = 0.019$). Conversely, higher

5-mC promoted TL elongation ($p = 0.001$), while longer baseline TL predicted more pronounced subsequent shortening ($p < 0.001$).

These findings support the potential utility of composite biomarkers integrating anthropometric and biochemical parameters for early detection and prediction of adverse epigenetic shifts in patients with SMD.

Conflict of Interest:

The authors declare no conflicts of interest related to the publication of this article.

Funding Statement:

The authors received no financial support for the research, authorship, and/or publication of this article.

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