

ORIGINAL RESEARCH

Plasma *PDGFR β* and *PSD4* methylation levels for non-invasive staging of liver fibrosis

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Highlights & Insights

- **Scientific Gap:** Liver biopsy remains an imperfect gold standard for fibrosis assessment due to sampling errors and inter-observer variability. Although non-invasive methods have improved evaluation, they cannot fully replace biopsy; accurate, timely fibrosis detection in MASLD remains needed.
- **Key Finding:** Machine learning using cell-free epigenetic markers and clinical data identified significant differences in plasma *PDGFR β* and *PSD4* methylation by MASLD status and fibrosis stage
- **Clinical Impact:** These methylation signatures may offer a promising non-invasive approach for assessing fibrosis in MASLD.

ABSTRACT

Background and Aim: While liver biopsy remains the reference standard for assessing hepatic fibrosis, the major prognostic factor in metabolic dysfunction-associated steatotic liver disease (MASLD), its inherent limitations have driven the search for innovative non-invasive diagnostic tests. In this study, we sought to evaluate the diagnostic accuracy of plasma *PDGFR β* and *PSD4* methylation levels, integrated within machine learning algorithms, for non-invasive staging of hepatic fibrosis in patients with MASLD, and to compare its performance against established non-invasive biomarkers.

Materials and Methods: Patients with biopsy-proven MASLD and healthy controls were recruited from the three institutions. Quantitative methylation of circulating cell-free DNA was assessed using bisulfite modification and pyrosequencing. The resulting data were used to develop linear discriminant analysis, random forest, and support vector machine algorithms to identify patients at different stages of fibrosis.

Results: The study included 234 patients with histologically confirmed MASLD and 43 healthy controls. Each dataset was validated using an independent cohort. Advanced fibrosis was associated with elevated plasma *PDGFR β* and *PSD4* methylation levels in both the discovery and validation cohorts. The integration of plasma DNA methylation markers with clinical parameters demonstrated performance comparable to that of existing noninvasive biomarkers for detecting both significant and advanced fibrosis, achieving area under the curve scores exceeding 0.75 across all models.

Conclusion: Supervised machine learning algorithms incorporating plasma *PDGFR β* and *PSD4* DNA methylation levels show promise as a non-invasive approach for assessing hepatic fibrosis in MASLD.

Keywords: Cell-free DNA, DNA methylation, liver fibrosis, machine learning algorithms, MASLD, predictive models.

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), is a complex and prevalent clinical condition, affecting over 30% of the global population.^[1] It is closely associated with obesity, type 2 diabetes, dyslipidemia and metabolic syndrome, which collectively contribute to hepatic injury and increase the risk of severe hepatic complications, including advanced fibrosis, cirrhosis and hepatocellular carcinoma (HCC).^[2,3] Notably, MASLD has emerged as the leading cause of chronic liver disease worldwide and is a significant contributor to liver-related morbidity and mortality.

Until recently, no pharmacological treatment had received regulatory approval for MASLD. However, the U.S. Food and Drug Administration (FDA) has recently granted accelerated approval to resmetirom for the treatment of Metabolic dysfunction-associated steatohepatitis (MASH) in patients with moderate-to-advanced liver fibrosis (stages F2 and F3). Notably, resmetirom, an oral thyroid hormone receptor- β -agonist, has demonstrated efficacy in reducing hepatic fibrosis and improving key histopathological features of MASH, including hepatic fat content and inflammation.^[4]

Fibrosis represents a critical prognostic determinant in chronic liver diseases of various etiologies. The early detection and accurate staging of hepatic fibrosis are essential to prevent the progression to more advanced stages, such as decompensated cirrhosis.^[5] While liver biopsy remains the reference standard for fibrosis assessment, its invasive nature, susceptibility to sampling errors, and inter-observer variability highlight the need for alternative diagnostic approaches.^[3,6–8] Non-invasive techniques (NIT), including serum biomarkers, routine laboratory tests, scoring systems (e.g., FIB-4 index, the NAFLD fibrosis score), and imaging-based methods such as vibration-controlled Transient Elastography (VCTE; FibroScan®, Echosens, Paris, France), have emerged as valuable and effective tools for excluding advanced fibrosis. Among these, VCTE is particularly effective for assessing liver stiffness and staging fibrosis in patients with MASLD. Nevertheless, limitations such as higher failure rates in obese individuals and practical challenges related to its application and stability restrict its broader feasibility.^[9] Despite their utility, NITs cannot fully replace liver biopsy in clinical practice.^[9,10]

The mechanisms driving the development and progression of MASLD are intricate and remain incompletely understood. Multiple factors, including dietary habits, lifestyle, genetic predisposition, and epigenetic modifications, have been implicated in the pathophysiology of MASLD.^[11,12] Among these, DNA methylation, a key epigenetic modification, predominantly occurs at cytosine-guanine dinucleotides, known as CpG sites, and is closely linked to alterations in gene expression. DNA methylation is mediated by DNA methyltransferases (DNMTs), which catalyze the addition of a methyl group to the fifth carbon of the cytosine ring, forming 5-methylcytosine. It plays a significant role in the pathogenesis of metabolic disorders including obesity, diabetes, and MASLD.^[13] Extensive research has demonstrated the association between DNA methylation patterns and liver diseases.^[3,14–17] For example, Gerhard

et al.^[14] identified the correlation between DNA methylation levels in patients with MASLD and key signaling pathways. Similarly, Xu et al.^[16] identified ten methylation markers for diagnosing HCC. Furthermore, our previous studies revealed significantly higher DNA methylation levels within the PPAR γ and PPAR α promoters, two genes with anti-fibrogenic effects, in patients with severe MASLD.^[17,18]

Predictive models are widely employed in the diagnosis and prognosis of liver diseases. For instance, Batista et al.^[19] applied Linear Discriminant Analysis (LDA) to develop a model for predicting fibrosis stages. Similarly, recent research has highlighted the effectiveness of the support vector machine (SVM) model in predicting fibrosis risk based on the patients' radiomics data.^[20] Building on the significance of predictive modeling, we evaluated whether methylation levels of circulating cell-free plasma DNA at specific CpG sites could serve as biomarkers to stratify fibrosis stages in MASLD. Two genes, *PDGFR β* and Pleckstrin and Sec7 Domain Containing 4 (*PSD4*), were selected due to their potential association with liver disease or fibrosis biology.^[16,21] Subsequently, three predictive models were developed and compared to identify the most effective approach for fibrosis stage stratification. These findings are anticipated to contribute to the development of novel NITs for liver fibrosis, offering valuable insights for future diagnostic advancements.

Material and Methods

Study Cohorts

The study was conducted on MASLD patients (Discovery Cohort n=115, Validation Cohort n=119) and healthy individuals (Discovery Cohort n=21, Validation Cohort n=22). Blood samples and clinical data were collected after obtaining informed consent from participants in compliance with ethical standards and approved by Koc University Research Ethics Committees [approval number: 2015.053. IRB1.014 (30.03.2015), 2016.024.IRB2.005 (15.02.2016), 2017.139. IRB2.048 (10.09.2017), 2022.246.IRB2.040 (30.07.2022), 09.2018.086 (24.09.2018)]. The study adhered to the ethical principles outlined in the Declaration of Helsinki. The diagnosis of MASLD was established based on the guidelines of American Association for the Study of Liver Diseases.^[22] The fibrosis severity in the study participants was assessed using histopathological evaluation.

DNA Methylation and Pyrosequencing Analysis

200 μ L of plasma was used for DNA extraction with the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The DNA was then treated with bisulfite using the EZ DNA Methylation Gold kit (Zymo Research, Irvine, CA, US) according to the manufacturer's instructions. The bisulfite-modified DNA was amplified using custom-designed primers. The primers were designed using PyroMark Assay Design Software 2.0, and their sequences are provided in Supplementary Table 1. Amplification was performed using the PyroMark-PCR kit (Qiagen) according to conditions in Supplementary Table 2. After thermal cycling, all samples were loaded onto a 1.5% agarose gel and run at 100 volts for 30 minutes. Samples were processed for the

Table 1. Demographic and clinical characteristics of patients

	Discovery cohort (n=136)		Validation cohort (n=141)	
	Healthy (n=21)	MASLD (n=115)	Healthy (n=22)	MASLD (n=119)
Male, n (%)	11 (52.3)	69 (60.0)	7 (31.8)	57 (47.9)
Female, n (%)	10 (47.6)	46 (40.0)	15 (68.2)	62 (52.1)
Age (years)	37 (24, 42)	55 (47, 61)	35 (25, 40)	53 (46, 59)
Diabetes, n (%)	0	69 (60.0)	0	85 (71.4)
BMI (kg/m ²)	23.9±2.1	29.5 (26.7, 34.2)****	23.86±2.4	33.3 (29.7, 36.6)
Albumin (g/dL)	4.82±0.1**	4.5 (4, 4.8)	4.65±0.1	4.4 (4.1, 4.7)
ALT (IU/L),	22.0±10.0	40 (25.5, 69.5)*	19.93±7.3	49 (31.0, 98.0)
AST (IU/L)	21.68±7.6	32.7 (25.0, 47.0)**	19.10±5.3	41 (29.0, 62.0)
Platelet (x10 ⁹),	258.5±56.4	213.5 (133.0, 285.0)	240.0±46.4	208.0 (148.0, 248.0)
FIB-4 Index	0.6±0.2	1.2 (0.7, 3.2)*	0.6±0.2	1.4 (1.0, 2.3)
Fibrosis stage, n (%)				
0	–	16 (13.91)	–	0 (0.00)
1	–	23 (20.00)	–	25 (21.00)
2	–	16 (13.91)	–	35 (29.41)
3	–	27 (23.48)	–	30 (25.21)
4	–	33 (28.70)	–	29 (24.37)
NAS score	–	3.0 (2.0, 4.0)****	–	6.0 (5.0, 7.0)

Categorical variables are shown as frequencies (percentages). Normally distributed data are presented as “mean±standard deviation” and non-normally distributed data are presented as “median (interquartile range)”. Comparisons between cohorts of each subgroup were made using the Student’s t-test for continuous and Fisher’s exact test for categorical variables. Statistically significant variables are annotated on the discovery cohort and p-values are given as p<0.05 (*), 0.01 (**), 0.0001 (****).

ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BMI: Body mass index; MASLD: Metabolic dysfunction-associated steatotic liver disease; NAS: NAFLD Activity score.

pyrosequencing experiment only after their band intensities shown in Supplementary Figure 1 had been checked. Sample preparation and pyrosequencing were subsequently carried out on the PyroMark Q96 ID instrument (Qiagen). Unmethylated and methylated EpiTect DNA controls (Qiagen) and negative PCR templates were used for each experiment. The average methylation percentages were analyzed using PyroMark software (version 2.5.8; Supplementary Fig. 2–6).

Predictive Models for Staging Fibrosis and Statistical Analysis

The discovery dataset was used to develop three binary classification models aimed at distinguishing between different stages of fibrosis. The first model was designed to differentiate patients with significant fibrosis ($F \geq 2$) from healthy subjects and those with mild or no fibrosis ($F \leq 1$). The second model focused on identifying patients with advanced fibrosis ($F \geq 3$), while the third model aimed to differentiate patients with cirrhosis ($F = 4$). To construct these models, three machine-learning algorithms were employed. Linear Discriminant Analysis was implemented using the LDA () function from the MASS package (version 7.3-60.0.1). The Random Forest algorithm was implemented using the random Forest () function in the randomForest R package (version 4.7-1.1). Finally, the SVM algorithm was implemented using the SVM () function from the

e1071 package (version 1.7-14). These algorithms were selected to assess their accuracy in classifying fibrosis stages using the provided data. Initially, the discovery dataset, consisting of 85 patients with MASLD and 21 healthy controls, was divided into a training set (70%) and a testing set (30%) using the create Data Partition () function from the caret R package (v 6.0-94). Hyperparameter optimization was performed through 5-fold cross-validation on the training set to maximize the area under the receiver operating characteristic (AUC ROC) curve. Once the models were trained and stabilized, the predict () function in caret was employed to estimate the class probabilities for the samples in the test set. The trained models were then independently validated on an external dataset comprising 103 patients with MASLD and 22 healthy controls. Samples with missing feature data were excluded from the model training and prediction procedures. The pROC R package (v 1.18.5) was used to visualize the ROC curve. Performance metrics, including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), precision, recall, and F1 scores, were calculated and presented in Supplementary Tables 3 and 4.

All statistical analyses were performed using GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA). For comparisons between cohorts, descriptive statistics were used. The Shapiro-Wilk test was used to check the normality of continuous

variables. Normally distributed variables were analyzed using the Student's t-test and presented as "mean±standard deviation" whereas non-normally distributed variables were analyzed using the Mann-Whitney U test and presented as median (interquartile range). Categorical variables were analyzed with Fisher's exact test and presented as frequencies (percentages). Two-tailed p-values <0.05 were considered statistically significant.

Results

The study cohort included 43 healthy subjects and 234 patients diagnosed with MASLD. The histological assessments and Fibroscan evaluations revealed that 115 patients exhibited mild fibrosis (stages F0-F2), whereas 119 had advanced fibrosis (stages F3-F4). The general characteristics of study participants are summarized in Table 1.

Higher plasma *PDGFRβ* and *PSD4* DNA methylation are associated with advanced hepatic fibrosis.

We investigated DNA methylation profiles at specific CpG sites within *PSD4*, a locus recently implicated in HCC, and *PDGFRβ*, a receptor for PDGF strongly expressed in hepatic stellate cells and activated myofibroblasts.^[16,21] Cell-free DNA was extracted from blood samples, and pyrosequencing assays were optimized for targeted CpG sites (Supplementary Table 1). Plasma DNA methylation levels were quantified at five CpG loci within the *PDGFRβ* promoter and three CpG loci within the *PSD4* promoter (Fig. 1, 2). The discovery cohort consisted of patients recruited from two centers. In this study, all CpG sites within *PDGFRβ* exhibited higher DNA methylation levels in patients with MASLD than in healthy controls (Fig. 1a-e). Notably, methylation levels at *PDGFRβ* CpGs 5 and 6 were significantly higher in patients with MASLD and advanced fibrosis (F3-F4) compared with those with mild fibrosis (F0-F2) and healthy controls (Fig. 1a, b). Other CpG sites (CpG 7–9) showed higher methylation levels in MASLD groups than in healthy controls (Fig. 1c–e). These findings were validated in an independent patient cohort recruited from the third center (Fig. 2). The elevated methylation levels at *PDGFRβ* CpG sites 5 and 6 in F3–4 MASLD remained significant but were less pronounced in the validation cohort (Fig. 2a, b).

For the *PSD4* promoter, plasma DNA methylation levels at all targeted CpG loci were considerably higher in patients with MASLD than in healthy controls in both the discovery and validation cohorts (p<0.001) (Fig. 1f–h, Fig. 2f–h). Remarkably, patients with advanced fibrosis exhibited higher methylation levels than those with mild fibrosis in both cohorts.

Machine Learning Algorithms for Accurate Staging of Liver Fibrosis

To identify fibrosis stages, we developed predictive models integrating plasma DNA methylation data and clinical parameters. Four diagnostic approaches were assessed across four distinct datasets: the FIB-4 index, clinical data alone, DNA methylation levels alone, and a combination of clinical data and DNA methylation levels. Patients were stratified into four fibrosis categories for comparison:

mild/no fibrosis (F0-1), significant fibrosis (≥F2), advanced fibrosis (≥F3), and cirrhosis (F4). To benchmark the models' performance, we conducted AUC analyses using three supervised machine learning approaches: (i) *lda()*, (ii) *randomForest()*, (iii) *svm()*.

Among the three supervised machine learning algorithms tested, SVM achieved the best diagnostic performance for detecting significant fibrosis, advanced fibrosis, and cirrhosis (Fig. 3). For identifying or excluding significant fibrosis, algorithms based on DNA methylation with or without clinical parameters performed comparably to the FIB-4 index, achieving AUC values of 0.84, 0.87, and 0.81 in the discovery cohort, and 0.63, 0.81, and 0.76 in the validation cohort, respectively (Fig. 3a, b). In detecting advanced fibrosis, SVM-based models outperformed the FIB-4 index, with higher positive predictive values of 0.87 and 0.74 compared with 0.66. For exclusion of advanced fibrosis, all three methods demonstrated excellent negative predictive values (Supplementary Tables 3 and 4). Additionally, models based on plasma DNA methylation levels of *PSD4* and *PDGFRβ* showed excellent diagnostic accuracy for detecting and ruling out stage 4 fibrosis (cirrhosis) (Supplementary Tables 3 and 4).

Overall, combining clinical data with DNA methylation levels yielded promising results for diagnosing or ruling out various stages of fibrosis, with AUC values consistently exceeding 0.75. Detailed comparisons of cohort performance across predictive models are presented in Figure 3 and Supplementary Figures 7 and 8; sensitivity and specificity metrics are provided in Supplementary Tables 3 and 4.

Discussion

Early and accurate identification of hepatic fibrosis is crucial for determining the clinical prognosis of chronic liver diseases and plays a vital role in preventing and treating advanced liver diseases.^[5] Significant efforts have been directed towards developing novel NITs, including blood-based biomarkers, for fibrosis assessment.^[9] In this study, we explored the potential of cell-free DNA methylation signatures as biomarkers for liver fibrosis in MASLD. Specifically, we analyzed DNA methylation levels in two candidate genes (*PDGFRβ* and *PSD4*) and identified significant differences across distinct fibrosis stages (significant fibrosis, advanced fibrosis, and cirrhosis).

PDGFRβ is the receptor for PDGF-B, a well-established pro-fibrotic growth factor predominantly produced by Kupffer cells, pericytes, and hepatic stellate cells. PDGF has a pivotal role in the extracellular matrix remodeling, particularly collagen synthesis and is implicated in various fibroproliferative disorders.^[23] In this study, a significant increase in *PDGFRβ* methylation levels at 5 CpG sites was observed in patients with mild or advanced fibrosis compared with healthy controls in the discovery cohort. Notably, CpG 5-6 methylation levels of *PDGFRβ* showed marked differences between MASLD F0-2 and MASLD F3-4 patients in the discovery data; in the validation cohort, the difference was modest but statistically significant. Previous research by Lambrecht et al.^[24] indicated that extracellular vesicles derived from activated murine and human hepatic stellate cells are enriched for *PDGFRβ* expression. Furthermore, circulating *PDGFRβ*

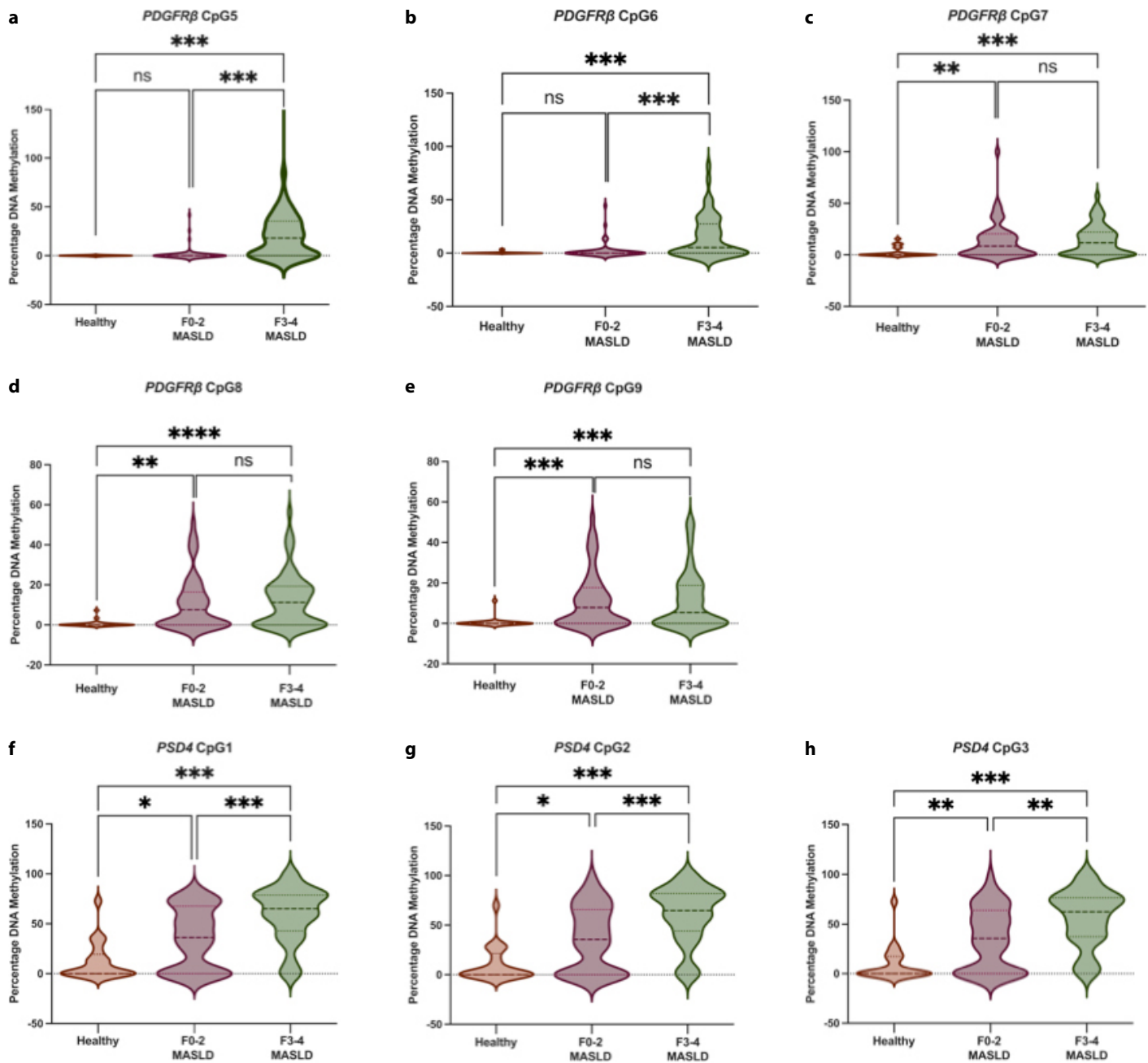


Figure 1. Plasma DNA methylation level of MASLD patients in the discovery cohort. Plots were shown as the correlation between the CpG methylation and fibrosis stage of the MASLD. The analysis examines the plasma DNA methylation levels across fibrosis stages F0-2 and F3-4, alongside those of healthy subjects. Bisulfite modification and pyrosequencing were used to assess the plasma CpG methylation at five loci in *PDGFRβ* and three loci in the *PSD4* gene. Changes in *PDGFRβ* (a–e) and *PSD4* (f–h) CpG methylation levels are associated with the fibrosis stage in the discovery cohort. DNA methylation is quantified and represented as a percentage.

*p<0.05, **p<0.01 and ***p<0.001.

protein levels, combined with a PDGFR β -based (PRTA) score, were identified as biomarkers of hepatic fibrosis, achieving AUROC values of 0.78 for significant fibrosis, 0.74 for advanced fibrosis, and 0.79 for cirrhosis across three different etiologies. In our study, apoptotic or necrotic myofibroblasts might have contributed to the circulating cell-free DNA methylation pool, thereby leading to an increase in *PDGFRβ* methylation in association with hepatic fibrosis.

PSD4 was previously identified as a prominent circulating cell-free methylation marker for HCC in a large cohort study.^[16] Additionally, prior research has demonstrated that TNF- α -induced phospho-p65 activation leads to the recruitment of DNMT1, resulting in hypermethylation of the *PSD4* promoter. This epigenetic modification is associated with carcinogenesis, particularly in individuals with excessive alcohol consumption.^[25] Based on these

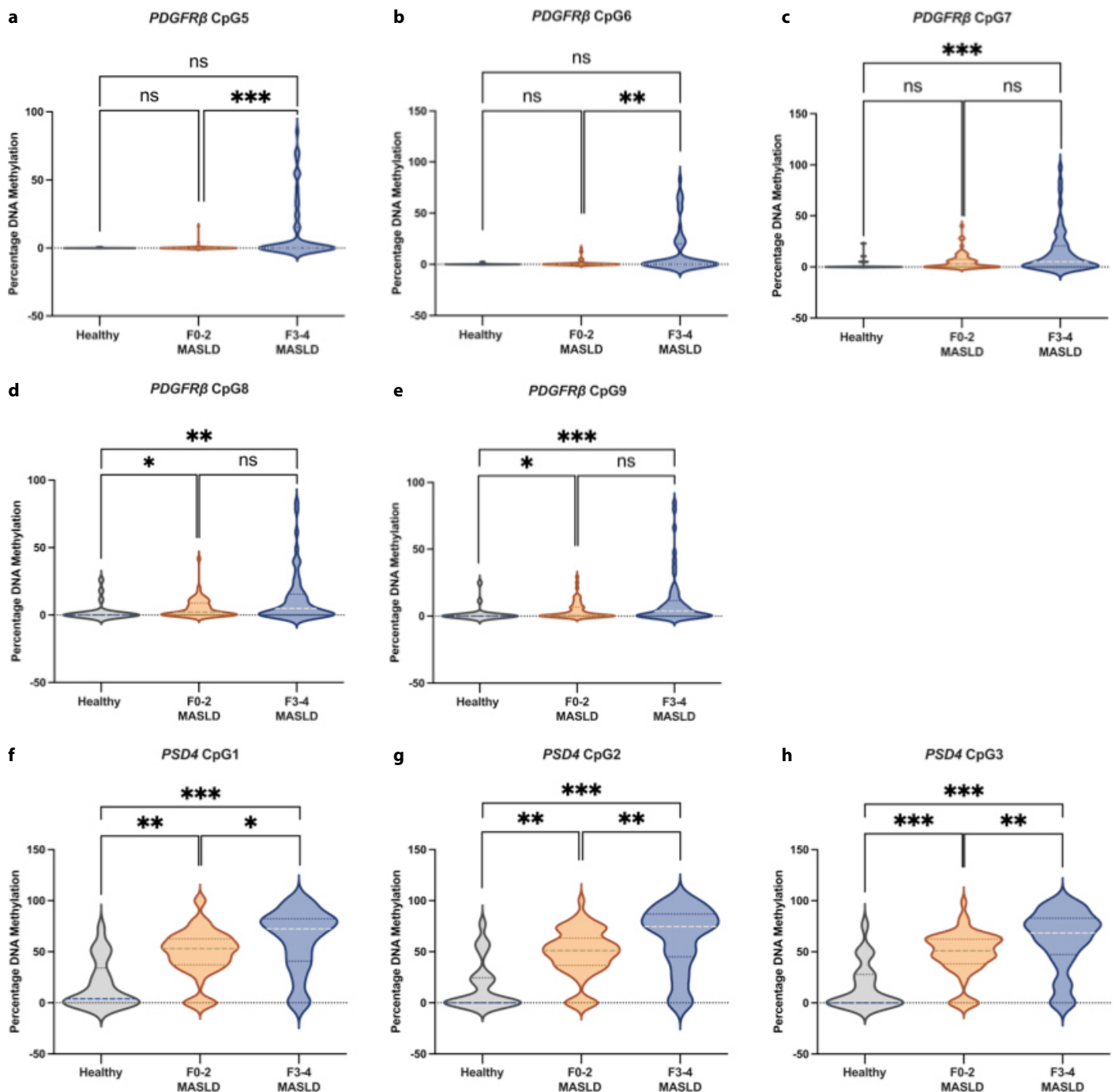


Figure 2. Plasma DNA methylation level of MASLD patients in the validation cohort. Plots were shown as the correlation between the CpG methylation and fibrosis stage of the MASLD. The analysis examines the plasma DNA methylation levels across fibrosis stages F0-2 and F3-4, alongside those of healthy subjects in the validation cohort. Bisulfite modification and pyrosequencing were used to assess the plasma CpG methylation at five loci in *PDGFRβ* and three loci in the *PSD4* gene. Changes in *PDGFRβ* (a–e) and *PSD4* (f–h) CpG methylation levels are associated with the fibrosis stage in the validation cohort. DNA methylation is quantified and represented as a percentage.

*p < 0.05, **p < 0.01 and ***p < 0.001.

findings, we investigated whether plasma methylation levels of the *PSD4* gene are associated with fibrosis stages in MASLD. Our analysis revealed that changes in cell-free DNA methylation within the *PSD4* promoter region were remarkably consistent across

cohorts and notably higher in MASLD patients than in healthy controls (Fig. 1f–h, Fig. 2f–h). These results suggest that *PSD4* methylation levels could serve as reliable predictors not only for hepatic fibrosis but also for HCC.

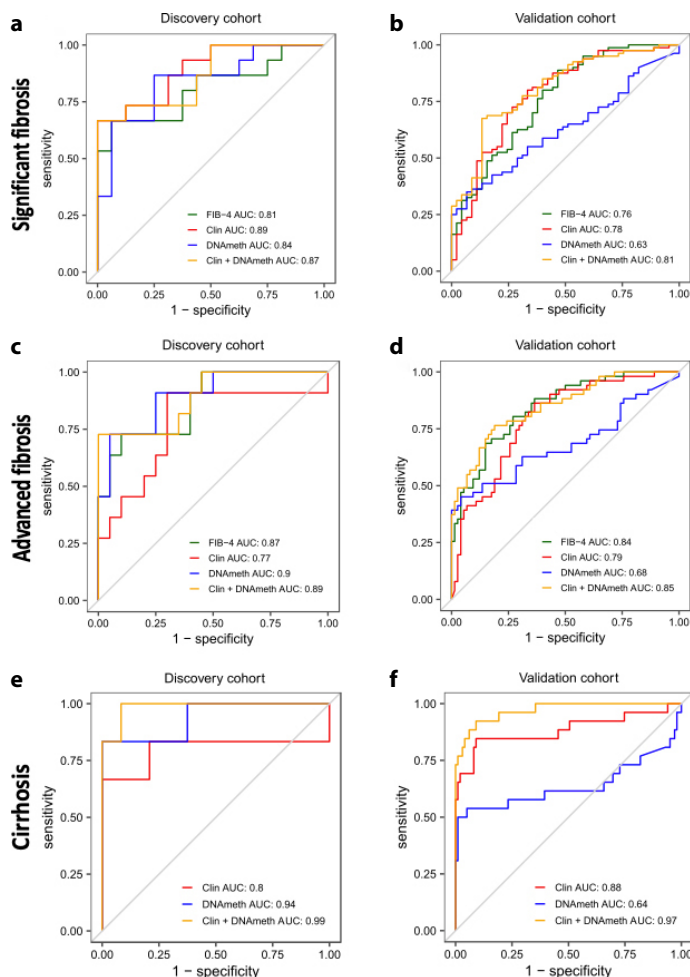


Figure 3. SVM analysis model in MASLD patients. This figure shows the SVM analysis in patients with different stages of fibrosis. **(a–b)** ROC curves of four markers (FIB-4, Clinical parameters, DNA Methylation, and Clinical Parameters + DNA methylation combination) in the discovery and validation cohort of patients with significant fibrosis. **(c–d)** ROC curves of four markers (FIB-4, Clinical parameters, DNA Methylation, and Clinical Parameters + DNA methylation combination) in the discovery and validation cohort of patients with advanced fibrosis. **(e–f)** ROC curves of four markers (FIB-4, Clinical parameters, DNA Methylation, and Clinical Parameters + DNA methylation combination) in cirrhotic patients' discovery and validation cohort.

We next evaluated whether DNA methylation markers outperformed other biomarkers, including the FIB-4 index. To this end, we applied three widely used supervised machine learning algorithms: LDA, Random Forest, and SVM.^[20,26,27] While differences were observed between cohorts for each marker, the AUROC scores consistently favored the combination of DNA methylation and clinical data in SVM analyses. The observed discrepancies between cohorts may be attributed to differences in sample size and fibrosis severity among patients. Notably, SVM results demonstrated that combining DNA methylation markers with clinical data yielded strong diagnostic performance, as reflected by AUROC scores and NPVs.^[28–30] Importantly, even DNA methylation-based algorithms without incorporating clinical parameters showed robust

performance in ruling out fibrosis. These algorithms achieved an NPV of 0.83 for significant fibrosis and an NPV of 1 for advanced fibrosis, highlighting their potential utility in primary care settings for stratifying MASLD patients and identifying those who require referral to specialized centers.

There are several limitations to this study. First, the relatively modest sample size may constrain the generalizability of our findings. Second, although multicenter, the data collection was limited to three medical centers within a single country, potentially introducing geographical and ethnic biases that may affect the generalizability of our results. Another significant limitation concerns participant selection: study included only a subset of metabolic profiles, which may not fully represent the diverse spectrum of metabolic manifestations observed in clinical practice. From an analytical perspective, although pyrosequencing provides highly accurate methylation analysis, its labor-intensive nature may present challenges for widespread clinical implementation. This methodological constraint could affect the immediate translation of our findings into routine clinical practice, particularly in resource-limited settings.

Conclusion

In conclusion, we explored the potential of machine learning algorithms as a non-invasive diagnostic tools for assessing fibrosis in patients with MASLD. Our findings demonstrate that methylation levels in circulating cell-free DNA are significantly altered in this patient group, aligning with prior research.^[3] Collectively, our findings underscore the potential of DNA methylation as a biomarker for fibrosis staging.

Online Appendix Link: <https://hepatologyforum.org/storage/upload/files/1773648500-appendix-en.pdf>

Ethics Committee Approval: Blood samples and clinical data were collected after obtaining informed consent from participants in compliance with ethical standards and approved by Koc University Research Ethics Committees [approval number: 2015.053.IRB1.014 (30.03.2015), 2016.024.IRB2.005 (15.02.2016), 2017.139.IRB2.048 (10.09.2017), 2022.246.IRB2.040 (30.07.2022), 09.2018.086 (24.09.2018)].

Informed Consent: Blood samples and clinical data were collected after obtaining informed consent from participants in compliance with ethical standards.

Conflict of Interest: Yusuf Yilmaz has received honoraria for lectures from Echosens and consulting fees from Zydus, Akero, and Novo Nordisk. Adil Mardinoglu is the founder of SZA Longevity, Trustlife Therapeutics, ScandiBio Therapeutics, and ScandiEdge Therapeutics. All other authors have no conflicts of interest to declare.

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