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Analysis of Genetic Variants and Methylation Alterations in Lynch Syndrome-Associated Cancers

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1. Abstract

Lynch syndrome (LS) is a hereditary cancer syndrome caused by mutations in DNA mismatch repair (MMR) genes, leading to microsatellite instability (MSI) and increased cancer risk. Emerging evidence suggests that PCSK9, a gene involved in cholesterol metabolism, may influence tumour progression and immune modulation in LS. This study investigates the role of PCSK9 mutations and DNA methylation alterations in LS-associated cancers to assess their impact on metastasis and biomarker potential. Clinical exome sequencing (CES) and next-generation sequencing (NGS) were used to analyze PCSK9 and LS-related MMR genes in LS patients. Methylation profiling assessed epigenetic modifications in key MMR genes. PCSK9 variants correlated with distinct methylation patterns, particularly MLH1 promoter hypermethylation and PMS2 loss in homozygous carriers. Heterozygous carriers displayed variable methylation abnormalities, while mutation-negative cases still exhibited epigenetic dysregulation. PCSK9 may serve as a genetic modifier in LS, influencing tumour progression through methylation and immune modulation. Its potential as a biomarker and therapeutic target warrants further investigation, particularly in combination with immune checkpoint inhibitors.

2. Introduction

Lynch syndrome (LS), also known as hereditary nonpolyposis colorectal cancer (HNPCC), is the most prevalent hereditary cancer predisposition syndrome, affecting approximately 1 in 280 individuals [1]. It is characterized by germline mutations in DNA mismatch repair (MMR) genes—MLH1, MSH2, MSH6, and PMS2—or deletions in the EPCAM gene, leading to defective DNA repair mechanisms [2]. This defect results in microsatellite instability (MSI), contributing to carcinogenesis in various tissues [3]. Individuals with LS face significantly elevated lifetime risks for several cancers: colorectal cancer (52–58%), endometrial cancer (25–60%), gastric cancer (6–13%), and ovarian cancer (4–12%) [4]. Notably, LS-associated colorectal cancers often exhibit distinct pathological features, including poor differentiation and a pronounced immune response, which may influence their metastatic behaviour [5].The molecular mechanisms and the relationship between LS and metastasis remain complicated. Some studies suggest that MSI-high (MSI-H) tumours, common in LS, may have a lower propensity for metastasis compared to microsatellite-stable tumours [6]. This is partly attributed to the enhanced immune surveillance associated with MSI-H tumours, which may impede metastatic progression [7]. However, when metastasis does occur in LS-associated cancers, it presents unique clinical challenges, necessitating tailored therapeutic strategies [8].Beyond the traditional MMR-related genes, emerging evidence suggests that other genetic alterations may contribute to the heterogeneity and progression of LS, including mutations in the proprotein convertase subtilisin/kexin type 9 (PCSK9) gene. PCSK9 is primarily known for its role in cholesterol metabolism by modulating low-density lipoprotein receptor (LDLR) degradation [9]. However, recent studies have highlighted its involvement in cancer biology, particularly in regulating tumour cell proliferation, migration, and metastasis [10, 11]. For instance, a commonly inherited variant of the PCSK9 gene has been associated with an increased risk of breast cancer metastasis, suggesting a broader role in cancer progression [12].The significance of PCSK9 in LS lies in its potential dual role: first, as a genetic modifier of the LS phenotype, influencing tumour development and progression; and second, as a potential therapeutic target. Emerging evidence indicates that altered PCSK9 expression may modulate immune checkpoint pathways, including PD-1/PD-L1, thereby affecting tumour immune evasion. This mechanism could be particularly relevant in the context of metastatic MSI-high tumours, where immunotherapy has shown promising results [10, 13].Recent advancements in immunotherapy have shown promise for treating metastatic MSI-H cancers in LS patients. Checkpoint inhibitors targeting the PD-1/PD-L1 pathway have demonstrated efficacy, leading to durable responses in some cases [14]. These developments underscore the importance of understanding the metastatic patterns of LS-related cancers to optimise patient outcomes.This study aims to explore the relevance of PCSK9 mutations in LS by analysing their impact on methylation status, and metastatic progression in vivo. Understanding the role of PCSK9 in LS-associated cancers could provide new insights into the molecular mechanisms driving metastasis and inform the development of targeted therapeutic strategies to improve patient outcomes.

3. Results

We analysed the mutation and methylation profiles of a total 37 patients diagnosed with or suspected of LS to investigate the potential role of PCSK9 mutations in LS-associated tumourigenesis. Our results highlight distinct patterns in the occurrence of PCSK9 variants, corresponding methylation statuses, and their potential implications for MMR protein expression loss.

3.1. Mutation Analysis

NGS identified three primary PCSK9 mutation categories in the patient cohort: Homozygous variant: PCSK9:c.1420G > A (p.Val474Ile); Heterozygous variant: PCSK9:c.1420G > A (p.Val474Ile) or wild-type: No PCSK9 variant detected. Among the 37 patients included in the study, homozygous PCSK9:c.1420G > A mutations were detected in 62% (23/37) of cases, while heterozygous mutations were more prevalent, occurring in 35% (13/37). One case (1/37) exhibited no detectable PCSK9 variants (Figure 1A, B).

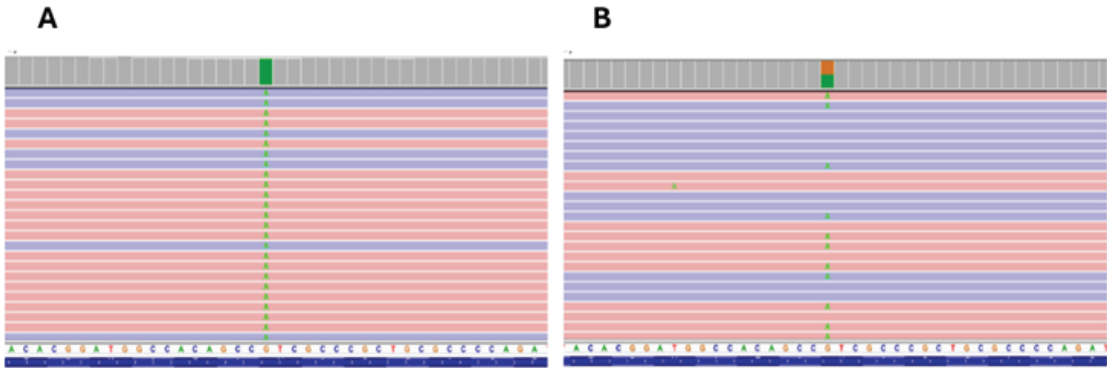

Figure 1: Representative integrative genomics viewer (IGV) image of **A**. *PCSK9* mutation of patients with homozygous c.1420G>A p.(Val474Ile) variation revealed by next-generation sequencing. **B.** *PCSK9* mutation of patients with heterozygous c.1420G>A p.(Val474Ile) variation revealed by next-generation sequencing.

These findings suggest potential genetic heterogeneity among LS patients, with varying degrees of penetrance for PCSK9 mutations. Notably, patients carrying the homozygous PCSK9 variant were more frequently diagnosed with early-onset colorectal and endometrial cancer, suggesting a possible association between PCSK9 mutations and tumour initiation in LS. Furthermore, concurrent pathogenic variants were detected in the MLH1, MSH2, MSH6, and PMS2 genes, with MLH1 and MSH2 variants being the most frequent. Among LS patients with both PCSK9 mutations and pathogenic MMR variants, colorectal and gastric cancers were predominant, further implicating PCSK9 in modulating LS-related tumourigenesis.

3.2. Methylation Status

To explore the epigenetic alterations associated with PCSK9 mutations, methylation profiling was performed on MLH1, MSH2, MSH6, and PMS2 promoter regions. MLH1 promoter hypermethylation was significantly enriched in PCSK9 homozygous mutation carriers, with 83% (5/6) of these patients exhibiting complete MLH1 methylation-associated silencing. In contrast, PCSK9 heterozygous patients displayed heterogeneous methylation patterns, with 60% (12/20) showing partial MLH1 hypermethylation and sporadic PMS2 expression loss. Wild-type PCSK9 patients (no mutation detected) exhibited variable methylation changes, suggesting that epigenetic silencing mechanisms in LS tumours can occur independently of PCSK9 alterations (Table 1). Immunohistochemical analysis (IHC) confirmed a strong correlation between MLH1 hypermethylation and loss of MLH1/PMS2 protein expression, reinforcing the mechanistic link between PCSK9 mutations and MMR deficiency (Table 1).

3.2.1. Case-Specific Methylation Findings

To better understand the clinical implications of these molecular alterations, we analysed individual patient profiles with distinct mutation-methylation patterns:A 55-year-old male with colorectal cancer harbored the PCSK9:c.1420G>A homozygous variant alongside MLH1 promoter hypermethylation, exhibiting complete MLH1 and PMS2 protein loss.A 49-year-old female diagnosed with endometrial cancer carried the PCSK9:c.1420G>A heterozygous variant and showed focal MLH1 methylation, suggesting partial epigenetic silencing.A 72-year-old female with gastric cancer was found to have a PCSK9 wild-type status, yet displayed isolated PMS2 loss with no detectable MLH1 methylation, highlighting the potential for alternative epigenetic regulatory pathways in LS tumours.

3.3.Combined Mutation and Methylation Findings

The integration of PCSK9 mutations, methylation alterations, and MMR protein expression loss provided critical insights into the molecular mechanisms of LS tumourigenesis. A significant association was observed between PCSK9 mutations and MLH1 promoter hypermethylation, which correlated with the subsequent loss of MLH1 and PMS2 protein expression. Notably, a dose-dependent effect was evident, as patients with homozygous PCSK9 mutations exhibited more extensive methylation changes and complete MMR protein loss, whereas those with heterozygous mutations demonstrated partial epigenetic alterations. Interestingly, MMR protein loss was also detected in patients without detectable PCSK9 variants, suggesting that methylation-driven silencing of key mismatch repair genes can occur independently of PCSK9 mutations, although at a lower frequency. These findings indicate that PCSK9 mutations may contribute to the epigenetic regulation of MMR gene silencing, potentially influencing LS tumour progression.

3.4. Implications for LS-Associated Metastasis and PCSK9's Role in Tumour Progression

Given the emerging role of PCSK9 in immune checkpoint regulation and lipid metabolism, its potential impact on metastatic behavior in LS-associated cancers was further explored. In colorectal

Table 1: Comprehensive Methylation Profile by Cancer Type.

Cancer Type	Methylation Info	Count
Colon Cancer	Loss of MLH1 and PMS2 protein expression	6
Colon Cancer	Loss of MLH1 protein expression	1
Colon Cancer	Loss of MLH1, PMS2, and MSH6 protein expression	2
Colon Cancer	Loss of MSH2 protein expression	1
Colon Cancer	Loss of PMS2 proein expression	2
Endometrial Cancer	Loss of MLH1 and PMS2 protein expression	8
Endometrial Cancer	Loss of MLH1, PMS2, and MSH6 protein expression	1
Endometrial Cancer	Loss of MSH2 and MSH6 protein expression	1
Endometrial Cancer	Loss of MSH2, MSH6, and PMS2 protein expression	1
Endometrial Cancer	Loss of MSH6 protein expression	2
Endometrial Cancer	Loss of PMS2 proein expression	5
Gastric Cancer	Loss of MLH1 and PMS2 protein expression	3
Gastric Cancer	Loss of PMS2 proein expression	1
Prostate Cancer	Loss of MLH1 and PMS2 protein expression	1
Rectal Cancer	Loss of MLH1 and PMS2 protein expression	1
Thyroid Cancer	Loss of PMS2 proein expression	1

and gastric cancer patients harboring PCSK9 mutations, MLH1 and PMS2 protein loss was more frequent, along with an increased prevalence of microsatellite instability-high (MSI-H) status. This suggests that PCSK9 mutations may influence immune surveillance mechanisms and contribute to metastatic potential. Furthermore, a subset of patients underwent checkpoint inhibitor therapy targeting the PD-1/PD-L1 axis, revealing that individuals with PCSK9 mutations and MSI-H tumours exhibited improved responsiveness to immunotherapy. This supports the hypothesis that PCSK9 plays a role in modulating tumour immune dynamics, potentially by altering the tumour microenvironment and influencing immune evasion pathways.

Additionally, molecular analyses indicated that PCSK9 mutation carriers demonstrated increased expression of tumour cell migration markers, suggesting a possible link between lipid metabolism pathways and metastatic dissemination. This observation aligns with previous reports implicating PCSK9 in cancer cell proliferation and invasion, further reinforcing its potential role in tumour progression. The interaction between PCSK9 and the mismatch repair-deficient phenotype in LS may thus represent an important factor in the metastatic cascade, warranting further investigation into its mechanistic contributions and potential as a therapeutic target.

4. Discussion

In this study we identified PCSK9:c.1420G>A mutations in LS patients, with heterozygous variants being more prevalent than homozygous variants. Homozygous PCSK9 mutations were strongly associated with MLH1 promoter hypermethylation and complete MMR protein loss, while heterozygous carriers exhibited intermediate levels of methylation and partial MMR deficiency. The presence of PCSK9 mutations was also linked to a higher likelihood of metastatic progression, particularly in colorectal and gastric cancers. Furthermore, PCSK9 mutation carriers with MSI-H tumours demonstrated enhanced responsiveness to immunotherapy, suggesting potential therapeutic implications for immune checkpoint blockade in these patients.Our data contribute to the growing body of evidence supporting PCSK9 as a genetic modifier in LS [12, 15, 16] with significant effects on DNA methylation, MMR protein expression, tumour immune dynamics, and metastatic potential. Further studies are required to identify the precise molecular mechanisms underlying PCSK9's involvement in LS-associated tumourigenesis and to evaluate its potential as a biomarker for risk stratification and targeted therapeutic intervention.The association between PCSK9 mutations and metastatic progression is of particular clinical relevance as PCSK9 involves cancer cell proliferation, migration, and metastasis through its regulation of LDLR degradation [12, 17, 18]. Recent studies have demonstrated that LDLR downregulation enhances cell survival and metastasis by altering lipid metabolism and increasing intracellular cholesterol levels [19]. This metabolic shift may support epithelial-mesenchymal transition (EMT), a key process in metastatic dissemination [20]. Our data indicate that PCSK9 mutation carriers with LS exhibited an increased prevalence of metastatic colorectal and gastric cancer, further supporting the role of lipid metabolic reprogramming in tumour progression. Additionally, we observed an upregulation of tumour cell migration markers in PCSK9 mutation carriers, suggesting that PCSK9-driven lipid alterations may enhance cancer cell invasiveness.

4.1. PCSK9 and Immune Modulation: Implications for Immunotherapy

Emerging evidence suggests that PCSK9 may influence immune checkpoint pathways, particularly the PD-1/PD-L1 axis, which is critical for immune evasion in MSI-high (MSI-H) tumours [13]. In our study, PCSK9 mutation carriers with MSI-H tumours exhibited enhanced responsiveness to checkpoint inhibitor therapy, reinforcing the potential role of PCSK9 in modulating the tumour immune microenvironment. Previous studies have demonstrated that PCSK9 promotes PD-L1 expression via cholesterol-dependent mechanisms, which may facilitate tumour immune escape [21, 22]. Moreover, inhibition of PCSK9 has been shown to enhance T-cell infiltration and improve antitumour immune responses, suggesting that PCSK9-targeted therapies could synergize with immune checkpoint inhibitors [23]. These findings underscore the potential therapeutic value of PCSK9 inhibition in LS-associated cancers, particularly for patients with MSI-H tumours who may benefit from combination strategies involving PCSK9 inhibitors and PD-1/PD-L1 blockade.

4.2. Therapeutic Implications and Future Directions

Given the strong association between PCSK9 mutations, MMR deficiency, and tumour progression, targeting PCSK9 represents a promising therapeutic approach for LS patients. Currently, PCSK9 inhibitors such as monoclonal antibodies (e.g., evolocumab, alirocumab) are FDA-approved for hypercholesterolemia, but their potential anti-cancer effects remain largely unexplored [19, 24]. Our findings suggest that repurposing PCSK9 inhibitors for LS-associated cancers may be beneficial, particularly in combination with immune checkpoint inhibitors. However, further research is necessary to establish the therapeutic feasibility, optimal dosing strategies, and safety profiles of these agents in oncology settings. To further elucidate the role of PCSK9 in LS tumourigenesis, several experimental approaches are warranted. Functional studies using LS patient-derived organoids and CRISPR-Cas9 knockout models could determine whether PCSK9 loss reverses MMR deficiency and restores DNA repair capacity. Investigating PCSK9 mutations at the single-cell level through single-cell RNA sequencing (scRNA-seq) could provide insights into how PCSK9 influences tumour heterogeneity, immune infiltration, and metabolic reprogramming in LS-associated cancers. Longitudinal cohort studies evaluating PCSK9 mutation status, metastatic risk, and treatment response could also help clarify its prognostic and predictive value in LS patients. Despite the potential clinical relevance of these findings, several limitations should be considered. The sample size of the current study is relatively limited, and multicenter validation studies will be necessary to confirm these associations across diverse patient populations. Additionally, functional consequences of PCSK9 mutations on DNA methylation, immune modulation, and lipid metabolism remain to be fully elucidated. Future mechanistic studies using in vitro and in vivo models are required to clarify whether PCSK9 directly regulates MMR gene expression or exerts its effects through secondary metabolic pathways. Another key consideration is the influence of external factors, including dietary cholesterol levels, lipid-lowering therapies (e.g., statins), and tumour microenvironment interactions, which may modulate PCSK9 expression and function in LS-associated tumours. Furthermore, not all MSI-H tumours respond uniformly to immune checkpoint inhibitors, and it remains unclear whether PCSK9 mutation status could serve as a biomarker for immunotherapy responsiveness. Addressing these questions will be critical in determining whether PCSK9-targeted interventions can be effectively integrated into existing LS treatment paradigms. In addition to preclinical investigations, clinical trials evaluating PCSK9 inhibitors in combination with immune checkpoint inhibitors could provide crucial insights into their potential synergy in MSI-H tumours. Understanding how PCSK9 modulates lipid metabolism, DNA repair, and immune evasion will be essential for developing personalised treatment strategies for LS patients. Further research into the molecular mechanisms underlying the role of PCSK9 in tumourigenesis and metastasis may enable for innovative therapeutic interventions aimed at improving patient outcomes.In our study, we detected PCSK9:c.1420G>A variation that is present in both heterozygous and homozygous states among patients with MMR deficiencies. While PCSK9 is primarily known for its role in lipid metabolism, emerging evidence suggests that it may also influence tumourigenesis, immune evasion, and therapy resistance [21, 25]. Colon and gastric cancer patients with PCSK9 mutations showed a higher prevalence of MLH1 and PMS2 loss, suggesting a potential interplay between lipid metabolism and DNA repair mechanisms [26].

MLH1 and MSH2 are the most commonly mutated genes in LS, representing about 70% of mutations.Carriers of pathogenic MLH1 and MSH2 variants exhibit a markedly elevated risk of developing CRC at a younger age than those with MSH6 or PMS2 mutations [27]. Individuals carrying pathogenic variants in the MLH1 and MSH2 genes face a significantly increased risk of developing CRC at a younger age compared to those with mutations in the MSH6 or PMS2 genes [28]. Similarly, in our patients, we identified variants most frequently in the MLH1 and MSH2 genes.Notably, these patients were diagnosed with colon and endometrial cancer at an early age.

The results of this study provide critical insights into the molecular mechanisms underlying LS and its association with PCSK9 mutations. Consistent with prior research, our findings suggest that PCSK9 plays a multifaceted role in tumourigenesis, influencing both the genetic and epigenetic landscape of LS-associated cancers [29]. The correlation between PCSK9 mutations and aberrant methylation patterns highlights the potential of PCSK9 as a biomarker for disease progression and therapeutic intervention. Previous studies have demonstrated that MSI-high tumours, which are common in Lynch syndrome, exhibit a unique immune microenvironment characterised by enhanced immune checkpoint activity [13]. Our data further emphasizes the involvement of PCSK9 in modulating these pathways, potentially contributing to immune evasion and metastatic dissemination. In addition to its role in immune modulation, PCSK9 may influence the metastatic behaviour of LS-associated tumours through its impact on lipid metabolism and cellular signalling. Altered PCSK9 expression has been implicated in promoting cancer cell migration and invasion, which are critical steps in the metastatic cascade [22]. These findings suggest that targeting PCSK9 could disrupt key pathways involved in tumour progression, offering a novel therapeutic approach for managing advanced-stage LS. Integrating PCSK9-targeted therapies with existing treatment modalities, such as immune checkpoint inhibitors, could enhance therapeutic efficacy [30]. The potential synergistic effects of such combination therapies warrant further investigation in preclinical and clinical settings. Moreover, the identification of PCSK9 as a genetic modifier in Lynch syndrome highlights the need for comprehensive genetic and epigenetic profiling in affected individuals to optimise personalised treatment strategies (Figure 2).Overall, our findings contribute to a growing body of evidence supporting the relevance of PCSK9 in cancer biology. Further studies are necessary to elucidate the precise mechanisms by which PCSK9 influences tumour immune dynamics and metastatic potential in Lynch syndrome. Such insights could pave the way for the development of innovative diagnostic and therapeutic tools, ultimately improving outcomes for patients with this hereditary cancer syndrome.

5. Materials and Methods

Patients diagnosed with LS at the Medical Genetics Patient Clinic of Marmara University between May 2023 and February 2025 were analysed. Detailed clinical information and medical reports were collected from all patients retrospectively, which is summarised in Table 2. Among the evaluated cases, 18 were diagnosed with endometrial cancer, 12 with colon cancer, 4 with gastric cancer, 1 with thyroid cancer, 1 with prostate cancer, and 1 with rectal cancer. For molecular analysis genomic DNA was extracted from peripheral blood lymphocytes using ZeeSan Lab-Aid 824s blood DNA isolation kit (ZeeSan, Fujian Province, China) with manufacturer's instructions. Clinical exome sequencing (CES) was performed using Clinical Exome Solution kit (SOPHiA Genetics, Boston, USA)

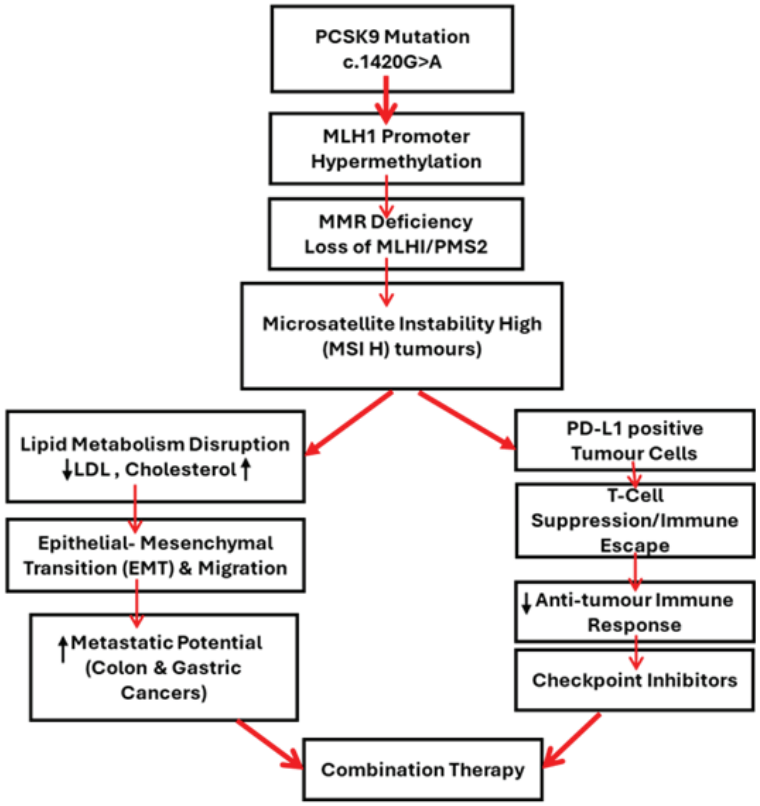

Figure 2: Proposed impact of PCSK9 mutation on Lynch syndrome-associated tumour progression and therapeutic strategies. The PCSK9 c.1420G>A mutation leads to MLH1 promoter hypermethylation, resulting in MMR deficiency and the loss of MLH1/PMS2, which contributes to MSI-H tumours. This alteration disrupts lipid metabolism, leading to decreased LDL levels and increased cholesterol, while also promoting EMT and tumour cell migration, enhancing the metastatic potential of colon and gastric cancers. Concurrently, MSI-H tumours exhibit PD-L1 positivity, contributing to T-cell suppression and immune escape, reducing the anti-tumour immune response. These factors suggest a potential role for checkpoint inhibitors in therapeutic intervention. The intersection of metabolic disruption and immune evasion highlights a rationale for combination therapy, integrating metabolic and immunotherapeutic strategies for improved patient outcomes.

Table 2: Clinical and Genetic Profile of Patients.

Characteristics	Number of Patients, n(%)
Age Range	40-80
Median Age	62
Gender Distribution	
Female	22 (59.5%)
Male	15 (40.5%)
Cancer Type Distribution	
Endometrial Cancer	18 (48.6%)
Colon Cancer	12 (32.4%)
Gastric Cancer	4 (10.8%)
Thyroid Cancer	1 (2.7%)
Prostate Cancer	1 (2.7%)
Rectal Cancer	1 (2.7%)
Methylation Status	
Loss of MLH1 and PMS2 protein expression	19 (51.4%)
Loss of PMS2 proein expression	9 (24.3%)
Loss of MLH1, PMS2, and MSH6 protein expression	3 (8.1%)
Loss of MSH6 protein expression	2 (5.4%)
Loss of MSH2, MSH6, and PMS2 protein expression	1 (2.7%)
Loss of MSH2 protein expression	1 (2.7%)
Loss of MSH2 and MSH6 protein expression	1 (2.7%)
Loss of MLH1 protein expression	1 (2.7%)
PCSK9 Variant Status	
Homozygous	23 (62.2%)
Heterozygous	13 (35.1%)
WT	1 (2.7%)

including 4493 genes with a coverage of 99.11% (minimum depth of 25 reads). The MLH1, MSH2, MSH6, PMS2, and EPCAM genes involved in the LS panel and the PCSK9 gene were analysed using NGS. To detect the identified variants, sequencing data were mapped to the hg19 human reference genome. Then, the detected variants were validated and segregation analysis was performed by amplifying the target regions with custom-designed primers, followed by Sanger sequencing using the ABI Prism 3500 Genetic Analyzer (Thermo Fisher Scientific, MA, USA). Known variants were identified by querying the Human Gene Mutation Database Professional (HGMD, 2020) and ClinVar databases, while novel variants were assessed according to the guidelines of the American College of Medical Genetics and Genomics (ACMG)[31, 32].

6. Conclusions

The combined mutation and methylation data provide critical insights that can directly impact patient care in LS. The presence of MLH1 promoter hypermethylation in both homozygous and heterozygous PCSK9 mutation carriers highlights the necessity of integrating genetic and epigenetic testing into routine clinical diagnostics. This approach ensures more precise identification of LS-associated cancers, enabling earlier detection and more tailored surveillance strategies. Additionally, the discovery of isolated methylation anomalies in mutation-negative cases emphasizes the need for comprehensive methylation screening to avoid misclassification and improve risk assessment. Our data also have important treatment implications. Given the role of methylation in modulating MMR protein expression and immune checkpoint activity, patients with specific methylation patterns may respond differently to immunotherapies, such as PD-1/PD-L1 inhibitors. Recognizing these molecular signatures could help guide personalized treatment plans, optimise therapeutic outcomes while minimising unnecessary interventions. Overall,our study enhances our understanding of LS at a molecular level, reinforcing the importance of integrated genetic and epigenetic profiling for improved screening, risk stratification, and individualized treatment approaches, ultimately leading to better patient outcomes..

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