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Original Article

Effects of the STING R232/H232 Variant on the Prognosis of Inflammatory Bowel Disease

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ABSTRACT

Aim: Inflammatory bowel disease (IBD) refers to a group of diseases which cause chronic and recurrent inflammation in different parts of the digestive tract, such as Crohn's disease (CD) or ulcerative colitis (UC). CD can affect both the large and small intestines, while UC usually affects only the large intestine. Recent studies in immunogenetics have revealed that the innate immune system is crucial in triggering gut inflammation, and rare variants in genes which function in this system are important risk factors for this disease. Stimulator of interferon genes (STING) is a nucleotide-binding endoplasmic reticulum protein involved in the innate immune response. This study aimed to analyse the R232/H232 variant in the *STING1* gene in pediatric patients diagnosed with IBD and to investigate whether this variant is associated with the prognosis of IBD.

Materials and Methods: Thirty-five pediatric patients admitted with a prediagnosis of IBD were included in this study. The R232/H232 variant was determined by end-point genotyping analysis after real-time reverse transcription-polymerase chain reaction (qRT-PCR) reactions using affinity probes. qRT-PCR analyses were performed to determine the mRNA expression levels of STING and interferon-induced genes in tissue samples. The western blotting method determined STING expression at the protein level.

Results: It was determined that 31.43% of the patients had heterozygous (R232/H232), and 68.57% had homozygous (H232/H232) genotypes. A significant difference was found between the genotype distribution and treatment stage. It was determined that 87.50% of the patients who started second-stage treatment had homozygous genotypes. It was also found that homozygous patients had longer durations of attacks than heterozygous patients.

Conclusion: R232/H232, the most common variant in the *STING1* gene, affects treatment response and attack duration in patients with IBD. Therefore, we suggest that variants in the *STING1* gene may be used to develop genetic-based personalized treatment strategies for IBD patients in the future.

Keywords: STING, R232/H232, inflammatory bowel disease, Crohn's disease, ulcerative colitis

Introduction

Inflammatory bowel disease (IBD) is a chronic gastrointestinal condition encompassing Crohn's disease (CD), ulcerative colitis (UC), and unclassified colitis (IBDU). The development of IBD is influenced by a combination of factors, including intestinal microbiota, environmental stimuli, immune responses, and genetic predisposition. CD is characterized by transmural inflammation and can occur anywhere in the gastrointestinal tract, from the oral cavity to the anus (1). UC is defined as a chronic inflammatory condition which causes continuous mucosal inflammation, affecting the rectum and various regions of the colon (2).

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IBDU refers to IBD cases confined to the colon which do not fit the specific characteristics of CD or UC (2). IBD can exhibit significant variability in terms of disease severity and prognosis; some patients may achieve remission, while others may experience relapse and progress to more complex phenotypes (3,4).

Currently, there is no definitive cure for IBD; therefore, the primary goal of treatment is to achieve long-term clinical remission without disease progression. Therapeutic strategies for CD aim to prevent complications such as structuring disease, penetrating disease, or the need for surgery. In UC, the focus is on reducing acute and chronic inflammation in order to prevent complications, avoid progression to surgery, and achieve remission (5).

The standard treatment approach involves a step-up therapy, where treatments are escalated based on disease severity. Initially, patients are treated with 5-aminosalicylic acids, such as mesalamine or sulfasalazine, or corticosteroids, including budesonide, prednisone, or dexamethasone. However, corticosteroids cannot be used continuously; therefore, in cases of disease worsening, the treatment plan may include immunosuppressive agents such as azathioprine (AZA) and mercaptopurine (MP), cyclosporine, methotrexate (MTX), or biological agents in order to maintain remission. However, all immunosuppressive medications have limited efficacy in inducing remission and are beneficial to fewer than half of those patients suffering from steroid dependency or resistance (6).

Future treatment strategies should incorporate therapeutic options tailored to the genetic profiles of the patients. Genome-wide association studies (GWAS) have identified over 163 single nucleotide variants (SNVs) associated with IBD (7,8). An example is a variant in the *neutrophil cytosolic factor 4* (*NCF4*) gene, which has been identified as a risk gene for CD. Although this SNV is not located in the coding region, it causes functional impairment in granulocytes (8). In CD patients with this variant, targeted treatments which stimulate granulocytes, such as sargramostim, have been shown to improve remission (9). Determining the correlations between genetic makeup, patient characteristics, and treatment responses will increase the likelihood of implementing personalized treatment strategies. Despite the availability of numerous drugs for IBD treatment and the introduction of new medications, none have proven universally effective for all IBD patients (10).

Stimulator of interferon genes (STING) is an endoplasmic reticulum protein involved in innate immune signalling. The STING protein, encoded by the *STING1* gene located on chromosome 5 (gene ID: 340061), is also known by names such as MITA, STING, hSTING, and TMEM173. It is a key inducer of type I interferons which are produced in response to cytosolic DNA or bacterial cyclic dinucleotides and play a canonical role in antiviral and antibacterial immunity (11). STING functions, such as being a fundamental mediator of innate immune responses to microbial and host-derived DNA, is crucial in sensing and regulating responses to infection, cellular stress, and tissue damage (12). Studies have also highlighted STING's essential role in gastrointestinal homeostasis and its significant overlap with IBD pathophysiology (13). Additionally, fundamental findings related to the structural and molecular biology of the cGAS-STING pathway have enabled the development of selective small molecular inhibitors with potential targeting capabilities for a range of inflammatory diseases in humans (14). A variant in the *STING1* gene, causing either arginine (R) or histidine (H) at position 232, has been identified (rs1131769). Cytosine is the most frequently observed nucleotide at this position (4425), making the R232 allele the wild-type variant (15). This study aimed to analyse the R232/H232 variant in pediatric patients diagnosed with IBD and to investigate whether this variant is associated with the prognosis of IBD.

Materials and Methods

Patient Population

This study, approved by our centre's Ethics Committee of Ege University (approval number: 21-12.1T/26, date: 21.04.2022), included a cohort of 35 pediatric patients, aged 0-18 years, diagnosed with IBD at the department of pediatric gastroenterology. During colonoscopy, biopsy specimens and 2 mL of blood samples were collected from each patient. The patients, diagnosed based on clinical and histopathological criteria, initially received steroid therapy aimed at inducing remission, followed by a maintenance regimen. Steroids were administered for 4-6 weeks, after which treatment transitioned to a maintenance phase with a gradual reduction in steroid dosage. Those patients demonstrating a positive response to the therapy were subsequently monitored with follow-up visits at 2, 4, and 8 weeks, and then at 3-month intervals.

Isolation Procedures

For DNA extraction from the blood samples, the Nucleic Acid Isolation Kit I (Roche Diagnostics, Mannheim, Germany) was employed. From 400 µL of blood placed into the MagNA Pure Compact (Roche Diagnostics, Mannheim, Germany) device, a total volume of 100 µL of DNA samples was obtained, with an average concentration of 80 ng/µL.

RNA isolation from the tissue samples was performed using tripure solution (Roche Diagnostics, Mannheim, Germany). A 5 mg tissue sample was homogenized in tripure solution. After the addition of chloroform, the mixture was centrifuged at 12,000 xg for 45 minutes to achieve phase separation. The upper clear phase was transferred to a separate Eppendorf tube for RNA precipitation using ethanol. The purity and concentration of the isolated nucleic acids were assessed using a Nanodrop spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA).

For protein isolation from the tissue samples, the complete™ Lysis-M kit (Roche Diagnostics, Mannheim, Germany) was used. Protein concentrations were determined spectrophotometrically using Bradford reagent and bovine serum albumin (BSA) standards (Fermentas, Massachusetts, USA).

Genotyping Analysis

The R232/H232 variant was determined using end-point genotyping analysis following real-time polymerase chain reaction (PCR) with affinity probes on the LightCycler 480 system (Roche Diagnostics, Mannheim, Germany). Primers and probes were obtained from Integrated DNA Technologies (IDT, Iowa, USA). The primer and probe sequences used in this analysis were as follows:

- **R232H Forward Primer:** 5'CGTTCTCCAGAAGCTCATAG3'
- **R232H Reverse Primer:** 5'CCCAACATTCGCTTCCT3'
- **Wild-Type Genotype Probe:** 56-FAM/CA+GC... G+T+CA/ 3IABkFQ
- **Mutant Genotype Probe:** 5HEX/AGC +A+C+G G+T+C A/3IABkQ

Genotype Validation via Sanger Sequencing

In order to validate the results obtained from the genotyping analyses, 1-2 samples from each genotype were selected for confirmation using the Sanger sequencing method. The specific region of the *STING1* gene containing the R232/H232 variant was amplified using specific primers (forward primer: 5' TCATCAGTGCTTGGCTAGG 3'; reverse primer: 5' CTTCCCTGCCTCAGAGCTPCR 3'). The amplified products were visualized through agarose gel electrophoresis. The PCR products were purified using the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel, Germany) and subjected to Big Dye PCR for Sanger sequencing. After a second purification of the PCR products, sequencing was performed.

Gene Expression Analyses via Real-time Reverse Transcription-PCR (qRT-PCR)

All RNA samples were diluted to a final concentration of 70 ng and subsequently reverse transcribed into cDNA using the iScript™ cDNA Synthesis Kit (Biorad, California, USA). To assess gene expression levels, qRT-PCR was performed on the LightCycler 480 system using iTaq Universal SYBR Green Supermix (Biorad, California, USA) and gene-specific primers. The primer sequences used for this analysis are provided in Table I.

Western Blot Analysis

In order to determine the expression of STING at the protein level, western blot analysis was performed following protein isolation from the tissue samples. Protein samples were separated on an SDS-PAGE gel at a concentration of 10 µg/mL. After the transfer and blocking steps, the membrane was incubated overnight with a primary STING antibody (#13647S, Cell Signaling, Massachusetts, USA) diluted 1:1000 in a 5% BSA solution. A horseradish peroxidase-conjugated anti-rabbit secondary antibody (#7074, Cell Signaling, Massachusetts, USA) was used at a 1:1000 dilution. Following several washes, the membrane was

Table I. Forward and reverse primer sequences used in gene expression analyses

Primer	Base sequence 5'-3'
STING-F	GCAGTGTGTGAAAAAGGGAAT
STING-R	CACCCCGTAGCAGGTTGTT
IFNβ-F	CAGCATCTGCTGGTTGAAGA
IFNβ-R	CATTACCTGAAGGCCAAGGA
IFIT2-F	GCGTGAAGAAGGTGAAGAGG
IFIT2-R	GCAGGTAGGCATTGTTTGGT
IFI44-F	GATGTGAGCCTGTGAGGTCC
IFI44-B	CTTTACAGGGTCCAGCTCCC
IL6-F	AGACAGCCACTCACCTCTTCAG
IL6-R	TTCTGCCAGTGCCTCTTTGCTG
ISG15-R	CAGCCATGGGCTGGGAC
ISG15-F	GCCGATCTTCTGGGTGATCT

STING: Stimulator of interferon genes, *IFNB*: Interferon beta, *IFIT2*: Interferon induced protein with tetratricopeptide repeats 2, *IFI44*: Interferon-induced protein 44, *IL6*: Interleukin 6, *ISG15*: Interferon-Stimulated gene 15

incubated with an enhanced chemiluminescence solution (Biorad, California, USA), and the signal was detected using a C-Digit blot scanner (Li-Cor Biosciences, Nebraska, USA).

This procedure enables the quantification and visualization of STING protein expression in tissue samples.

Statistical Analysis

The patients were stratified based on their genotypes, and subsequent comparisons were made across various prognostic markers including gene/protein expression levels, treatment response, frequency and duration of relapses, and time to remission. Statistical analyses were conducted using SPSS software (version 26). Fisher's exact test was employed to compare allele frequencies between the groups. The distribution of gene expression levels was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests to evaluate normality. For non-normally distributed data, differences between groups were analysed using the Mann-Whitney U test. A p-value of less than 0.05 was considered statistically significant.

Results

Clinical Data

This study included a total of 35 patients, comprising 16 males (45.71%) and 19 females (54.29%). Based on their clinical findings, laboratory results, endoscopy, and colonoscopy outcomes, 30 patients (85.71%) were diagnosed with UC and 5 patients (14.29%) were diagnosed with CD. Clinical characteristics, including follow-up durations, relapse frequencies and durations, treatments, treatment responses, and times to remission, are summarized in Table II.

Genotype Findings for R232/H232

The genotype analysis revealed that 15.71% of the patients carried the C allele, whereas 84.29% carried the T allele. Consequently, among the 35 patients, 11 were identified as heterozygous (CT, R232/H232), representing 31.43% of the cohort, and 24 were homozygous (TT, H232/H232), accounting for 68.57%. These genotype classifications were corroborated through Sanger sequencing, which confirmed the results obtained using the LightCycler 480 device, as illustrated in Figure 1.

Upon stratifying the IBD patients by disease type and genotype, it was found that all individuals with CD exhibited the homozygous genotype (H232/H232, 100%). In contrast, among those patients with UC, 11 individuals were heterozygous (R232/H232, 36.67%), while 19 were homozygous (H232/H232, 63.33%) (Table III).

Table II. Clinical data of the included patients

Gender	Female	19 (54.29%)
	Male	16 (45.71%)
Etiology	UC	30 (85.71%)
	CD	5 (14.29%)
Follow-up duration	<1 year	4 (11.43%)
	1-3 years	21 (60.00%)
	4-5 years	7 (20.00%)
	>5 years	3 (8.57%)
Treatment response	First stage	19 (54.29%)
	Second stage	16 (45.71%)
Attack duration	1-3 day	17 (48.57%)
	≥4 day	18 (51.43%)
Number of attacks	1	13 (37.14%)
	2	5 (14.29%)
	3	4 (11.43%)
	≥4	13 (37.14%)
Time to remission	1 day	7 (20.00%)
	2 days	3 (8.57%)
	3 days	15 (42.86%)
	≥4 days	10 (28.57%)

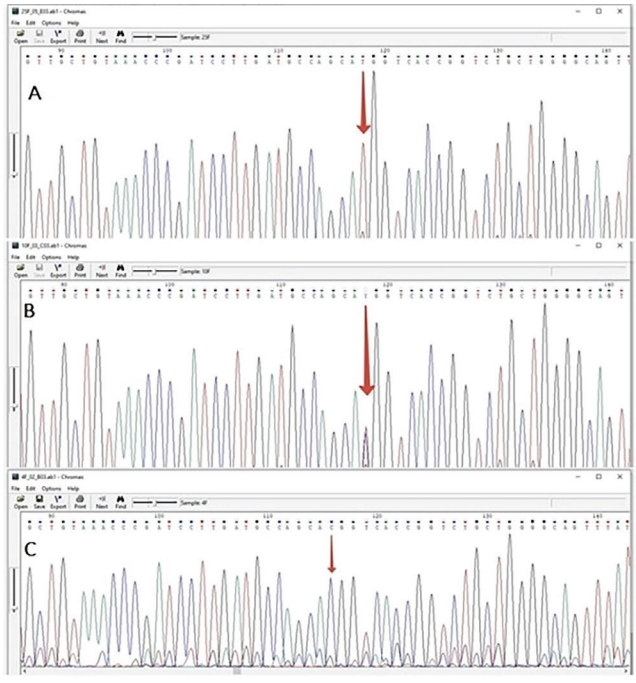

Figure 1. Genotype validation by sanger sequencing A: Wild-type (R232/R232), B: Heterozygous (R232/H232), C: Homozygous (H232/H232)

Statistical analysis revealed no significant differences in the distribution of heterozygous and homozygous genotypes across the various disease subgroups.

Comparison of R232/H232 Genotype with Prognostic Markers

After stratifying the patients according to their genotypes, prognostic markers such as treatment response, the number of relapses within one year, relapse duration, and time to remission following treatment were evaluated and subjected to statistical analysis.

In our study, when comparing the patients undergoing first and second-phase treatments based on their genotypes, it was observed that a significant proportion of those advancing to the second-phase treatment (87.5%) had the homozygous genotype. This analysis, which included all patients, found a significant difference in treatment response based on the R232/H232 genotype ($p<0.027$) (Table IV).

Comparison of Relapse Duration and Frequency Based on R232/H232 Genotype

A comparison of relapse durations across R232/H232 genotypes revealed a statistically significant difference ($p<0.009$) (Table V). Specifically, among those patients experiencing relapses lasting four days or more, 11.11% had the heterozygous genotype, whereas 88.89% had the homozygous genotype.

When the analysis was restricted to patients with UC, a similar trend was observed: 84.61% of those with relapses lasting four days or more carried the homozygous genotype. However, the p-value was 0.058, which is on the threshold of statistical significance (Table VI).

Further analysis of relapse frequency throughout the year did not reveal significant differences based on the R232/H232 genotype, indicating no meaningful variation in relapse counts between heterozygous and homozygous cases. Additionally, no significant differences were found between the time to remission and the distribution of R232/H232 genotypes.

Effects of R232/H232 Genotype on cGAS/STING Pathway Components

In our study, the expression of STING at both mRNA and protein levels was analysed in the biopsy tissues from the patients with IBD. Comparative analysis of STING expression between heterozygous and homozygous genotypes revealed no significant differences (Figure 2, Table VII). Our findings indicate that STING protein is expressed in a significant proportion of IBD cases. However, very low levels of STING protein expression were observed in a small subset of patients (samples 7, 8, and 29) (Figure 2).

Additionally, expression levels of interferon-stimulated genes (ISGs) including IFNB, IFIT2, IFI44, IL6, and ISG15 were assessed. No significant differences in the expression of these genes were found between heterozygous and homozygous

Table III. Distribution of genotypes and disease subgroups among IBD cases

Disease type	Heterozygous R232/H232 (n/%)	Homozygous H232/H232 (n/%)	Total (n/%)	p*
Crohn disease	0 (0.0)	5 (100)	5 (100)	<0.157
Ulcerative colitis	11 (36.67)	19 (63.33)	30 (100)	

*Fisher's exact test, IBD: Inflammatory bowel disease

Table IV. Comparison of treatment responses based on genotype

Treatment	Heterozygous R232/H232 (n/%)	Homozygous H232/H232 (n/%)	Total (n/%)	p*
First stage	9 (47.36)	10 (52.63)	19 (100)	**<0.027**
Second stage	2 (12.50)	14 (87.50)	16 (100)	

*Fisher's exact test

Table V. Relapse durations among all patients by genotype

Duration of attack (days)	Heterozygous R232/H232 (n/%)	Homozygous H232/H232 (n/%)	Total (n/%)
1	0 (0.0)	2 (100.0)	2 (100)
2	4 (66.67)	2 (33.33)	6 (100)
3	5 (55.56)	4 (44.44)	9 (100)
>4	2 (11.11)	16 (88.89)	18 (100)

p<0.009 (Fisher's exact test)

Table VI. Relapse durations among ulcerative colitis patients by genotype

Duration of attack (days)	Heterozygous R232/H232 (n/%)	Homozygous H232/H232 (n/%)	Total (n/%)
1	0 (0.0)	2 (100)	2 (100)
2	4 (66.67)	2 (33.33)	6 (100)
3	5 (55.56)	4 (44.44)	9 (100)
>4	2 (15.38)	11 (84.61)	13 (100)

p<0.058 (Fisher's exact test)

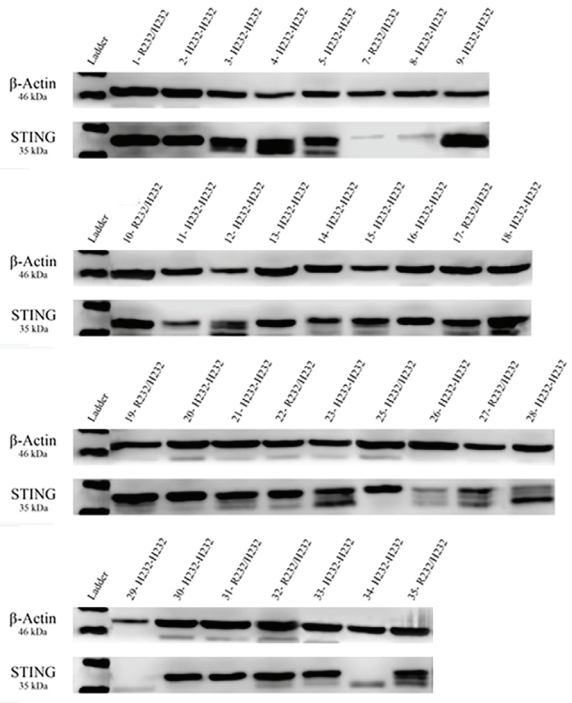

Figure 2. Western blot gel images for B-Actin and STING protein in biopsy samples from IBD patients. The first line indicates the ladder. Genotypes are indicated on the top of the gels and each line represents a patient
STING: Stimulator of interferon genes, IBD: Inflammatory bowel disease

Table VII. Comparison of gene expression levels between heterozygous and homozygous genotypes in all IBD patients

Gene	Genotype	Expression level (mean ± SD)	Minimum	Maximum	p*
STING	R232/H232	1.29±0.89	0.42	3.13	0.734
	H232/H232	1.16±0.89	0.22	4.24	
IFNB	R232/H232	3.047±4.858	0.18	13.93	0.913
	H232/H232	2.224±3.521	0.13	16.01	
IFIT2	R232/H232	1.467±1.814	0.02	5.77	0.722
	H232/H232	2.881±4.783	0.16	20.64	
IFI44	R232/H232	2.066±1.599	0.18	5.01	0.160
	H232/H232	1.267±1.567	0.20	6.17	
IL6	R232/H232	3.440±5.616	0.08	18.65	0.806
	H232/H232	3.491±6.186	0.01	24.78	
ISG15	R232/H232	1.274±0.856	0.29	2.93	0.663
	H232/H232	1.983±2.966	0.17	3.18	

*: Mann-Whitney U test, IBD: Inflammatory bowel disease, SD: Standard deviation, *STING*: Stimulator of interferon genes, *IFNB*: Interferon beta, *IFIT2*: Interferon induced protein with tetratricopeptide repeats 2, *IFI44*: Interferon-induced protein 44, *IL6*: Interleukin 6, *ISG15*: Interferon-Stimulated gene 15

genotypes (Table VII). These results suggest that variations in the R232/H232 genotype do not substantially affect the overall expression of STING or related ISGs in the patient cohort studied.

Discussion

In this study, we explored the effects of the R232/H232 single nucleotide variant in the *STING1* gene on the prognosis of IBD. It is well-established that genetic factors play a significant role in IBD, with familial clustering observed in approximately 5-10% of patients. SNVs can influence disease development by affecting the production or function of proteins, which in turn can impact cellular functions, innate immune responses, and consequently both disease activity and treatment response (16,17). Despite the identification of numerous genes involved in the development of IBD, the precise mechanisms by which the SNVs in these genes affect cellular functions or contribute to IBD pathogenesis remain unclear.

GWAS have identified over 163 SNVs associated with IBD (7,18). The incidence of these SNVs in IBD populations differs from that in the general population, and the precise mechanisms by which these variants influence cellular functions or contribute to IBD pathogenesis remain inadequately understood. Some genes, such as *NOD2*, *ATG16L1*, *IL23R*, and *IRGM*, have had their cellular effects elucidated, including their impacts on innate immune cell functions, autophagy processes, and bacterial clearance (19-21).

In IBD management, steroids are not used for maintenance therapy due to their limitations. Consequently, first-line treatments often involve immunosuppressive AZA, MP, or MTX. These agents are employed to induce and maintain remission. However, their efficacy in achieving remission induction is limited, and they benefit fewer than half of those patients suffering from steroid dependence or resistance (6). Anti-tumor necrosis factor (TNF) α therapies, employed in the second phase of treatment, have demonstrated considerable effectiveness in IBD. Nevertheless, anti-TNFα therapy is not effective in approximately 30% of IBD patients, and a significant number of patients may experience loss of response or adverse effects. This often necessitates surgical intervention (22,23).

Mutations in the *STING1* gene are associated with a severe autoinflammatory disease known as STING-associated vasculopathy with onset in infancy (SAVI), with this being a life-threatening condition (24). The human *STING1* gene exhibits substantial heterogeneity and population-level

variability (24). The H232 variant, first identified in the *STING1* gene, features a histidine residue at position 232 (25-27). This H232/H232 genotype has been reported in approximately 30% of East Asians and 10% of Europeans. Structural and functional studies of the human STING protein have predominantly used the H232 allele, which is suggested to be a minor allele which may lead to functional impairment (28). *In vitro* studies have demonstrated that cells carrying the H232 allele respond less effectively to cyclic dinucleotides (29,30). Specifically, the STING protein carrying the H232 variant binds metazoan 2'3'-cGAMP but exhibits a diminished interferon response to bacterial c-di-GMP, and shows a complete loss of response to c-di-AMP and 3'3'-cGAMP (15,31).

The most prevalent allele in the population, R232, contains an arginine amino acid at position 232 and is considered the "wild-type" *STING1* isoform, found naturally in approximately 60% of the population (15). The R232/R232 genotype is dominant in European populations. In studies involving approximately 1,000 Americans, about 45% were found to have the R232/R232 genotype, whereas only about 2% carried the H232/H232 genotype (28). Additionally, over 50% of Americans possess at least one non-R232 *STING1* allele, indicating substantial heterogeneity in the *STING1* gene across human populations (28). The R232 amino acid is located within the loop region of STING which forms the binding pocket for c-di-GMP. This allele allows the binding of various cyclic dinucleotides, including c-di-GMP, c-di-AMP, and both 2'3'- and 3'3'-cGAMP (15,31). In contrast, the H232 variant, which has been associated with functional impairment of STING, results in a reduced response to cyclic dinucleotides, particularly affecting the protein's ability to activate downstream signalling pathways (24).

In our study, we found that all five patients with CD had the homozygous genotype (H232/H232) and carried the TT allele, while among the 30 patients with UC, 11 (36.67%) had the heterozygous CT (R232/H232) genotype and 19 (63.33%) had the homozygous TT (H232/H232) genotype. Despite the R232/R232 genotype being referred to as the wild-type in the literature (24), none of the IBD cases included in our study exhibited this genotype. In order to ensure the accuracy of the genotyping method used, validation studies were conducted using Sanger sequencing. The sequence analysis confirmed that the genotypes identified using affinity probes were accurate.

Study Limitations

A significant limitation of our study was the absence of a control group, which prevented us from determining whether the higher prevalence of the H232 variant is a general population phenomenon or specific to our patient cohort. Due to budget constraints and technical limitations, a control group could not be included in our study. However, we aim to continuing to collect samples from IBD patients in other projects in order to analyse *STING1* variants in larger case-control groups so as to better understand their prevalence and impact.

When comparing patients undergoing first and second-line therapies by genotype, it was notable that a significant proportion of patients advancing to second-line therapy (87.5%) were found to have the H232/H232 genotype. Furthermore, the significant difference observed between the H232/H232 genotype and the duration of flare-ups daily suggests that this variant may influence both treatment response and prognosis in IBD. This finding highlights the potential role of the *STING1* gene variant in modulating disease progression and therapeutic outcomes.

In the intestine, as in most tissues, basal levels of type I interferon are typically low. However, in response to infection or cellular damage, there is a rapid induction of type I interferon. Interestingly, a deficiency in type I interferon signalling during mucosal inflammation has been associated with increased cytokine release by effector T-cells (32). This suggests a potentially altered adaptive immune response in IBD as a result of variable type I interferon levels. While genotypes associated with excessive STING activity have been linked to autoinflammatory disorders, no specific *STING1* variants have been identified in GWAS as being directly associated with IBD (33). Nevertheless, it has been reported that the *STING1* gene is hypomethylated in the intestinal epithelium of a pediatric IBD cohort (33). This finding may account for the observed overexpression and potential hyperactivation of STING in the epithelial cells of IBD patients. In line with this, IBD patients exhibit a signature of interferon-regulated genes, and elevated levels of interferon are associated with a lack of therapeutic response. Additionally, the interferon-stimulated gene *ISG15* is highly expressed in IBD patients with active inflammation (34). However, our study did not find significant differences in the mRNA expression levels of ISGs such as IFNB, IFIT2, IFI44, IL6, and ISG15 between the heterozygous and homozygous genotypes.

This lack of observed difference in gene expression levels might be attributed to the fact that the patients were receiving immunosuppressive therapy at the time of biopsy. Therefore, we believe that evaluating gene expression in treatment-naive newly diagnosed patients may provide a

clearer understanding of the impact of *STING1* genotypes on interferon-regulated gene expression.

Conclusion

In summary, genetic variants in genes associated with IBD may be responsible for previously unidentified disease phenotypes and could offer opportunities for the development of new therapeutic strategies. The genetic profiles of IBD patients are hoped to be able to assist clinicians in making decisions regarding personalized treatment approaches in the future. For instance, modifications to treatment protocols could include more frequent clinical monitoring for those patients carrying variants associated with poor prognosis, earlier initiation of second-line therapy in those patients with prolonged time to remission, or even pre-emptive transition to second-line therapy before disease flare-ups occur. Such changes could lead to secondary benefits, such as reduced hospital admission durations, increased school attendance and academic performance, and/or decreased malnutrition.

Further research in larger patient-control cohorts is needed in order to elucidate the effects of these findings and to identify the corresponding disease phenotypes.

Ethics

Ethics Committee Approval: This study, approved by our centre's Ethics Committee of Ege University (approval number: 21-12.1T/date: 21.04.2022).

Informed Consent: His parents or legal guardians of patients provided signed informed consent.

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Footnotes

Authorship Contributions

Surgical and Medical Practices: M.K., D.B., V.B., Concept: G.A., D.B., V.B., Design: G.A., V.B., Data Collection or Processing: G.A., T.K., V.B., Analysis or Interpretation: M.K., T.K., V.B., Literature Search: G.A., M.K., V.B., Writing: G.A., M.K., V.B.

Conflict of Interest: No conflict of interest was declared by the authors.

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