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Association of genetic polymorphism of NUDT15, TPMT and ITPA gene in the toxicity and efficacy of azathioprine-based regimen in Egyptian inflammatory bowel disease patients

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Abstract

Background Inflammatory Bowel disease (IBD) is a chronic progressive condition that prompts generous physical and mental morbidity. Choosing the best kind of management and medication dosage prevents new episodes of high disease activity during therapy because of adverse drug reactions (ADRs). This can lead to cessation or inefficacy of the treatment, or complete non-responsiveness to specific medications. Pharmacogenetics (PGx) is a well-established aspect in IBD. One of the exemplary instances of PGx is thiopurines, which are frequently utilized as IBD therapy. This study aimed to evaluate specific gene polymorphism involved in the toxicity and efficacy of Azathioprine (AZA) use in the management in Egyptian patients and to find the correlation between the polymorphism of Nudix Hydrolase15 (NUDT15) gene (rs116855232), The Thiopurine methyltransferase (TPMT) gene (rs1800460) and Inosine Triphosphatase (ITPA) gene (rs1127354) which are involved in the metabolism of the medications utilized in IBD management.

Methods This prospective study was performed in 150 patients with IBD. All patients were treated with 2 mg/kg per day AZA (Imuran, GlaxoSmithKline®) for at least 3 months at therapeutic doses to induce remission. Subsequent treatment of AZA. The minimum follow-up period for those who did not experience ADR was one year. Among the studied patients, one hundred twenty-nine patients were treated with combination regimen of steroids (oral prednisone 1 mg/kg/day).

Also, treatment failure was considered among the patients who could not tolerate AZA side effects, or there was no improvement after dose modification.

Results The most identifiable adverse effect among the studied population was anemia followed by leukopenia and myelosuppression. SNPs genotype TPMT (rs1800460) and ITPA gene (rs1127354) were significantly related to adverse effects among IBD patients receiving Azathioprine treatment. There was a lack of any variants in the NUDT15 genotype among the Egyptian population.

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Conclusion Further research is required in to clarify the relationship between NUDT15 PGx and AZA-ADRs. The effect of NUDT15 PGx on toxicity and ADRs as yet necessitates to be elucidated. Studies with a larger sample size and involving different ethnicities are also necessary.

Keywords Pharmacogenetic, Inflammatory bowel disease, Azathioprine, Crohn's disease, Ulcerative colitis, SNPs, Genotype, Thiopurine, NUDT15, (rs116855232), TPMT (rs1800460), ITPA, (rs1127354)

1 Background

IBD is an idiopathic chronic inflammatory disease, which is associated with dysregulation of the gut immune response [1]. IBD is classified as Crohn's disease (CD) and ulcerative colitis (UC), which differs in the anatomic site and the inflammatory pattern, as well as the involved gut layers. The IBD's etiology remains unknown; however, environmental stimuli seem to result in an overactivity of the immune system among vulnerable subjects [1]. Exposure to pathogens enables the immune system to protect against foreign agents. A hygienic environment is therefore important to avoid excess stimuli that mature the immune system, thus reducing immune tolerance [2].

The IBD disease severity can be classified based on many available scoring systems. Such systems, though have some limitations by subjective definitions, have the ability to monitor disease progression over time. The Simple Colitis Clinical Activity Index [SCCAI] as well as the Pediatric Ulcerative Colitis Activity Index [PUCAI] are reliable scoring systems, which have clear definitions for clinical response and disease remission. The Mayo Clinic Score and Ulcerative Colitis Disease Activity Index [UCDAI] are a composite tool for assessing the IDB manifestations [Defecation frequency and bleeding per rectum] and endoscopic severity. While such scoring systems are not validated, Mayo Score is simple to be applied and can be utilized to assess the endpoints of therapy in clinical trials [3].

There's no cure for UC, the therapy aims at induction and maintaining of remissions, and improving the quality of life. Amino salicylates, Corticosteroids, Immunomodulators, Janus kinase (JAK) inhibitors, Biologics all are treatment options available. The biological treatment is safe and effective; however, its clinical use is limited being expensive and also it should be administered by parenteral route [4].

Thus, immunomodulatory agents, especially thiopurine drugs, are more frequently utilized as a maintenance treatment for IBD patients. In spite of the widespread utilization of thiopurines [1], they cause many ADRs and are associated with therapeutic failures. The variability of responsiveness to thiopurines is because of inter-individual differ in pharmacokinetics [5].

TPMT is a cytoplasmic enzyme which is responsible for catalysis of the rate-limiting step in thiopurines' metabolism [6]. TPMT is coded by TPMT gene, and its effected are mediated by S-adenosyl-L-methionine as the S-methyl donor and S-adenosyl-L-homocysteine as a by-product. Thiopurines, primarily 6-mercaptopurine (6-MP), and its prodrug AZA, exert cytotoxic and immunosuppressive effects in IBD treatment [7]. Though the exact mechanism of action of thiopurine drugs remains unknown, they are supposed to incorporate Thioguanine nucleotides (6-TGNs) into the cell DNA, leading to inhibition of DNA synthesis and cellular death [8]. Based on enzyme activity, the TPMT gene has genetic heterogeneity and thus, is associated with significant inter-individual variability as regards the clinical effectiveness as well as toxicity profiles [8].

Unfortunately, thiopurine drugs can cause many adverse effects such as nausea, emesis, rash, along with other more severe complications such as myelosuppression, hepatic toxicity and pancreatitis. These ADRs limit thiopurines usage and can result in their withdrawal in about 33% of cases [6].

The enzyme Nudix Hydrolase 15 (NUDT15) was also recognized as a relevant predictor of thiopurine tolerance [9]. It converts thioguanine triphosphate (TGTP) metabolites into thioguanine monophosphate which are less toxic [10]. In patients with reduced NUDT15 activity, more TGTP incorporates into cellular DNA causing toxicity and cell death [11]. So far, 3 clinically relevant genetic variants were described in NUDT15 gene: rs116855232, rs746071566 and rs186364861. A meta-analysis demonstrated a significant correlation between NUDT15 gene variants and thiopurines-related bone marrow depression [9] Fig. 1

ITPA-rs1127354 and rs7270101 are two ITPA gene variants which are linked to 25–60% reduction in ITPase activity [13]. In cases having acute lymphoblastic leukemia, adjusted thiopurine doses based their ITPA genotype was associated with fewer adverse effects [14]. ITPA-rs1127354 was reported as a determinant of febrile neutropenia during therapy based on TPMT genotype and TGNs concentrations [15]. But, so far, there is no available evidence supporting the usefulness of a pharmacogenetic testing for ITPA in clinical settings [10]. Figure 2

Genetic, transcriptomic, epigenetic, and miRNAs studies have focused on recognizing novel genetic risk factors

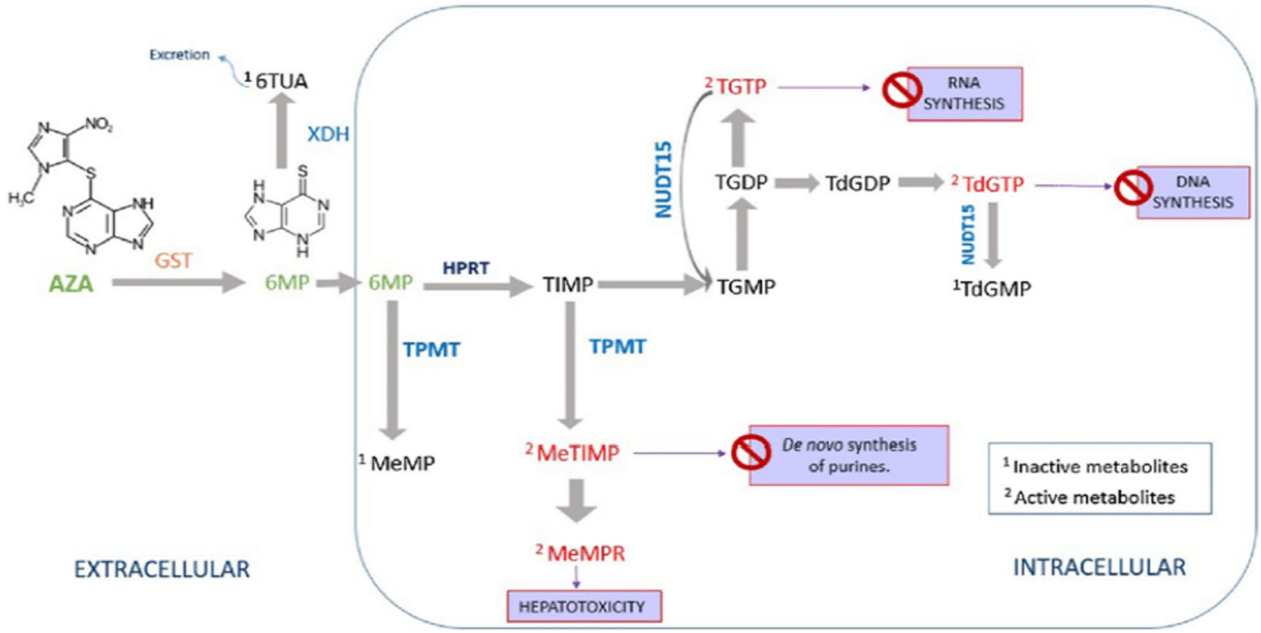

Fig. 1 AZA metabolic pathway mediated by TPMT and NUDT15. AZA azathioprine, GST glutathione-Stransferase, 6-MP 6-mercaptopurine, XDH xanthine oxidase enzyme, 6TUA thiouric acid, HPRT hypoxanthine phosphoribosyltransferase, TIMP thioinosine monophosphate, TPMT thiopurine methyltransferase, MeMP methylmercaptopurine, MeTIMP methylthioinosine [12]

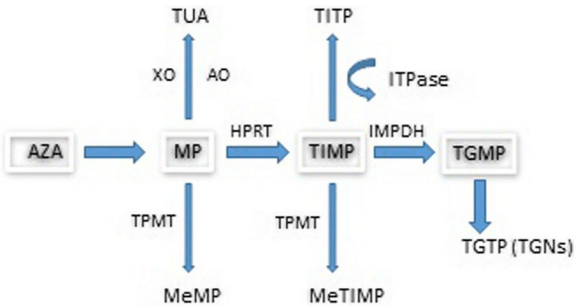

Fig. 2 Metabolism of AZA involves three pathways: the first is degradation to TUA which is then excreted, then through methylation by TPMT into MeMP, and breakdown of MP into TIMP catalyzed by HPRT. Then, TIMP is further metabolized via IMPDH into TGMP. Kinases convert this into the TGNs. Approximately 15–20% of patients with IBD demonstrate hypermethylation when treated with thiopurines. That means that during thiopurine metabolism, methylated thiopurine metabolites are preferentially produced instead of TGNs [16]

and their clinical utility. These factors include prognostic factors that affected disease course and therapeutic responsiveness [17]. Pharmacogenetics (PGx) studies the influence of variations in the DNA sequence on drug response [10]. The use of biological drugs Adalimumab and Infliximab as one of the lines of treatment for IBD has greatly improved the outcomes but has also shown great variability in response among patients [6].

Myelosuppression is the commonest adverse effect related to azathioprine use. It could be reversed after dose reduction [18]. While those who carry 2 non-functional TPMT alleles can develop severe bone marrow depression because of elevated TGNs concentrations, patients who carry single non-functional TPMT allele might have no ability to tolerate azathioprine and thus, the dose should be reduced [18]. Adverse reactions related to azathioprine can also occur in few cases that have no abnormality in TPMT activity, suggesting that other factors, such as other genetic variants, might result in these adverse reactions [19].

2 Aim of the study

This study evaluated specific gene polymorphism involved in the toxicity and efficacy of (AZA) use in the management of Egyptian patients and to find a correlation between the polymorphism of (NUDT15) gene (rs116855232), (TPMT) gene (rs1800460), and (ITPA) gene (rs1127354) which has a role in the metabolism of the medications utilized in IBD management.

3 Methods

3.1 Study design

This prospective study was performed in 150 patients with IBD (CD or UC) admitted to the Specialized Mansoura Hospital from 1 May 2019 to 1 May 2020 with ethical approval number (REC-H-PHBSU-20003). The age of

the enrolled cases ranged from 18 to 80 years. The decision of initiating azathioprine will be taken at the discretion of treating clinician at Mansoura center, prior usage of thiopurines or biological treatment.

3.2 Inclusion and exclusion criteria

After screening 300 patients, 150 only fulfilled the inclusion criteria. (i) Diagnoses CD and UC were based on clinical, radiologic, histologic, and endoscopic features. A detailed history was taken; physical exam and laboratory workup, including a CBC, chemistry profile and serology, were performed to confirm the IBD diagnosis.

(ii) Patient included was screened for Genotyping. (iii) Patients with severe UC either steroid-dependent or steroid-refractory need AZA. (iv) patients with severe CD responded to AZA (according to European Crohn's and Colitis Organization (ECCO) Guideline/Consensus Paper parts (1 and 2) were included in this study.[23];[24]

Patients excluded from this study were (i) patients under the age of 18 years old and over 80. (ii) patients with mild and moderate ulcerative colitis UC and CD responding to 5 ASA according to ECCO Guideline/Consensus Paper. (iii) patients suffering from malignancy and organ failure were excluded from this study. (iv) No history of blood transfusion or administration any drugs which may influence azathioprine metabolism including cyclosporine, methotrexate or allopurinol, or a liver enzyme inducer or inhibitor.

3.3 Treatment protocol and follow-up

All patients were treated with 2 mg/kg per day AZA (Imuran, GlaxoSmithKline®) for at least 3 months at therapeutic doses to induce remission. Subsequent treatment of AZA and regimens will be discontinued if ADR-related occurs. The minimum follow-up period for those who did not experience ADR was one year.

Since there is genetic difference in the metabolism of thiopurines, the optimum dosage is variable. Azathioprine doses are often decreased by 10-folds and administered 3 times weekly (not daily) in the homozygous phenotype, whereas standard doses are provided at 2 mg/kg per day in the wild phenotype. Empirical dosage based on body weight adjustment is generally accepted: azathioprine is initiated at low dose (1 mg/kg/d) to avoid the development of serious ADRs, then gradually titrated to 2 mg/kg as a maximum dose.

Among the studied patients, one hundred twenty-nine patients were treated with combination regimen of steroids (oral prednisone 1 mg/kg/day).

Following complete remission, the corticosteroid dose was tapered gradually by 2.5 (< 20 mg/day) or 5 mg/week (≥ 20 mg/day). Corticosteroids underwent withdrawal at week 12 in the majority of cases. Following withdrawal of steroids, patients continued azathioprine for maintaining of remissions.

For cases in which corticosteroids were not withdrawn at week 12, corticosteroids were administered till week 16. After that, if the corticosteroids dose was ≤ 50% of the initial dosing regimen and ≤ 10 mg/day, partial remission was considered in these cases and they continued to administer steroids. Otherwise, "treatment failure" was considered in them.

Also, treatment failure was considered among the patients who couldn't tolerate AZA side effects, or there was no improvement after dose modification.

None of the 150 studied patients had a history of blood transfusion or administration any drugs which may influence azathioprine metabolism including cyclosporine, methotrexate or allopurinol, or a liver enzyme inducer or inhibitor, for the past 90 days before the study.

3.4 Laboratory examination

Initial investigations included CBC, CRP, ESR, stool markers (fecal calprotectin) and for follow-up and Liver function test baseline and follow-up.

Physical examination included Vital signs and endoscopy performed to confirm the diagnosis.

3.5 Adverse effect management and follow-up

To evaluate the ADRs of azathioprine, anemia was diagnosed in IBD patients, based on the World Health Organization recommendations, as follows: Hb < 13.0 g/dL among adult men, < 12.0 g/dL among adult non-pregnant females, and < 11.0 g/dL among pregnant females. Leucopenia and neutropenia were defined as WBCs < 3.5 * 109/L and neutrophils < 1.5 * 10^9/L, respectively. Influenza-like manifestations were defined as fever and headache. GI disturbances included nausea, emesis and pain [25].

Patients who could not tolerate AZA were converted into 5-aminosalicylic acid (5-ASA) 2–4.8 gm/day oral or to infliximab infusion by escalated doses.

3.6 Genotyping study of NUDT15 gene: rs116855232, The TPMT gene; rs1800460 and ITPA gene variant rs1127354

Peripheral blood samples (6 ml) were obtained from enrolled patients and genomic and genomic DNA underwent extraction utilizing a commercially available kit (DNeasy Plant Mini Kit, Qiagen, Valencia, US) based on manufacturer guidelines. DNA adsorbs to DNeasy membrane in the existence of a high concentration of chaotropic salt, which removes water from hydrated particles in solution. During DNA extraction, buffer conditions were adjusted to permit an adsorption process specific to

silica-gel membrane and provide effective elimination of carbohydrate, polyphenol and other plant metabolites.

Genotyping for the three studied genotypes polymorphisms was carried out utilizing TaqMan® SNP Genotyping Assays (Thermofisher, Waltham, MA, US) which utilized the 5′ nuclease activity of Taq polymerase for generating a fluorescent signal during polymerase chain reaction. For each SNP, 2 TaqMan® probes were utilized, which were different in sequence only at SNP, with one probe complementary to wild-phenotype allele and the other to variant allele.

3.7 Statistical analyses

Data were analyzed by SPSS win statistical package V21 (SPSS Inc., Chicago, IL). Numerical data were represented as means and standard deviations (SDs), medians, and ranges as appropriate. Qualitative data were represented as numbers and percentages. Chi-square (Fisher's exact) test was utilized for examination of the correlation between qualitative variables. For quantitative data, comparison between 2 groups was performed by either a student *t*-test or Mann–Whitney test (nonparametric *t*-test). Testing for normality will be done utilizing the Kolmogorov–Smirnov test and the Shapiro–Wilk test. All tests will be 2-tailed, and a p value ≤ 0.05 was considered significant.

Sample size underwent calculation using PGA: power calculator [20]. Considering IBD prevalence of 31.6% as reported by [21]. And by considering the expected odds ratio of 0.2, using previous reports by [22, 23]. Marker allele frequency of 7.5% and 3.4 as reported disease allele frequency of 3.3%, a minimal sample size of 130 cases was required with a power of 80% and a significance level of 5%. We conducted our study on 150 IBD cases.

3.8 Study diagram

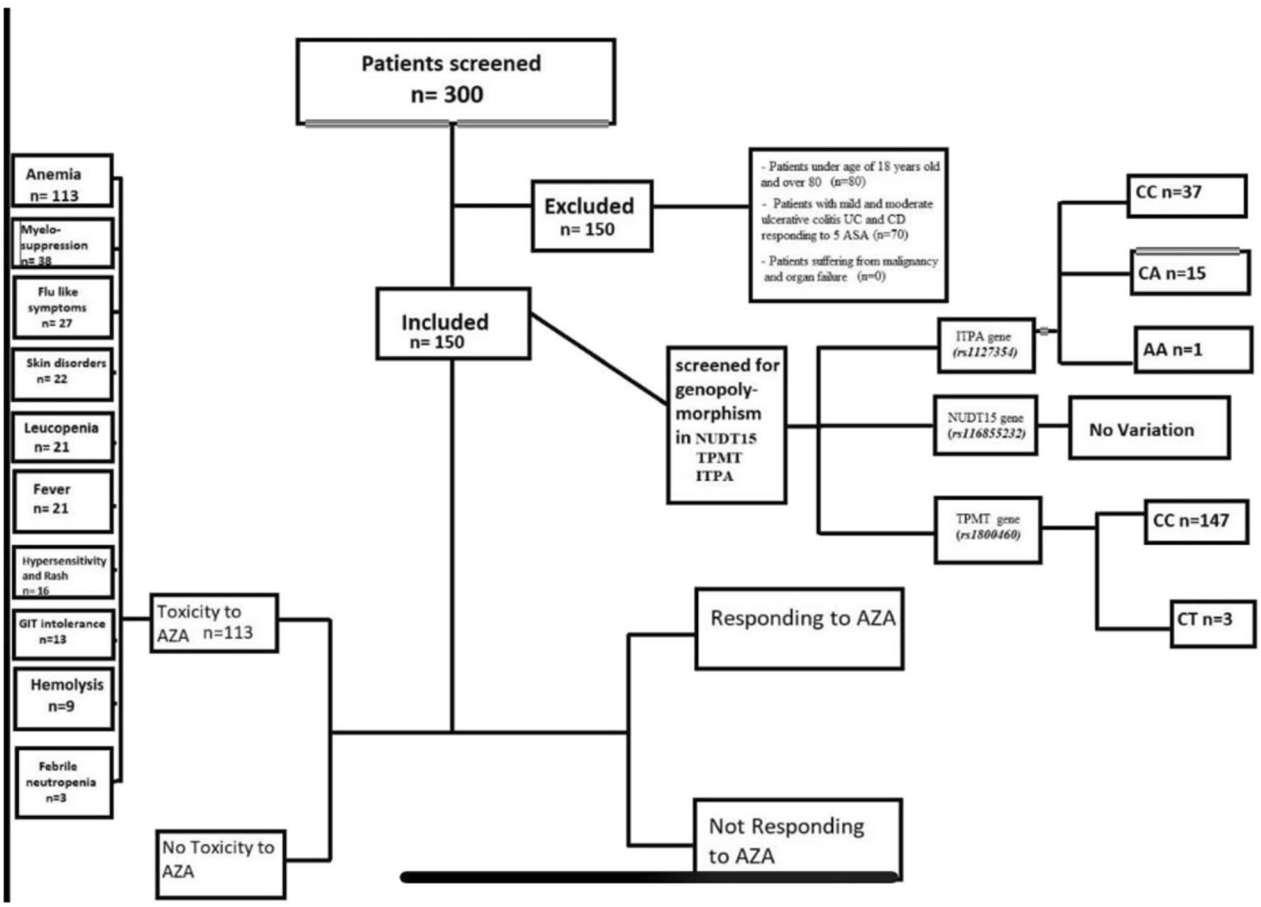

Table 1 Patients characteristics

Parameter	IBD *N* = 150
Age (years) Mean ± SD	35.2 ± 10.8
Males (*N*), %	65 **(43.3%)**
Females (*N*), %	85 (56.7%)
Smoking (Mean ± SD)	24 (16%)
Weight (kg) (Mean ± SD)	72 (± 9.3)
Height (cm) (Mean ± SD)	169.6 (± 8.8)
UC *N*, %	116 (77.3%)
CD *N*, %	34 (22.7%)
Site of affection	
Extensive *N*, %	46 (30.7%)
Left-sided *N*, %	24 (16%)
Rectosigmoid *N*, %	17 (11.3%)
Chronic *N*, %	15 (10%)
Proctitis *N*, %	15 (10%)
Pancolitis *N*, %	14 (9.3%)
Active *N*, %	13 (8.7%)
Enteritis *N*, %	6 (4%)
Duration (years) Median, range	4 (0.5–25)

UC Ulcerative Colitis, *CD* Crohn's Disease

Bold values indicate significant results

4 Results

4.1 Patients characteristics

Demographic data Among the studied, male patients were 43.3% while female patients were 56.7%, and the mean age of the whole participants was 35.2 ± 10.8 years. The mean body weight was 72 kg (± 9.3 kg), and the mean patient height was 169.6 cm (± 8.8 cm). 16% of the patients were smokers. UC patients were 77.3%, and CD patients were 22.7%. The median duration was 4Y ranging from 0.5 to 25 Y. The most identifiable adverse effect among the studied population was Anemia in 75% of the studied patients, patient characteristics shown in Tables 1, 2.

4.2 Frequency of treatment toxicity

Patients exposed to treatment toxicity were 37 (24.6%) Table 2, the reason of treatment toxicity was severe diarrhea and active bleeding which was detected in 40 patients (26.7%), in addition of 7 patients (4.7%) with same condition and developed fever, vomiting and rashes, other causes like Herpes Zoster, Glaucoma + Bradycardia, splenectomy, Psoriasis, Shingles' herpes virus and Ulcer's mucosa.

4.3 Association of rs1800460 and rs1127354 genotypes with adverse effects.

We further studied the genotype of ITPA gene ***rs1127354***; it showed that AA type was found in 1 patient who did not show any significant adverse effects.

CC major variant was found in 94% of the patients, of which 75.2% showed toxicity symptoms. Fever in 14.2% patients, hypersensitivity and rash in 10.6%.hemolysis 6.4%, febrile neutropenia 6.4%, myelosuppression 22.0%, GIT intolerance 9.2%, flu-like symptoms 18.4%, skin disorders 14.9%, anemia 62.4%, leucopenia 15.6%, neutropenia 14.2%, lymphopenia 27.7%.

CA minor variant was found in 8 patients, of which 87.5% showed toxicity symptoms. fever 12.5%, hypersensitivity and rash 12.5%, myelosuppression 87.5%, flu-like symptoms in 12.5%), skin disorders 12.5%, anemia 25%, leucopenia 87.5%, neutropenia 12.5%, lymphopenia 62.5%, no patients showed hemolysis, febrile neutropenia, or GIT intolerance.

We found that minor variant CA of SNPs ITPA genotype ***rs1127354*** was significantly related to adverse effects among IBD patients receiving treatment, regarding myelosuppression, anemia, and leucopenia, while we did not find any significant difference regarding fever, hypersensitivity and rash, hemolysis, febrile neutropenia and other parameters between the variant types of ***rs1127354***.

Evaluation of the TPMT genotype of ***rs1800460*** in our study showed that the CC major variant was found in 147 patients, of whom 74.8% showed toxicity symptoms. fever 14.3%, hypersensitivity and rash 10.2%, hemolysis 5.4%, febrile neutropenia in 6.1%, myelosuppression 23.8%, GIT intolerance in 7.5%, flu-like symptoms 17.7%, skin disorders in 14.3%, anemia 59.2%, leucopenia 16.7%, neutropenia 14.3%, lymphopenia 28.6%.

CT minor variant was found in 3 patients, all of them showed toxicity symptoms, hypersensitivity, and rash in 33.3%, GIT intolerance 66.7%, myelosuppression 100%, flu-like symptoms 33.3%, skin disorders 33.3%, anemia 100%, leucopenia 100%, lymphopenia 66.7%, no patients showed Hemolysis, fever, febrile neutropenia or neutropenia.

Table 2 Frequency of treatment toxicity

Parameter	IBD *N* = 150
Toxicity *N*, %	113 (75.3%)
Fever *N*, %	21 (14.0%)
Hypersensitivity and Rash *N*, %	16 (10.7%)
Hemolysis *N*, %	9 (6.0%)
Febrile neutropenia *N*, %	3 (2.0%)
Myelosuppression *N*, %	38 (25.3%)
GIT intolerance *N*, %	13 (8.7%)
Flu-like symptoms *N*, %	27 (18.0%)
skin disorders N, %	22 (14.7%)
Anemia *N*, %	113 (75.3%)
Leucopenia *N*, %	21 (14.0%)

We found that minor variant CT of SNPs TPMT genotype ***rs1800460*** was significantly related to adverse effects among IBD patients receiving treatment, regarding myelosuppression, GIT intolerance, and leucopenia, while we did not find any significant difference regarding fever, hypersensitivity, and rash, hemolysis, febrile neutropenia and other parameters between the variant types of ***rs18004604***. Our results showed no variants of NUDT15 genotype ***rs116055332*** were found among the studied population. (Table 3).

4.4 Significant toxicity according to SNPs' genotypes

The main toxic adverse effects of TPMT genotype ***rs18004604*** and ITPA genotype ***rs1127354*** are shown in Fig. 5.

4.5 Time to failure according to rs1127354 genotypes:

Our result showed that the overall cumulative probability of time to failure according to studied SNPs rs1127354 as predictive time failure. Figure 3, 5-year failure cumulative rate were 26.9% and 35.7% with a mean time of failure of 112 months and 75.9 months for the rs1127354 variants with 95% CI of 91.9–132 for wild variant and 58.7–93 for heterozygous and 36 for the mutant variant with no significant difference. Table 4.

4.6 Time to failure according to rs1800460 genotypes

The studied SNPs rs1800460 showed that 5 years predictive time failure rate was 25% for wild type and 100% for heterozygous with a mean time of failure of 112 months and 40 months for the with 95% CI of 92.9–132.7 for wild variant and 19.3–60.7 for heterozygous with significant difference (p value 0.003) Fig. 4.

4.7 Regression analysis of failure

We studied other factors as a predictive failure for the treatment, among the studied parameters as shown in Table 3 BMI, Comorbidities, and rs1800460 were significantly related to the studied outcome. Table 5

5 Discussion

Pharmacogenomics (PGx) is well-established in the field of IBD. One of the exemplary instances of PGx is thiopurines that are frequently utilized in IBD management [10]. In the current study, we gave an outline about the usefulness of PGx in treating IBD in clinical practice. The wide inter-individual variability in the responsiveness to pharmacotherapy (as regards effectiveness and toxic effects) results in significant therapeutic failures during IBD treatment [24]. Better stratification of the IBD patients is required to maximize their benefits from the drugs and to reduce drug-related ADRs. Pharmacogenomic testing that is performed before therapy initiation can help optimize the selection of appropriate drugs and their doses, as well as reduce their ADRs [24] Fig. 5.

Thiopurine drugs, such as AZA, 6-MP and tioguanine, are long-standing treatments, which are commonly utilized for IBD management [25]. Thiopurines have demonstrated efficacy in maintaining remissions among IBD cases and also were associated with decreases in the necessity for surgical interventions, post-surgical recurrence and the risk of

Table 3 Association of rs1800460 and rs1127354 genotypes with adverse effects

Parameter		ITPA *(rs1127354)*							TPMT *(rs1800460)*				
		CC $N=141$		CA $N=8$		AA $N=1$		p	CC $N=147$		CT $N=3$		p
Toxicity	***N*, %**	106	75.2%	7	87.5%	0	0%	0.247	110	74.8%	3	100.0%	1
Fever	***N*, %**	20	14.2%	1	12.5%	0	0%	0.913	21	14.3%	0	0.0%	0.480
Hypersensitivity and Rash	***N*, %**	15	10.6%	1	12.5%	0	0%	0.929	15	10.2%	1	33.3%	0.289
Hemolysis	***N*, %**	9	6.4%	0	0%	0	0%	0.737	8	5.4%	1	33.3%	0.170
Febrile neutropenia	***N*, %**	9	6.4%	0	0%	0	0%	1	9	6.1%	0	0.0%	0.658
Myelosuppression	***N*, %**	31	22.0%	7	87.5%	0	0%	**<0.001**	35	23.8%	3	100.0%	**0.015**
GIT intolerance	***N*, %**	13	9.2%	0	0.0%	0	0%	1	11	7.5%	2	66.7%	**0.020**
Flu-like symptoms	***N*, %**	26	18.4%	1	12.5%	0	0%	1	26	17.7%	1	33.3%	0.451
skin disorders	***N*, %**	21	14.9%	1	12.5%	0	0%	1	21	14.3%	1	33.3%	0.381
Anemia	***N*, %**	88	62.4%	2	25.0%	0	0%	**0.036**	87	59.2%	3	100.0%	0.275
leucopenia	***N*, %**	22	15.6%	7	87.5%	0	0%	**<0.001**	26	17.7%	3	100.0%	**0.007**
Neutropenia	***N*, %**	20	14.2%	1	12.5%	0	0%	1	21	14.3%	0	0.0%	1
Lymphopenia	***N*, %**	39	27.7%	5	62.5%	0	0%	0.066	42	28.6%	2	66.7%	0.206

OR odds ratio; *CI* confidence interval; *GIT* Gastrointestinal tract

Bold values indicate significant results < 0.05

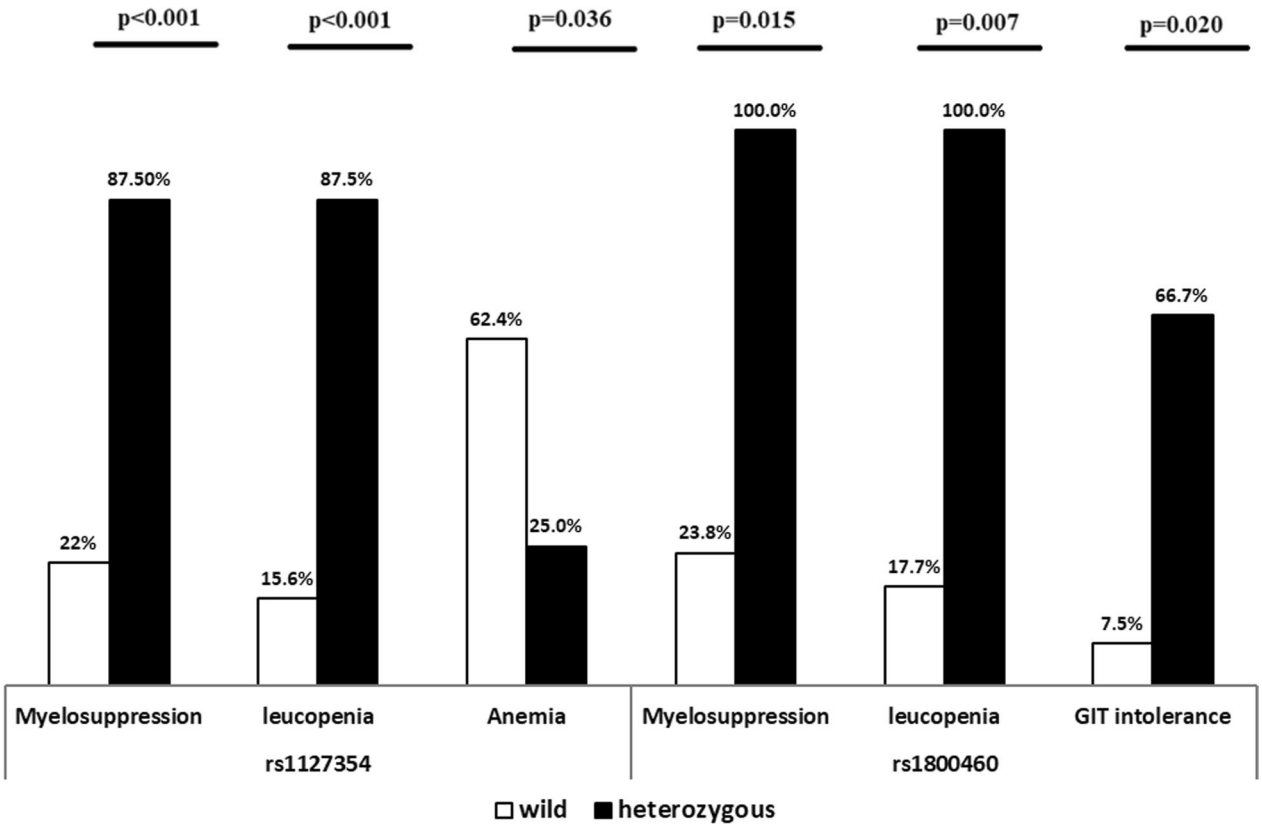

Fig. 3 Significant toxicity according to SNPs' genotypes

Table 4 Association of studied SNPs with time to failure

		Genotypes			*p*
		Wild	**Heterozygous**	**Mutant**	
rs1127354	5-year failure cumulative rate (%)	26.9	35.7	100	0.216
	time to failure (months)	mean	112	75.9	36
		95% CI	91.9–132	58.7–93	36–36
rs1800460	5-year failure cumulative rate (%)	25.9	100	–	**0.003**
	time to failure (months)	mean	112.8	40	–
		95% CI	92.9–132.7	19.3–60.7	–
	time to failure (months)	mean	114.7	106.6	105
		95% CI	85.1–144.3	79.5–133.7	40.2–169.8

CI confidence interval

Bold values indicate significant results < 0.05

IBD-associated colorectal cancer [26]. Also, thiopurines are associated with the improvement of pharmacokinetics of anti-TNF agents when combined with them [25].

Indeed, thiopurines are commonly utilized in treating CD and UC being effective, administered by oral route, cheap and several clinicians are familiar and have experience with their usage [27]. But, as shown in current study 52% of the studied population showed no response to AZA therapy, in previous studies reported up to 60% of cases either showed inadequate response or developed thiopurine toxicity, leading to their withdrawal or dose adjustment. With the continues introduction of novel targeted biological agents, the role of thiopurines drugs in the current era is, thus, being questioned [28].

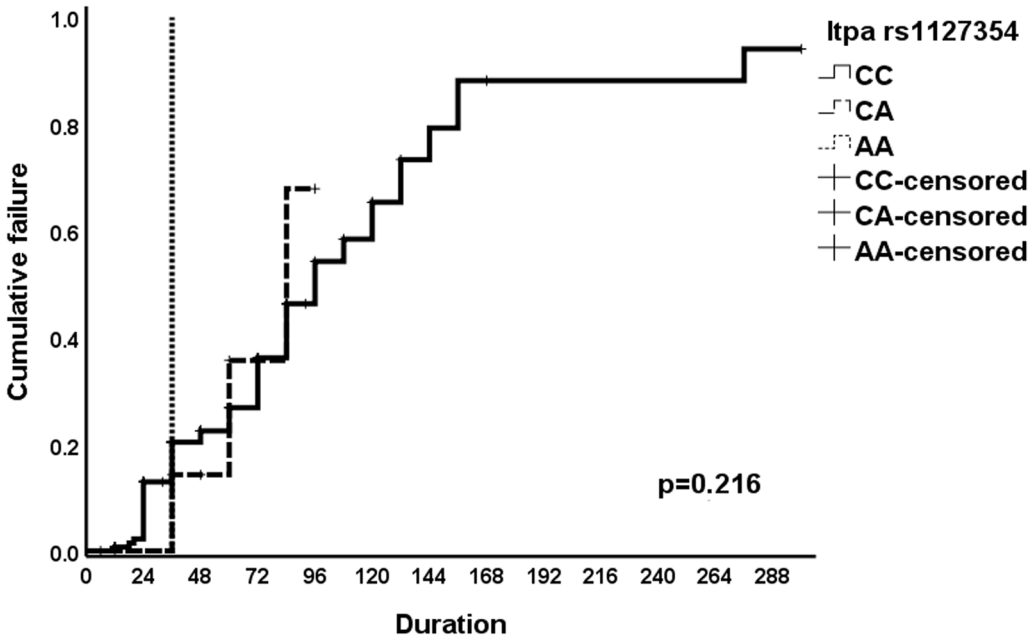

Fig. 4 Time to failure according to rs1127354 genotypes

Table 5 Cox regression analysis for prediction of failure to treatment

	Univariable				Multivariable			
	p	OR	95% CI		*p*	OR	95% CI	
Age	0.668	0.995	0.973	1.018				
Gender	0.605	1.140	0.694	1.871				
Smoking	0.152	0.403	0.162	1.007				
BMI	**0.043**	1.075	1.002	1.153	0.286	1.042	0.966	1.124
Comorbidities	**0.037**	1.645	1.167	2.798	0.053	1.702	0.993	2.918
Initial TLC	0.638	1.023	0.930	1.127				
Initial ANC	0.696	0.977	0.868	1.099				
Initial ALC	0.317	0.898	0.728	1.108				
Initial platelets	0.982	1.022	0.997	1.067				
Fecal Calprotectin	0.409	1.074	0.989	1.132				
Association of AZA with steroids	**< 0.001**	3.633	2.019	6.539	< 0.001	3.402	1.877	6.166
rs1127354	0.517	1.402	0.504	3.898				
rs1800460	**0.009**	4.879	1.490	15.976	0.019	4.214	1.270	13.981

OR odds ratio; *CI* confidence interval

Bold values indicate significant results < 0.05

Our results showed significant reduction in hemoglobin and RBCs indices. A large number of reports have suggested that monitoring of hemoglobin and RBCs indices may allow for adjustments in drug dose to reduce the incidence of adverse reactions in patients receiving these agents and the clinical efficacy of biological treatment [29] However, a few reports did not find any significant difference regarding leucocytes count [30].

In this study, we investigated the frequency of ITPA genotype rs1127354, TPMT genotype rs1800460, and, NUDT15 genotype rs16855232, SNPs genotypes polymorphism, and their related effects on the AZA, and possible relation to adverse effects.

We reported that the TPMT genotype (rs1800460) was the common finding of the study with rare heterozygosity among the studied population. Moradveisi

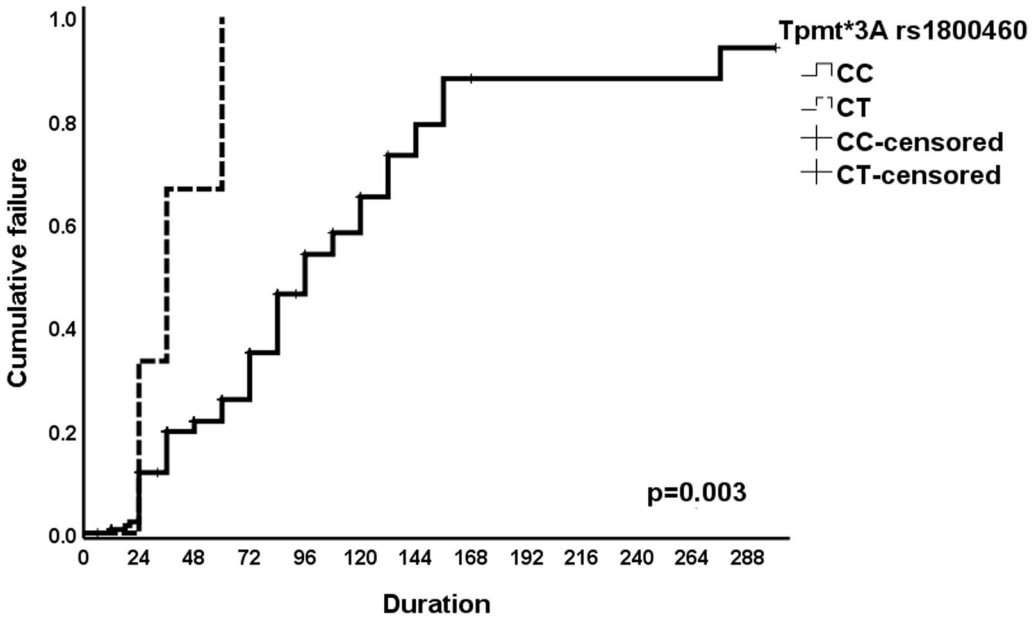

Fig. 5 Time to failure according to rs1800460 genotypes

et al. [31] reported that in heterozygotes, TPMT*3A was the most common mutant allele (85%), whereas TPMT*2 and TPMT*3C were rare.

Luber et al. [25] reported that typical dosing regimen of thiopurines in heterozygous or TPMT-deficient individuals resulted in an accumulation of 6-thioguanine nucleotide within the bone marrow with a subsequent severe myelosuppression. We found that minor variant CT of SNPs genotype TPMT genotype (rs1800460) was significantly related to adverse effects among IBD patients receiving treatment, regarding myelosuppression, GIT intolerance, and leucopenia.

TPMT activity itself varies among races, and it has been reported that the enzyme activity of TPMT in the Japanese population is about 50% and is approximately 70% lower than in Jewish and white Americans, respectively. Therefore, screening for the TPMT gene mutation alone may be inappropriate for predicting azathioprine/6-MP-induced adverse reactions [32].

Most studies on the correlation between TPMT polymorphism and adverse effects are in patients with IBD, mainly in Westerners in which the TPMT gene predicts hematological adverse effects, in 5–10% of patients treated with thiopurine drugs. Other studies have demonstrated that only 29% of leukopenic patients had mutant polymorphisms and indicated that only one-third of myelosuppressive episodes can be attributed to genetic polymorphism [32].

A publication describes a meta-analysis investigating the associations between TPMT polymorphisms and AZA-induced adverse events in patients with autoimmune diseases. The results showed that TPMT polymorphisms were significantly associated with AZA-induced overall adverse effects, bone marrow toxicity, and gastric intolerance. However, the subgroup analysis according to ethnicity showed a significant association between TPMT polymorphisms and AZA-induced bone marrow toxicity in Asian populations, but not in Caucasian populations [33].

Our results showed that variant in rs1800460 was significantly related to leucopenia, but there was no significant differences among the whole population regarding the adverse effect as mentioned before.

The remaining causes may be mediated by immune mechanisms or by other variables affecting the metabolism of the drug. TPMT activity does not usually predict azathioprine-induced hepatotoxicity or gastrointestinal upset [34].

We did not detect any variation of NUDT15 genotype rs16855232 among the studied population. Many authors reported that NUDT15 variants were associated with an alteration in the metabolism of thiopurines [35]. Mutations of NUDT15, which occur more frequently in the East Asian population explaining our finding among the Egyptian population, cause a reduction in enzymatic activity in a TGN-independent way [25].

Roberts and Barclay [36] showed a result of an analysis of 436,011 imputed SNPs in 978 patients and described a polymorphism SNP in rs16855232, which

was significantly associated with adverse effects. The risk allele was found in 89.4% of patients with the adverse effect but was only found in 6.8% (43/632) of controls.

NUDT15 variants were linked to an enhanced risk of Thiopurine-mediated myelosuppression in IBD cases of European ancestry [27]. Moreover, the occurrence of Thiopurine-induced myelotoxicity was faster in IBD cases having mutations in both TPMT and NUDT15 [37]. However, the current results showed no variants were found among the Egyptian population in contrast to previous authors. These results reflect the unique circumstance of the Egyptian population and the need to find its polymorphism, and this will help in the individualized treatment plan.

The distribution of minor variant polymorphism of rs 1127354 ((Pro32Thr/P32T, 94C > A) is a SNP within the ITPA (Inosine triphosphate pyrophosphatase) gene. It was 5.3%, and the wild variant was 0.7%. This frequency was lower than findings obtained by [38], who reported that the distribution of minor variant for rs1127354 polymorphisms was 9.9%, reported that the distribution of C/A variant for rs1127354 was 20%, much higher than our results and the A/A variant was 1.7%, similar to our finding [39].

We found that minor variant ITPA gene 94C > A (rs1127354) is significantly related to the adverse effect from drugs including Myelosuppression, leucopenia and anemia [40]. In addition, ITPA gene variants can predict ADRs [41]. The leukocyte and erythrocyte highly express the ITPA, which causes hydrolysis of ITPA to prevent 6-TITP accumulation [14]. Similar to 6-T(d)GTP of the 6-TGNs, 6-TITP could incorporate into DNA and RNA to compete with nucleotides [40]. SNPs 94C > A (rs1127354) and IVS2 + 21A > C, which is linked to ITPA deficiency, were also shown to be linked to ADRs caused by thiopurines including leukopenia, influenza-like manifestations as well as pancreatitis [31].

In consistent with our results, Wang et al. 2014 [42] reported that ITPA-deficient patients experienced more toxicity related to thiopurines. ITPA converts 6-TITP to phosphorylated 6-thio IMP, an intermediate metabolite of AZA/6-mercaptopurine. In ITPA-deficient patients, 6-TITP is accumulated leading to ADRs like myelosuppression [43].

Usually, the therapeutic choices for IBD are based upon disease activity. But, complete implementation of the therapy necessitates (A) predictors of response to therapies which have different mode of actions to direct the correct treatment to the right patients and (B) predictors of the pharmacokinetic and the pharmacodynamics properties for optimization of a specific treatment and for ensuring the sustained effects of this treatment [44].

Identifying the patient who is at high risk of therapeutic failure could properly optimize the escalation therapy for better controlling of IBD based on risk stratification [3].

As shown in our study, rs 1800460 was significantly related to TIM which often develop over a few weeks after initiating thiopurines, however, could develop at any time during the course of treatment. The majority of cases experience no symptoms; however, severe opportunistic infections can happen resulting in about 1% mortality and higher incidence for treatment failure. According to Voskuhl et al. 2019, a significant evidence that links TPMT variant rs 1800460 to TIM does exist [24].

Cytotoxic metabolites of 6-TG are responsible for the therapeutic effects of thiopurine drugs; however, they can cause bone marrow suppression. About 10% of individuals of European ancestry carry TPMT genetic alleles. Three TPMT genetic variants account for 90% of the TPMT deficiency in Europeans, which together explain 25% of TIM. On the other hand, only 3% of Asian population carry TPMT genetic variants in spite of a greater incidence of TIM among Asians populations [24].

A patient who has loss-of-function *TPMT* allele is at enhanced risk of myelosuppression after receiving the standard dose of thiopurines. Genetic variation of TPMT is defined by a star (*) alleles. Each of these star alleles is defined by the genotype at ≥ 1 locus within the gene and is utilized to annotate TPMT enzyme activity levels and thus guide dosing regimens. Recently, dosing recommendations based upon TPMT have been published by CPIC [45].

In brief, decreasing the initial doses (30–80% of target dose) must be considered in patients who are TPMT intermediate metabolizers, whereas significantly decreased dosage regimen (10% of target dose) or using another drug must be considered in patients who are TPMT poor metabolizers.

A study by Sicilia et al. 2021 [46] concluded that a total of 23% of IBD received at least one course of steroid treatment. CD cases commonly stepped up to biologic drugs and/or surgery compared to UC cases. In CD cases, behaviors pattern was an important risk factor. Following a single steroid course, 35% of IBD cases maintained remissions without requiring treatment escalation.

IBD cases can be influenced by other non-related comorbid conditions which include any secondary health condition affecting a patient who suffers from a primary disease.

As proved in this study, patients who respond to AZA are associated with multiple side effects including, but not limited to, myelosuppression and liver

toxicity. McLean and cross [47] reported the same effect of co-morbidities on treatment outcome.

The IBD is commonly associated with other nonrelated diseases that can affect the therapy and prognosis. Also, making a decision is complex step since the current evidence does not usually apply, because in the majority of clinical trials these patients are ruled out. In contrast, the presence of such nonrelated diseases requires consideration of the likely consequences of management on other comorbidities in terms of drug interactions or facilitating side effects. Prognosis can also be changed, particularly in the existence of cardiovascular disease that causes higher morbidity and mortality. Eventually, such associated diseases necessitate cooperation and coordination between clinicians for collaborative decision making, and appropriate medical care [48].

Our study showed variant type TMPT gene rs 1800460 was responsible for increased risk of AZA toxicity which predicts treatment failure as shown by Ansari et al. [49]. The same results obtained by Grover et al. 2021 showed that polymorphisms in the TPMT gene have been identified as a significant cause of thiopurines-associated leukopenia and failure of the treatment.

Wang et al. 2014 reported that ITPA gene variant rs4848306 was the commonest among cases complaining of nausea/emesis and having slowly developing side effects (67%), followed by cases complaining of nausea/emesis without having slowly appearing side effects (50%) and cases with no nausea/emesis, however, experiencing slowly appearing side effects (25%). Authors concluded that ITPA polymorphisms might be a significant predictor of treatment failure [42].

As proved in our study, ITPA-deficient cases showed more thiopurines toxicity. ITPA catalyzes 6-TITP to phosphorylated 6-thio IMP, an intermediate metabolite of AZA/6-mercaptopurine. In ITPA-deficient patients, 6-TITP is accumulated leading to development of ADRs, which include myelosuppression. Five functional ITPA gene SNPs have been identified [50].

While a systematic review demonstrated that the enzymatic tests have the ability to identify those who are at risk for AZA toxicity and for the failure of treatment prediction as the reduced enzymatic activity could also be due to any known or unknown mutation or external trigger (for example: drug interaction, blood transfusion), these tests are limited by a recent blood transfusion, other drugs, alcoholic, and food [42].

6 Conclusion

We found that variants of TPMT genotype and, the ITPA gene were significantly related to adverse effects among IBD patients regarding myelosuppression, GIT intolerance, leucopenia, and anemia.

However, we have found no significant association between the NUDT15 polymorphism and the adverse effects of AZA. Further research is required in this area to clarify the relationship between NUDT15 polymorphism and AZA-induced adverse effects in the Egyptian population.

The effect of NUDT15 polymorphism on toxicity and ADRs still requires explanation. Studies involving a larger sample size and different ethnicities are necessary for detecting any association between NUDT15 gene variants and long-term overall survival, their effects at the molecular level and whether they have the ability to predict relapses; as seen in case of ITPA/TPMT genotypes.

BMI is a significant predictor for no-remission after treatment; also, there was a significant relation between Fecal Calprotectin levels and the patient's response to treatment, in our study AZA exhibited the same significant relation with steroids.

7 Limitation

Further analyses will be needed to determine how and which variants should be genotyped, taking into account the allele frequencies in the target population. The costs and benefits of genotyping also require consideration. These results suggest that further population-specific pharmacogenetic studies are indicated. Most studies investigating differences in drug response as a result of genetic polymorphisms.

Straight clinical recommendations on how to individualize therapy based on pharmacogenetic test are essential; however, the study design of most pharmacogenetic studies is inadequate to draw these sorts of conclusions. The study design of most pharmacogenetic studies is inadequate to draw these sorts of conclusions. The cost effectiveness of genotyping patients before the start of treatment should also be evaluated.

We can therefore conclude that pharmacogenetics remains a promising field that already contributed to better understanding the molecular mechanisms of some of the drugs used in IBD.

In the future, it is necessary to organize studies in well characterized patients who have been uniformly treated and systematically evaluated in order to quantitate drug response and side effects more objectively. Also the prize of genotyping should be compared with the money saved by the prevention of side effects or treatment of non-responders.

An effort should be made to collect genomic DNA from all patients enrolled in clinical drug trials after informed consent for future pharmacogenetic studies.

Abbreviations

IBD	Inflammatory bowel disease
ADRs	Adverse drug reactions
PGx	Pharmacogenetics
AZA	Azathioprine
NUDT15	Enzyme Nudix Hydrolase 15
NUDT15	Nudix Hydrolase15 gene
TPMT	Thiopurine methyltransferase gene
ITPA	Inosine triphosphatase gene
CBC	Complete blood count
CRP	C-reactive protein
ESR	Erythrocyte sedimentation rate
CD	Crohn's disease
UC	Ulcerative colitis
SCCAI	Simple colitis clinical activity index
PUCAI	The paediatric ulcerative colitis activity index
UCDAI	The Mayo Clinic score and ulcerative colitis disease activity index
JAK	Janus kinase
6-MP	6-Mercaptopurine
6-TGNs	Thioguanine nucleotides
DPWG	Dutch Pharmacogenetics Working Group
CPIC	Clinical Pharmacogenetics Implementation Consortium
6-TITP	6-Thioinosine triphosphate
SNPs	Single nucleotide polymorphisms
PK	Pharmacokinetics
PD	Pharmacodynamic
TNF	Anti-tumor necrosis factor
SNPs	The single nucleotide polymorphisms

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Availability of data and materials
The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
Ethics approval was obtained from Faculty of Pharmacy, Beni-Suef University, reference number (REC-H-PHBSU-20003). Informed written consent to participate in the study was provided by all participants.

Consent for publication
Not applicable.

Competing interests
Dr Mohamed EA Abdelrahim is a co-author of this study and an Associate Editor for the journal. He was not involved in handling this manuscript during the submission process. The rest of the authors have no conflict of interest to declare.

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References

1. Antonioli L, Fornai M, Romano B, Pellegrini C, Blandizzi C (2020) Editorial: IBD management-novel targets and therapeutic perspectives. Front Pharmacol 11:448
2. Weingarden AR, Vaughn BP (2017) Intestinal microbiota, fecal microbiota transplantation, and inflammatory bowel disease. Gut Microbes 8(3):238–252
3. Peyrin-Biroulet L, Sandborn W, Sands BE, Reinisch W, Bemelman W, Bryant RV et al (2015) Selecting therapeutic targets in inflammatory bowel disease (STRIDE): determining therapeutic goals for treat-to-target. Am J Gastroenterol 110(9):1324–1338
4. Na SY, Moon W (2019) Perspectives on current and novel treatments for inflammatory bowel disease. Gut Liver 13(6):604–616
5. Lee KM, Kim YS, Seo GS, Kim TO, Yang SK (2015) Use of thiopurines in inflammatory bowel disease: a consensus statement by the Korean Association for the study of intestinal diseases (KASID). Intest Res 13(3):193–207
6. Abaji R, Krajinovic M (2017) Thiopurine S-methyltransferase polymorphisms in acute lymphoblastic leukemia, inflammatory bowel disease and autoimmune disorders: influence on treatment response. Pharmgenomics Pers Med 10:143–156
7. Kotur N, Dokmanovic L, Janic D, Stankovic B, Krstovski N, Tosic N et al (2015) TPMT gene expression is increased during maintenance therapy in childhood acute lymphoblastic leukemia patients in a TPMT gene promoter variable number of tandem repeat-dependent manner. Pharmacogenomics 16(15):1701–1712
8. Murdaca G, Spanò F, Contatore M, Guastalla A, Magnani O, Puppo F (2014) Pharmacogenetics of etanercept: role of TNF-α gene polymorphisms in improving its efficacy. Expert Opin Drug Metab Toxicol 10(12):1703–1710
9. van den Bosch BJ, Coenen MJ (2021) Pharmacogenetics of inflammatory bowel disease. Pharmacogenomics 22(1):55–66
10. Ramos KN, Gregornik D, Ramos KS (2021) Pharmacogenomics insights into precision pediatric oncology. Curr Opin Pediatr 33(6):564–569
11. Coenen MJH (2019) NUDT15 genotyping in Caucasian patients can help to optimise thiopurine treatment in patients with inflammatory bowel disease. Transl Gastroenterol Hepatol 4:81
12. Casajús A, Zubiaur P, Méndez M, Campodónico D, Gómez A, Navares-Gómez M et al (2022) Genotype-guided prescription of azathioprine reduces the incidence of adverse drug reactions in TPMT intermediate metabolizers to a similar incidence as normal metabolizers. Adv Ther 39(4):1743–1753
13. Steponaitiene R, Kupcinskas J, Survilaite S, Varkalaite G, Jonaitis L, Kiudelis G et al (2016) TPMT and ITPA genetic variants in Lithuanian inflammatory bowel disease patients: prevalence and azathioprine-related side effects. Adv Med Sci 61(1):135–140
14. Burgis NE (2016) A disease spectrum for ITPA variation: advances in biochemical and clinical research. J Biomed Sci 23(1):73
15. Yamamoto-Furusho JK, Fonseca-Camarillo G (2015) Genetic markers associated with clinical outcomes in patients with inflammatory bowel disease. Inflamm Bowel Dis 21(11):2683–2695

16. Warner B, Johnston E, Arenas-Hernandez M, Marinaki A, Irving P, Sanderson J (2018) A practical guide to thiopurine prescribing and monitoring in IBD. Frontline Gastroenterol 9(1):10–15
17. Devasia AJ, Illangeswaran RSS, Raj IX, George B, Balasubramanian P (2020) NUDT15 polymorphism explains serious toxicity to azathioprine in Indian patients with chronic immune thrombocytopenia and autoimmune hemolytic anemia: a case series. Drug Metab Pers Ther. https://doi.org/10.1515/dmpt-2020-0128
18. Ailing Z, Jing Y, Jingli L, Yun X, Xiaojian Z (2016) Further evidence that a variant of the gene NUDT15 may be an important predictor of azathioprine-induced toxicity in Chinese subjects: a case report. J Clin Pharm Ther 41(5):572–574
19. Sturm A, Maaser C, Calabrese E, Annese V, Fiorino G, Kucharzik T et al (2019) ECCO-ESGAR guideline for diagnostic assessment in IBD part 2: IBD scores and general principles and technical aspects. J Crohns Colitis 13(3):273–284
20. Menashe I, Rosenberg PS, Chen BE (2008) PGA: power calculator for case-control genetic association analyses. BMC Genet 9:36
21. Okeke EN, Ladep NG, Adah S, Bupwatda PW, Agaba EI, Malu AO (2009) Prevalence of irritable bowel syndrome: a community survey in an African population. Ann Afr Med 8(3):177–180
22. Liu Z, Wang S, Qi W, Wang X, Sun D, Wang H et al (2017) The relationship between ITPA rs1127354 polymorphisms and efficacy of antiviral treatment in Northeast Chinese CHC patients. Medicine 96(29):e7554
23. Thiesen S, Yin P, Jorgensen AL, Zhang JE, Manzo V, McEvoy L et al (2017) TPMT, COMT and ACYP2 genetic variants in paediatric cancer patients with cisplatin-induced ototoxicity. Pharmacogenet Genom 27(6):213–222
24. Voskuil MD, Bangma A, Weersma RK, Festen EAM (2019) Predicting (side) effects for patients with inflammatory bowel disease: the promise of pharmacogenetics. World J Gastroenterol 25(21):2539–2548
25. Luber RP, Honap S, Cunningham G, Irving PM (2019) Can we predict the toxicity and response to thiopurines in inflammatory bowel diseases? Front Med (Lausanne) 6:279
26. Raghu Subramanian C, Triadafilopoulos G (2016) Care of inflammatory bowel disease patients in remission. Gastroenterol Rep (Oxf) 4(4):261–271
27. Atreya R, Neurath MF, Siegmund B (2020) Personalizing treatment in IBD: Hype or reality in 2020? Can we predict response to Anti-TNF? Front Med (Lausanne) 7:517
28. Hanauer SB, Sandborn WJ, Lichtenstein GR (2019) Evolving considerations for thiopurine therapy for inflammatory bowel diseases—a clinical practice update: commentary. Gastroenterology 156(1):36–42
29. Kuhn V, Diederich L, Keller TS, Kramer CM, Lückstädt W, Panknin C et al (2017) Red blood cell function and dysfunction: redox regulation, nitric oxide metabolism, Anemia. Antioxid Redox Signal 26(13):718–742
30. Odagiri K, Uehara A, Mizuta I, Yamamoto M, Kurata C (2011) Longitudinal study on white blood cell count and the incidence of metabolic syndrome. Intern Med (Tokyo, Japan) 50:2491–2498
31. Moradveisi B, Muwakkit S, Zamani F, Ghaderi E, Mohammadi E, Zgheib NK (2019) ITPA, TPMT, and NUDT15 genetic polymorphisms predict 6-mercaptopurine toxicity in middle eastern children with acute lymphoblastic leukemia. Front Pharmacol 10:916
32. Kasirer Y, Mevorach R, Renbaum P, Algur N, Soiferman D, Beeri R et al (2014) Thiopurine S-methyltransferase (TPMT) activity is better determined by biochemical assay versus genotyping in the Jewish population. Dig Dis Sci 59(6):1207–1212
33. Liu YP, Xu HQ, Li M, Yang X, Yu S, Fu WL et al (2015) Association between *Thiopurine S-Methyltransferase* polymorphisms and azathioprine-induced adverse drug reactions in patients with autoimmune diseases: a meta-analysis. PLoS ONE 10(12):e0144234
34. Rudin S, Marable M, Huang RS (2017) The promise of pharmacogenomics in reducing toxicity during acute lymphoblastic leukemia maintenance treatment. Genom Proteom Bioinform 15(2):82–93
35. Moriyama T, Nishii R, Perez-Andreu V, Yang W, Klussmann FA, Zhao X et al (2016) NUDT15 polymorphisms alter thiopurine metabolism and hematopoietic toxicity. Nat Genet 48(4):367–373
36. Roberts RL, Barclay ML (2015) Update on thiopurine pharmacogenetics in inflammatory bowel disease. Pharmacogenomics 16(8):891–903
37. Walker GJ, Harrison JW, Heap GA, Voskuil MD, Andersen V, Anderson CA et al (2019) Association of genetic variants in NUDT15 With thiopurine-induced myelosuppression in patients with inflammatory bowel disease. JAMA 321(8):773–785
38. Pineda-Tenor D, García-Álvarez M, Jiménez-Sousa MA, Vázquez-Morón S, Resino S (2015) Relationship between ITPA polymorphisms and hemolytic anemia in HCV-infected patients after ribavirin-based therapy: a meta-analysis. J Transl Med 13(1):320
39. Hai H, Tamori A, Enomoto M, Morikawa H, Uchida-Kobayashi S, Fujii H et al (2014) Relationship between inosine triphosphate genotype and outcome of extended therapy in hepatitis C virus patients with a late viral response to pegylated-interferon and ribavirin. J Gastroenterol Hepatol. https://doi.org/10.1111/jgh.12376
40. Kakuta Y, Kinouchi Y, Shimosegawa T (2018) Pharmacogenetics of thiopurines for inflammatory bowel disease in East Asia: prospects for clinical application of NUDT15 genotyping. J Gastroenterol 53(2):172–180
41. Milosevic G, Kotur N, Krstovski N, Lazic J, Zukic B, Stankovic B et al (2018) Variants in TPMT, ITPA, ABCC4 and ABCB1 genes as predictors of 6-mercaptopurine induced toxicity in children with acute lymphoblastic leukemia. J Med Biochem 37(3):320–327
42. Citterio-Quentin A, Moulsma M, Gustin MP, Lachaux A, Boulieu R (2018) ITPA activity in children treated by azathioprine: relationship to the occurrence of adverse drug reactions and inflammatory response. Basic Clin Pharmacol Toxicol 122(6):588–595
43. Yang X, Li Q, He Y, Zhu Y, Yang R, Zhu X et al (2022) Individualized medication based on pharmacogenomics and treatment progress in children with IgAV nephritis. Front Pharmacol 13:956397
44. Colman RJ, Dhaliwal J, Rosen MJ (2021) Predicting therapeutic response in pediatric ulcerative colitis—a journey towards precision medicine. Front Pediatr 9:634739
45. Relling MV, Schwab M, Whirl-Carrillo M, Suarez-Kurtz G, Pui CH, Stein CM et al (2019) Clinical Pharmacogenetics Implementation Consortium guideline for thiopurine dosing based on TPMT and NUDT15 genotypes: 2018 update. Clin Pharmacol Ther 105(5):1095–1105
46. Sicilia B, Arias L, Hontoria G, García N, Badia E, Gomollón F (2021) Are steroids still useful in immunosuppressed patients with inflammatory bowel disease? A retrospective, population-based study. Front Med (Lausanne) 8:651685
47. McLean LP, Cross RK (2014) Adverse events in IBD: to stop or continue immune suppressant and biologic treatment. Expert Rev Gastroenterol Hepatol 8(3):223–240
48. Román AL, Muñoz F (2011) Comorbidity in inflammatory bowel disease. World J Gastroenterol 17(22):2723–2733
49. Ansari A, Hassan C, Duley J, Marinaki A, Shobowale-Bakre EM, Seed P et al (2002) Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. Aliment Pharmacol Ther 16(10):1743–1750
50. van Bodegraven AA, Wijmenga C (2010) Chapter 45—genomic assessment of inflammatory bowel disease. In: Ginsburg GS, Willard HF (eds) Essentials of genomic and personalized medicine. Academic Press, San Diego, pp 575–589

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