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Identification of Patients With Variants in *TPMT* and Dose Reduction Reduces Hematologic Events During Thiopurine Treatment of Inflammatory Bowel Disease

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See editorial on page 850.

BACKGROUND & AIMS: More than 20% of patients with inflammatory bowel disease (IBD) discontinue thiopurine therapy because of severe adverse drug reactions (ADRs); leukopenia is one of the most serious ADRs. Variants in the gene encoding thiopurine S-methyltransferase (TPMT) alter its enzymatic activity, resulting in higher levels of thiopurine metabolites, which can cause leukopenia. We performed a prospective study to determine whether genotype analysis of *TPMT* before thiopurine treatment, and dose selection based on the results, affects the outcomes of patients with IBD. **METHODS:** In a study performed at 30 Dutch hospitals, patients were assigned randomly to groups that received standard treatment (control) or pretreatment screening (intervention) for 3 common variants of *TPMT* (*TPMT**2, *TPMT**3A, and *TPMT**3C). Patients in the intervention group found to be heterozygous carriers of a variant received 50% of the standard dose of thiopurine (azathioprine or 6-mercaptopurine), and patients homozygous for a variant received 0%–10% of the standard dose. We compared, in an intention-to-treat analysis, outcomes of the intervention (n = 405) and control groups (n = 378) after 20 weeks of treatment. Primary outcomes were the occurrence of hematologic ADRs (leukocyte count $< 3.0*10^9$/L or reduced platelet count $< 100*10^9$/L) and disease activity (based on the Harvey–Bradshaw Index for Crohn's disease [n = 356] or the partial Mayo score for ulcerative colitis [n = 253]). **RESULTS:** Similar proportions of patients in the intervention and control groups developed a hematologic ADR (7.4% vs 7.9%; relative risk, 0.93; 95% confidence interval, 0.57–1.52) in the 20 weeks of follow-up evaluation; the groups also had similar mean levels of disease activity ($P = .18$ for Crohn's disease and $P = .14$ for ulcerative colitis). However, a significantly smaller proportion of carriers of the *TPMT* variants in the intervention group (2.6%) developed hematologic ADRs compared with patients in the control group (22.9%) (relative risk, 0.11; 95% confidence interval, 0.01–0.85). **CONCLUSIONS:** Screening for variants in *TPMT* did not reduce the proportions of patients with hematologic ADRs during thiopurine treatment for IBD. However, there was a 10-fold reduction in hematologic ADRs among variant carriers who were identified and received a dose reduction, compared with variant carriers who did not, without differences in treatment efficacy. ClinicalTrials.gov number: NCT00521950.

Keywords: Leukocyte; Adverse Event; Pharmacogenetic; Side Effect.

Thiopurines are effective to induce and maintain long-term remission in up to 70% of patients with inflammatory bowel disease (IBD) (Crohn's disease [CD] and ulcerative colitis [UC]).[1] Azathioprine and 6-mercaptopurine are inactive prodrugs that need to undergo intracellular conversion to pharmacologically active 6-thioguanine nucleotides before exerting their cytotoxic action on (overactive) immune cells. Thiopurine S-methyltransferase (TPMT) metabolizes thiopurines to inactive metabolites, leaving less

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Abbreviations used in this paper: ADR, adverse drug reaction; CD, Crohn's disease; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HBI, Harvey–Bradshaw Index; IBD, inflammatory bowel disease; RBC, red blood cell; TOPIC, Thiopurine response Optimization by Pharmacogenetic testing in Inflammatory bowel disease Clinics; TPMT, thiopurine S-methyltransferase; UC, ulcerative colitis.

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parent drug to be metabolized to active 6-thioguanine nucleotides.[2,3] Approximately 10% of Caucasians carry a genetic variant in the *TPMT* gene, resulting in decreased TPMT enzyme activity and consequently higher 6-thioguanine nucleotide levels and a higher risk of potentially life-threatening myelosuppression during thiopurine treatment.[4]

More than 20% of IBD patients discontinue thiopurine treatment owing to (serious) adverse drug events.[5,6] Current guidelines for thiopurine treatment mandate regular hematologic monitoring to detect (severe) myelotoxicity, most commonly presenting as leukopenia and to a lesser extent as thrombocytopenia.[1] However, this is not a complete safeguard because myelotoxicity can develop suddenly at any time point during treatment, and patients with bone marrow suppression have a higher cumulative incidence of infections, mortality, and death.[1,7] This underscores the importance of treating patients as safely as possible (ie, based on genotype) from treatment start. Pharmacogenetic testing for *TPMT* has been advocated for a long time to optimize the safety of thiopurine treatment, but clinical use of pretreatment *TPMT* testing has been low, and effectiveness data are lacking.[8] To date, 2 TPMT-related, randomized, controlled trials have been performed, one including patients with a range of inflammatory conditions, but mainly IBD (85% of the patients included), the other study including 29 IBD patients. Definitive conclusions could not be drawn from either study.[9,10] A recent meta-analysis (n = 4306 patients) suggested that IBD patients with decreased TPMT activity are indeed at increased risk of developing leukopenia compared with patients with normal TPMT activity.[3]

In general, pharmacogenetic testing to optimize treatment is applied only on a limited scale in clinical practice to date because large-scale, randomized, controlled trials proving the effectiveness of available tests largely are lacking.[11–13] This also is hampering for the clinical uptake of *TPMT* testing before thiopurine treatment. In this randomized controlled trial (Thiopurine response Optimization by Pharmacogenetic testing in Inflammatory bowel disease Clinics [TOPIC]), we investigated whether pretreatment *TPMT* genotyping followed by personalized dosing results in a reduced incidence of hematologic adverse drug reactions (ADRs). In addition, we evaluated the influence of this safety optimizing strategy on clinical outcome and other ADRs.

Materials and Methods

Patients

Patients were assessed for eligibility by their gastroenterologist. Patients who met the inclusion criteria were assigned randomly to pretreatment *TPMT* genotyping (intervention group) or standard treatment (control group). Inclusion criteria were as follows: age older than 18 years and a diagnosis of IBD. Exclusion criteria were as follows: previous use of azathioprine or 6-mercaptopurine, co-treatment with allopurinol, leukocyte count less than $3.0*10^9$/L, liver test abnormalities (liver enzyme levels [alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and/or γ-glutamate transpeptidase] $\geq$2 times normal upper limit), reduced renal function (creatinine serum level $\geq$2 times normal upper limit), known TPMT enzyme activity or genotype, and current pregnancy.

The study protocol was approved by the Institutional Review Board of the Radboud university medical center (Commissie Mensgebonden Onderzoek Regio Arnhem Nijmegen, protocol number: 13171), approval for inclusion of patients in other institutes was obtained from institutional ethics committee. All patients provided written informed consent. This study is registered at clinicaltrials.gov (NCT00521950).

Study Design

Thirty Dutch hospitals participated in the parallel randomized controlled trial. Patients were enrolled by the TOPIC recruitment team (see Acknowledgment section for a list of TOPIC collaborators). Randomization was based on a computer-generated schedule per participating center with a block size of 4 (developed by C.J.v.M.). Gastroenterologists and patients were blinded to randomization. All authors had access to the study data and have reviewed and approved the final manuscript.

Blood samples for *TPMT* genotyping and enzyme measurements were collected from every patient before treatment initiation, and were numbered upon arrival at the laboratory. Only patients assigned to the intervention group underwent pretreatment testing for 3 common *TPMT* variants (TPMT*2 [238G>C (rs1800462)], TPMT*3A [460G>A (rs1800460) and 719A>G (rs1142345)], and TPMT*3C [rs1142345]), accounting for approximately 95% of the variant alleles observed in Caucasians.[5,14,15] The turn-around time for genotyping results (intervention group) and dosing advice (all patients) was 5 working days. Patients in the control group and patients who did not carry a *TPMT* variant were treated according to standard IBD guidelines (2–2.5 mg/kg/day azathioprine or 1–1.5 mg/kg/day 6-mercaptopurine). Patients in the intervention group who carried a genetic variant received 50% (heterozygotes) or 0%–10% (homozygotes) of the standard thiopurine dose according to the evidence-based guidelines of the Dutch Pharmacogenetics Working Group.[16] For all patients (intervention and control groups) a letter containing the dose advice was sent to the gastroenterologist. The majority of the patients (n = 705; 90%) received advice for the standard dose according to the Dutch guidelines. The study was not blinded. Gastroenterologists were allowed to change the thiopurine dose or stop treatment when a side effect occurred. The following guidelines were provided: consider a dose reduction by a count of $4*10^9$/L or less and a fast decrease of leukocyte count, dose reduction of 50% by a leukocyte count of $3*10^9$/L or less, and treatment stop by a leukocyte count of less than $1*10^9$/L. Treatment re-challenge was at the discretion of the gastroenterologist.

The primary outcome of the study was the development of a hematological ADR. Secondary outcomes based on blood levels were signs of hepatotoxicity, pancreatitis, or anemia. Secondary outcomes reported by clinicians included general side effects (dizziness, shivers, fever, general malaise), gastrointestinal side effects (stomach ache, diarrhea, reduced appetite, nausea, and vomiting), hepatic side effects (cholestasis, cholangitis, hepatitis, and steatosis), dermatological side-effects (hair loss, warts, and skin rash), myalgia, and arthralgia. Included patients were followed up for 20 weeks after thiopurine treatment initiation.

Blood for biochemical measurements was collected at least 1 week before study start and at weeks 1, 2, 4, 6, 8, and 20. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) levels were measured before treatment start and at 20 ± 6 weeks. Leukocytes, thrombocytes, hemoglobin, hematocrit, mean corpuscular volume, liver enzymes (alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and γ-glutamate transpeptidase), and amylase or lipase were measured at every visit. At week 8, blood samples were collected for 6-thioguanine nucleotide and 6-methylmercaptopurine ribonucleotide metabolite measurement; metabolites were assessed after follow-up evaluation.

Clinical evaluation was performed before study start and at week 20 ± 6 weeks to determine disease location and activity. During the follow-up period, clinical information (complications and changes in treatment such as changes of azathioprine/6-mercaptopurine dose or co-medication) was collected when patients had contact with the gastroenterologist, timing and intervals of which were at the discretion of the clinician. Patients received questionnaires concerning disease activity (number of [liquid] stools, abdominal pain, fever, use of antidiarrheal medication, general well-being) 1 week before treatment initiation and at week 20. These data, in combination with clinical measures, were used to calculate the disease activity (Harvey–Bradshaw Index [HBI] for CD and partial Mayo for UC). For the HBI, we used abdominal mass measured before treatment initiation in case this measure was missing at 20 weeks.

Genotyping

Genetic analysis was performed in a ISO15189-accredited laboratory (Human Genetics Department, Radboud university medical center, Nijmegen, The Netherlands). Genotyping of 3 common *TPMT* variants (TPMT*2, TPMT*3A, and TPMT*3C, UCSC Genome Browser [genome.ucsc.edu] accession number: NM_000367.3) was performed using TaqMan SNP genotyping assays according to the manufacturer protocol (Life Technologies, Bleiswijk, The Netherlands: rs1800462: assay-ID:C__12091552_30; rs1800460: assay-ID:C__30634116_20; and rs1142345: assay-ID:C____19567_20). Signals were detected with the 7500 Fast Real-Time Polymerase Chain Reaction System (Life Technologies) and subsequently analyzed using Allelic Discrimination software version 1.4 (Life Technologies). All patients in the intervention arm were genotyped in triplicate. Patients assigned to the control group were genotyped in one batch in duplicate after the follow-up period of 20 weeks. Each genotyping experiment contained at least 4 positive controls for each *TPMT* variant. Five percent of samples were genotyped in duplicate (within or between plates); all genotypes were concordant. Sequencing of the protein coding part of the *TPMT* gene was performed in a subset of patients (Supplementary Table 1).

Enzyme and Metabolite Measurements

TPMT enzyme activity and thiopurine metabolites were assessed after the follow-up period had ended. Blood for TPMT enzyme activity measurements was collected before treatment initiation and stored at -80°C until further processing. Enzyme activity was measured in red blood cells (RBCs) using a high-performance liquid chromatography method described previously.[17]

Blood samples for 6-thioguanine nucleotide and 6-methylmercaptopurine ribonucleotide measurement were stored immediately at 2°C–8°C and sent to the Department of Clinical Pharmacy and Toxicology (Orbis Medical Centre, Sittard-Geleen, The Netherlands), where samples were processed and stored at -20°C until required. 6-Thioguanine nucleotides and 6-methylmercaptopurine ribonucleotides levels were determined with a modified high-performance liquid chromatography method as published previously.[18] Lower limits of quantification for 6-thioguanine nucleotide and 6-methylmercaptopurine ribonucleotide metabolite levels were 40 pmol/8 × 10^8 RBCs and 300 pmol/8 × 10^8 RBCs, respectively. Interassay variability for both 6-thioguanine nucleotides and 6-methylmercaptopurine ribonucleotides was less than 10%.

Statistical Analysis

The study was designed to have 80% power with inclusion of 388 patients per treatment arm and a reduction in hematologic ADR rate of 50% (hematologic ADR rate of 11% in the nongenotyped group and 5.5% in the genotyped group; 2-sided *P* value threshold was .05).[5]

The data set was analyzed on an intention-to-treat basis after exclusion of patients who were lost to follow-up evaluation (Supplementary Figure 1). Differences in baseline variables and ADRs between patients grouped as intervention or control were assessed using the Pearson X^2 test, the Fisher exact test, the Student *t* test, the Mann–Whitney *U* test, or an independent sample Kruskal–Wallis test, as appropriate. Hardy–Weinberg equilibrium was assessed using a X^2 test. Primary outcomes of the study were as follows: (1) occurrence of hematologic ADRs defined as a leukocyte count of 3.0*10^9/L or less (which is indicative of an increased risk for serious systemic infections) within the follow-up period of 20 ± 6 weeks, or platelet count less than 100*10^9/L; and (2) clinical outcome based on disease activity scores. Secondary outcomes were the occurrence of other (severe) ADRs. Post hoc comparisons for patients with and without a variant between the intervention and control groups were performed for the primary outcome hematologic ADRs using the Pearson X^2 test. Analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (release 20.0.0.1; IBM Corp, Armonk, NY).

Results

Patients were included from October 2007 until December 2010 and followed up for a period of 20 weeks; 796 eligible patients were randomized (Supplementary Figure 1). Final analyses included 405 patients from the intervention group and 378 patients from the control group (Supplementary Figure 1). Baseline characteristics of the intervention and control groups did not show statistically significant differences (Table 1 and Supplementary Table 2) except for the biologics used as co-medication at the study start (intervention group, 3.7%; control group, 7.4%; P = .027). Steroid use, the main co-medication for patients with IBD, was similar for both groups during follow-up evaluation (Supplementary Table 3). Overall, the thiopurine dose was similar for the intervention and control groups (Supplementary Table 4). Fifty-five (13.6%) patients in the

Table 1. Characteristics of the Study Population

	Total	Intervention group	Control group
Total	783 (100%)	405 (100%)	378 (100%)
Male, n	354 (45.2%)	186 (45.9%)	168 (44.4%)
Age, *y* (SD)	41.0 (15.8)	41.6 (15.9)	40.5 (15.8)
Weight, *kg* (SD)	74.3 (16.2)[a]	73.9 (16.3)	74.7 (16.2)
Age of disease onset, *y* (SD)	35.7 (15.1)[a]	36.3 (15.4)	35.0 (14.8)[b]
Disease duration until treatment start, median (minimum–maximum), *y*	1.2 (0–49.7)[a]	1.3 (0–45.0)	1.1 (0–49.7)[b]
Medication, n			
Azathioprine	503 (64.2%)	256 (63.2%)	247 (65.3%)
6-mercaptopurine	279 (35.6%)	148 (36.5%)	131 (34.7%)
None started[c]	1 (0.1%)	1 (0.3%)	0 (0%)
Drug dose start, *mg/kg*			
Azathioprine[c]	2.0 (0–3.1)	2.1 (0–2.7)	2.2 (0–3.1)
6-mercaptopurine	1.1 (0–2.2)	1.2 (0–2.2)	1.2 (0–2.0)
Drug dose 20 weeks, *mg/kg*			
Azathioprine	2.1 (0.5–3.1)[d]	2.1 (0.5–2.7)[e]	2.2 (0.6–3.1)[f]
6-mercaptopurine	1.0 (0.3–1.5)[g]	1.1 (0.3–1.5)[h]	1.1 (0.4–1.5)[h]
Co-medication, n			
Corticosteroids	640 (81.7%)	336 (83.0%)	304 (80.4%)
Mesalamine	388 (49.6%)	198 (48.9%)	190 (50.3%)
Biologicals	43 (5.5%)	15 (3.7%)	28 (7.4%)
TPMT genotype, n			
*1/*1	705 (90.0%)	365 (90.1%)	340 (89.9%)
*1/*2	7 (0.9%)	4 (1.0%)	3 (0.8%)
*1/*3A	58 (7.4%)	31 (7.7%)	27 (7.1%)
*1/*3C	12 (1.5%)	4 (1.0%)	8 (2.1%)
*3A/*3A	1 (0.1%)	1 (0.2%)	0 (0%)
Baseline ESR	15 (1–109)[i]	14 (1–109)[j]	15 (1–102)[k]
CD patients	16 (1–109)[l]	16 (1–109)[m]	16 (1–102)[n]
UC patients	12.5 (1–95)[o]	14 (1–95)[p]	10 (2–85)[q]
Increased baseline ESR	270 (42.7%)[i]	141 (42.1%)[j]	129 (43.3%)[k]
CD patients	176 (46.1%)[l]	91 (44.4%)[m]	85 (48.0)[n]
UC patients	90 (37.2%)[o]	50 (39.1%)[p]	40 (35.1%)[q]
Baseline CRP	8 (0–284)[r]	8 (0.6–214)[s]	7 (0–284)[t]
CD patients	9 (0.6–284)[u]	8 (0.6–91)[v]	10 (0.6–284)[w]
UC patients	6 (0–214)[x]	7 (1–214)[y]	5 (0–180)[z]
Increased baseline CRP	277 (37.4%)[aa]	144 (37.4%)[bb]	133 (37.5%)[cc]
CD patients	194 (43.2%)[dd]	96 (40.9%)[ee]	98 (45.8%)[ff]
UC patients	78 (27.8%)[gg]	48 (32.7%)[hh]	30 (22.4%)[ii]

NOTE. Table data show means (SD), n (percentage), or medians (minimum–maximum) for disease duration.
[a]n = 781. [b]n = 376. [c]Patient was homozygous for a *TPMT* variant and did not start thiopurine medication in agreement with therapeutic recommendations, this patient was included in the azathioprine group for start dose because this was the medication planned. Other patients who did not start medication also were included in the planned medication group. [d]n = 323. [e]n = 162. [f]n = 161. [g]n = 208. [h]n = 104. [i]n = 633. [j]n = 335. [k]n = 298. [l]n = 382. [m]n = 205. [n]n = 177. [o]n = 242. [p]n = 128. [q]n = 114. [r]n = 564. [s]n = 294. [t]n = 270. [u]n = 356. [v]n = 185. [w]n = 171. [x]n = 199. [y]n = 107. [z]n = 92. [aa]n = 740. [bb]n = 385. [cc]n = 355. [dd]n = 449. [ee]n = 235. [ff]n = 214. [gg]n = 281. [hh]n = 147. [ii]n = 134.

intervention group and 41 (10.8%) patients in the control group did not start with the advised dose, all but 1 patient started with a lower dose (Supplementary Figure 1 and Supplementary Table 5). Two patients (1 each in the intervention and control groups) started treatment before the dose was provided and 12 patients (5 in the intervention group and 7 in the control group) did not start treatment at the planned time point. In addition, 1 patient in this study was homozygous for a *TPMT* variant and did not start treatment according to the dose advice of 0–10% of the standard thiopurine dose. The azathioprine starting dose was not different between the intervention and control groups, but a significant difference was observed in the 6-mercaptopurine starting dose (Supplementary Table 4) ($P = .045$), this could be attributed to our intervention because the dose difference was evident only in patients with a genetic variant in *TPMT* ($P < .004$), patients without a variant were started on similar 6-mercaptopurine doses as patients in the control group ($P = .27$). Thiopurine treatment was discontinued at similar rates in the intervention (n = 170; 42.0%) and control (n = 143; 37.8%) groups; 266 (65.7%) and 262 (69.3%) of the patients were using thiopurines for up to 20 weeks in the intervention and control groups, respectively (Supplementary Table 4).

Table 2. Overview of the Primary and Secondary Adverse Effects That Occurred in the Study Population

	Total population		Intervention group		Control group		
	n (%)	N total	n (%)	N total	n (%)	N total	RR (95% CI)
Primary outcome							
Hematologic ADR	58 (7.5)	783	30 (7.4)	405	30 (7.9)	378	0.93 (0.57–1.52)
Secondary outcomes based on blood levels							
Signs of hepatotoxicity	203 (26.6)	762	106 (26.7)	397	98 (25.9)	371	1.01 (0.80–1.28)
Signs of pancreatitis	187 (25.0)	749	106 (27.2)	389	84 (22.2)	365	1.18 (0.92–1.52)
Signs of anemia	474 (62.1)	763	246 (61.8)	398	231 (61.1)	371	0.99 (0.89–1.11)
Secondary outcomes reported by clinicians							
General	324 (41.4)	783	161 (39.8)	405	163 (43.1)	378	0.92 (0.78–1.09)
Dizziness	125 (16.0)	783	59 (14.6)	405	66 (17.5)	378	0.83 (0.60–1.15)
Shivers	67 (8.6)	783	35 (8.6)	405	32 (8.5)	378	1.02 (0.65–1.61)
Fever	104 (13.3)	783	57 (14.1)	405	47 (12.4)	378	1.13 (0.79–1.62)
General malaise	213 (27.2)	783	109 (26.9)	405	104 (27.5)	378	0.98 (0.78–1.23)
Gastrointestinal	559 (71.4)	783	290 (71.6)	405	269 (71.2)	378	1.01 (0.92–1.10)
Stomach ache	395 (50.4)	783	205 (50.6)	405	190 (50.3)	378	1.01 (0.88–1.16)
Diarrhea	235 (30.0)	783	123 (30.4)	405	112 (29.6)	378	1.03 (0.83–1.27)
Reduced appetite	160 (20.4)	783	82 (20.2)	405	78 (20.6)	378	0.98 (0.74–1.29)
Nausea	317 (40.5)	783	160 (39.5)	405	157 (41.5)	378	0.95 (0.80–1.13)
Vomiting	120 (15.3)	783	63 (15.6)	405	57 (15.1)	378	1.03 (0.74–1.43)
Infections	30 (3.8)	783	13 (3.2)	405	17 (4.5)	378	0.71 (0.35–1.45)
Hepatic	54 (6.9)	783	27 (6.7)	405	27 (7.1)	378	0.93 (0.56–1.56)
Cholestasis	16 (2.0)	783	8 (2.0)	405	8 (2.1)	378	0.93 (0.35–2.46)
Cholangitis	3 (0.4)	783	1 (0.2)	405	2 (0.5)	378	0.47 (0.04–5.13)
Hepatitis	41 (5.2)	783	21 (5.2)	405	20 (5.3)	378	0.98 (0.54–1.78)
Steatosis	3 (0.4)	783	0 (0)	405	3 (0.8)	378	0.13 (0.01–2.57)
Dermatologic	171 (21.8)	783	83 (20.5)	405	88 (23.3)	378	0.88 (0.68–1.15)
Hair loss	52 (6.6)	783	26 (6.4)	405	26 (6.9)	378	0.93 (0.55–1.58)
Warts	9 (1.1)	783	5 (1.2)	405	4 (1.1)	378	0.93 (0.32–4.31)
Skin rash	136 (17.4)	783	65 (16.0)	405	71 (18.8)	378	0.85 (0.63–1.16)
Myalgia	114 (14.6)	783	62 (15.3)	405	52 (13.8)	378	1.12 (0.79–1.57)
Arthralgia	132 (16.9)	783	70 (17.3)	405	62 (16.4)	378	1.05 (0.77–1.44)

NOTE. The following reference values were used for the side effects based on blood levels: hematologic ADR: leukocyte count $\leq 3.0*10^9$/L and/or platelet count $<100*10^9$/L; signs of hepatotoxicity: at least 1 liver enzyme (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, and/or γ-glutamate transpeptidase) more than 2$\times$ the upper limit reference value; and signs of pancreatitis: amylase and/or lipase blood level higher than the reference value. Fourteen patients (7 in each group) with signs of pancreatitis developed pancreatitis on the initially started thiopurine. Pancreatitis is defined as the presence of 2 of 3 criteria for pancreatitis (amylase or lipase levels more than 3$\times$ the upper limit reference value, stomach ache, or radiologic discrepancies). Signs of anemia were a hemoglobin level lower than the reference value.
Clinicians scored the patients for the presence of ADRs, and these were scored as present or absent on case report forms during every hospital visit of the patients. A patient was included once, in case a specific ADR was reported more than one time. In the overall groups (general, gastrointestinal, hepatic, and dermatologic side effects) presented in the table, patients might be counted more than once if they had more than one ADR in the specific group.
CI, confidence interval; RR, relative risk.

ADR frequencies in the first 20 weeks after thiopurine initiation are summarized in Table 2. The main outcome measure of our study, hematologic ADRs (leukocyte count $\leq 3.0*10^9$/L or platelet count $<100*10^9$/L), was observed in 30 patients in the intervention and control groups. Overall, no significant differences in ADR frequencies were observed between both groups (Table 2). Two patients died. One patient was a heterozygous TPMT*3A carrier allocated to the intervention group, starting treatment according to dose advice (1.2 mg/kg), in addition to using mesalamine and prednisone. CRP was increased from 6 to 29 mg/L and the leukocyte count decreased from $5.1*10^9$/L to $3.6*10^9$/L in the 3 days before treatment initiation. Three days after treatment initiation the leukocyte count had decreased to $2.9*10^9$/L, and the patient died from leukopenia resulting from *Escherichia coli* sepsis with pneumonia. The other patient (without a TPMT variant) started azathioprine in addition to infliximab and corticosteroids. The leukocyte count decreased to $2.5*10^9$/L on day 16, and azathioprine treatment was stopped. From that moment on the patient used prednisone, methotrexate, and infliximab as immunosuppressive treatment. On day 27 the patient was hospitalized with *Pneumocystis carinii* pneumonia (leukocyte count, $8.0*10^9$/L) and died on day 48.

Observed allele frequencies were comparable with reported frequencies (Table 1).[15] One person (0.1%) was homozygous for *TPMT**3A and 77 patients (9.8%) were heterozygous carriers of a *TPMT* variant. Enzyme activity measurements showed that patients carrying a genetic variant had a lower TPMT enzyme activity than patients without a variant (Supplementary Figure 2). Twelve patients without one of the pretested variants had low TPMT enzyme activity (<60 mg 6-methylguanine/mmol hemoglobin per hour), one of the patients developed leukopenia. Complete sequencing of the *TPMT* gene coding region showed a known silent variant (rs2842934; Ile158Ile, TPMT*1S) in 4 of the 12 patients.[19] Only 1 of these patients developed a hematologic ADR.

Thirty patients in each group developed a hematologic ADR, the majority, 29 in each group, developed leukopenia. A reduced platelet count was observed in 3 patients (2 patients also developed leukopenia) in the intervention group and in 2 patients (1 patient also developed leukopenia) in the control group. The intention-to-treat analysis showed no difference in the occurrence of hematologic ADRs between the intervention and control groups (7.4% vs 7.9%; relative risk, 0.93; 95% confidence interval, 0.57–1.52) (Table 2). An analysis excluding patients on biologicals was performed because biological use differed between the intervention and control groups at baseline. This did not show any difference in hematologic ADRs between both groups (7.4% vs 6.7%). Limiting the analysis to only those patients who actually started treatment showed similar results (7.5% vs 8.1%). In addition, we did not observe significant differences in the median time to a hematologic ADR (in those patients who developed an ADR) between the intervention (42 days; interquartile range, 69 days) and control (56 days; interquartile range, 58 days) groups, the number of patients who developed an ADR in the first 8 weeks did not differ between groups (18 in each group). Post hoc analysis of the subgroup of patients carrying a *TPMT* variant (*2, *3A, or *3C) , which included only those patients who started treatment at the study start, showed that a personalized dose regimen based on pretreatment genotyping resulted in a statistically significant decrease in hematologic ADR occurrence ($P = .011$; relative risk, 0.11; 95% confidence interval, 0.01–0.85) (Table 3). In the intervention group only 1 of 39 patients carrying a *TPMT* variant (2.6%) developed a hematologic ADR compared with 8 of 35 patients in the control group (22.9%). Analysis of the subset of patients with a genetic variant who had not received biologicals (n = 69) also showed fewer instances of hematologic ADRs ($P = .011$). No difference in the occurrence of hematologic ADRs between the intervention and control groups was found for the patients without a *TPMT* variant ($P = .47$) (Table 3).

Several approaches were used to investigate whether a thiopurine dose reduction in the *TPMT* variant carriers in the intervention arm resulted in effective treatment. First, the disease activity was assessed. We did not observe statistically significant differences in clinical outcome (disease activity) between both groups at baseline in an intention-to-treat analysis ($P = .13$ for HBI; $P = .83$ for partial Mayo) and 20 weeks after treatment initiation ($P = .18$ for HBI; $P = .14$ for partial Mayo). A decrease in the median disease activity scores after 20 weeks was observed in both treatment groups, and in patients with and without the genetic variant (Figure 1). Both groups also showed similar rates of clinical remission (Supplementary Table 6). To assess treatment efficacy the change in ESR and CRP between treatment start and 20 weeks was evaluated (Supplementary Table 6). This showed a statistically significant difference for the absolute ESR change in patients with a genetic variant ($P = .042$, for the benefit of patients in the intervention group). Besides clinical outcome we also evaluated treatment efficacy by measuring 6-thioguanine nucleotide and 6-methylmercaptopurine ribonucleotide metabolite levels at week 8. This was performed to investigate whether thiopurine dose reduction in *TPMT* variant carriers in the intervention arm resulted in effective treatment (Figure 2 and Supplementary Figure 3). Comparison of patients with a *TPMT* variant showed that a reduced thiopurine dose resulted in 6-thioguanine nucleotide levels within the therapeutic range, whereas a standard dose resulted in clearly increased 6-thioguanine nucleotides levels (Figure 2*A*). In addition, 6-thioguanine nucleotides and 6-methylmercaptopurine ribonucleotide concentrations in patients without a genetic variant did not differ between the intervention and control

Table 3. Secondary Analysis: Hematologic ADR Occurrence in the Intervention and Control Groups

	Intervention	Control	RR (95% CI)
Total, n	399	370	
Hematologic ADR			
Total	29 (7.2%)	29 (7.8%)	
TPMT variant carriers	1 of 39 (2.6%)[a]	8 of 35 (22.9%)	0.11 (0.01–0.85)
No *TPMT* variant	29 of 360 (8.1%)	22 of 335 (6.6%)	1.2 (0.72–2.09)

NOTE. No deviations from Hardy–Weinberg equilibrium were observed (238G>C, $P = .9$; 460G>A, $P = .93$; 719A>G, $P = .67$). The patient homozygous *TPMT*3A* was randomized to the intervention group, the other patients in the intervention group were heterozygous for *TPMT*2* (n = 4), *TPMT*3A* (n = 30), or *TPMT*3C* (n = 4). The following genotypes were observed in the control group, all patients were heterozygous, *TPMT*2* (n = 3), TPMT*3A (n = 26), and *TPMT*3C* (n = 6).
CI, confidence interval; RR, relative risk.
[a]Patient died from leukopenia caused by *E coli* sepsis with pneumonia 3 days after thiopurine treatment start.

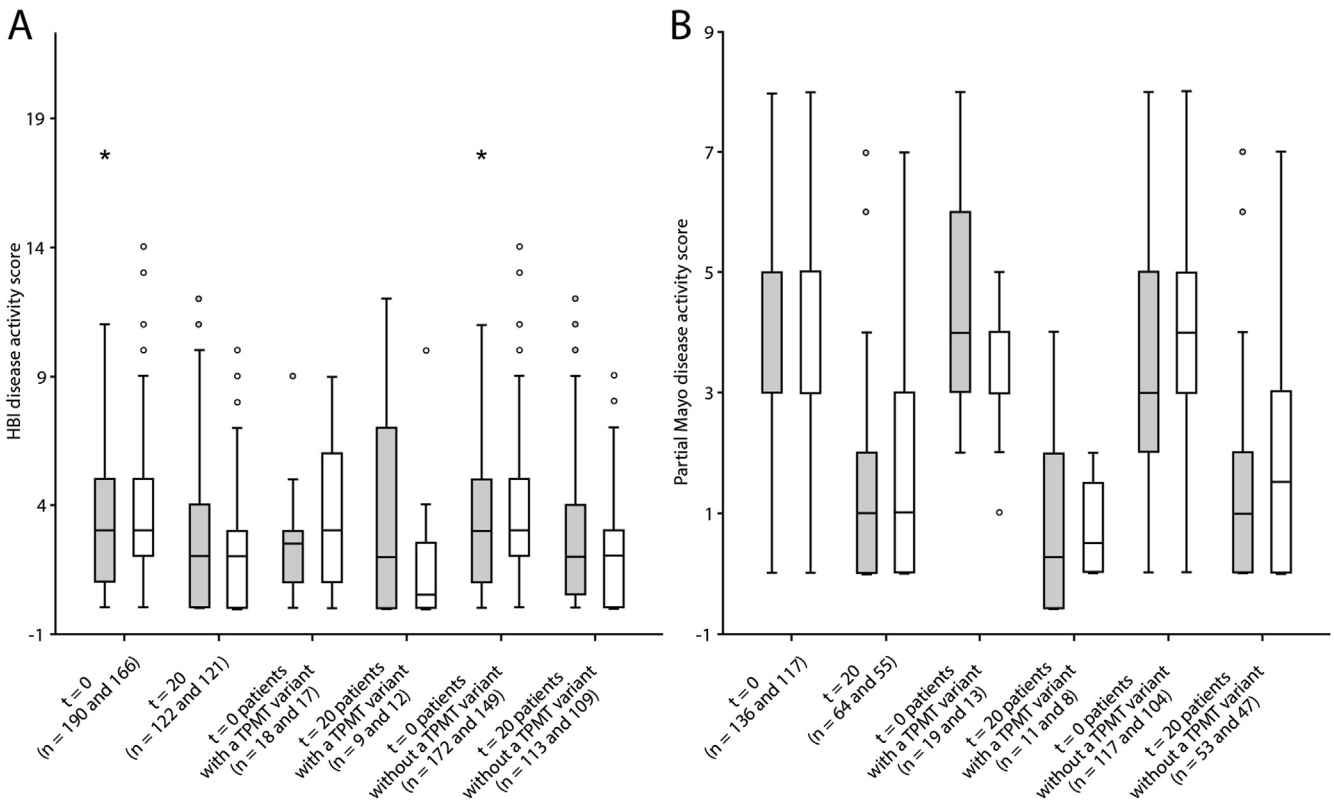

Figure 1. Box-plots for disease activity scores for patients with (*A*) Crohn's disease (HBI) and (*B*) ulcerative colitis (partial Mayo). Disease activity scores are shown for the intervention (*grey bars*) and control (*white bars*) groups. The HBI can range from 0 to 19 and the partial Mayo score can range from 0 to 9, a higher score means higher disease activity. The numbers indicated for each set of boxes indicates the number of patients for the intervention and control groups, respectively. The *boxes* indicate the 25th to 75th percentiles and the medians are indicated by a *horizontal line* in the box. *Whiskers* indicate the 1.5 interquartile range. *Open circle* indicates outliers (>1.5 interquartile range), and extreme outliers (>3 interquartile range) are indicated by an *asterisk*. The x-axis indicates the number of patients analyzed.

groups, indicating that both groups were equally adherent to treatment. The highest 6-methylmercaptopurine ribonucleotides concentrations were, as expected, observed in patients without a genetic *TPMT* variant (Figure 2*B*), followed by patients with a variant on standard thiopurine dose (control group); lowest levels were observed in patients treated with a reduced thiopurine dose. Six patients (1 in the intervention and 5 in the control group) had undetectable metabolite levels at week 8 after thiopurine initiation, suggesting noncompliance.

We also explored whether in addition to group allocation other baseline factors (co-medication, sex, age, and weight) were associated with the development of a hematologic ADR. We observed more hematologic ADRs in patients using biologics ($P = .002$).

Discussion

The TOPIC trial, a large randomized controlled trial studying the effect of *TPMT* genotyping before thiopurine treatment in IBD patients, showed no significant difference in the risk of a hematologic ADR or treatment efficacy between the intervention and control groups. Post hoc analysis indicated that TPMT screening significantly reduced the risk of a hematologic ADR in the subgroup of patients with a genetic variant.

Forty percent of the patients discontinued thiopurine treatment because of adverse effects, which is relatively high compared with previous reports.[6] Taking the patients with a successful re-challenge into account, we observed drop-out rates consistent with those in the literature. Despite the high discontinuation rate, we did not observe increased frequencies of hematologic ADRs in our population.[1] Thus, the TOPIC trial accurately reflects the general IBD population treated with thiopurines.

We could not show a difference in the risk for occurrence of a hematologic ADR between the intervention and control groups. Other ADRs commonly observed in patients treated with thiopurines also showed comparable frequencies in the 2 groups. This latter finding was in line with expectations, because, for example, hepatotoxicity, malaise, and pancreatitis do not seem to be linked to low TPMT activity, as a meta-analysis of 1309 patients confirmed.[20] The meta-analysis, however, showed a higher rate of bone marrow toxicity and overall ADR development (ie, all ADRs that required dose reduction).

A subgroup analysis in patients with a variant in the *TPMT* gene showed that the intervention strongly reduced

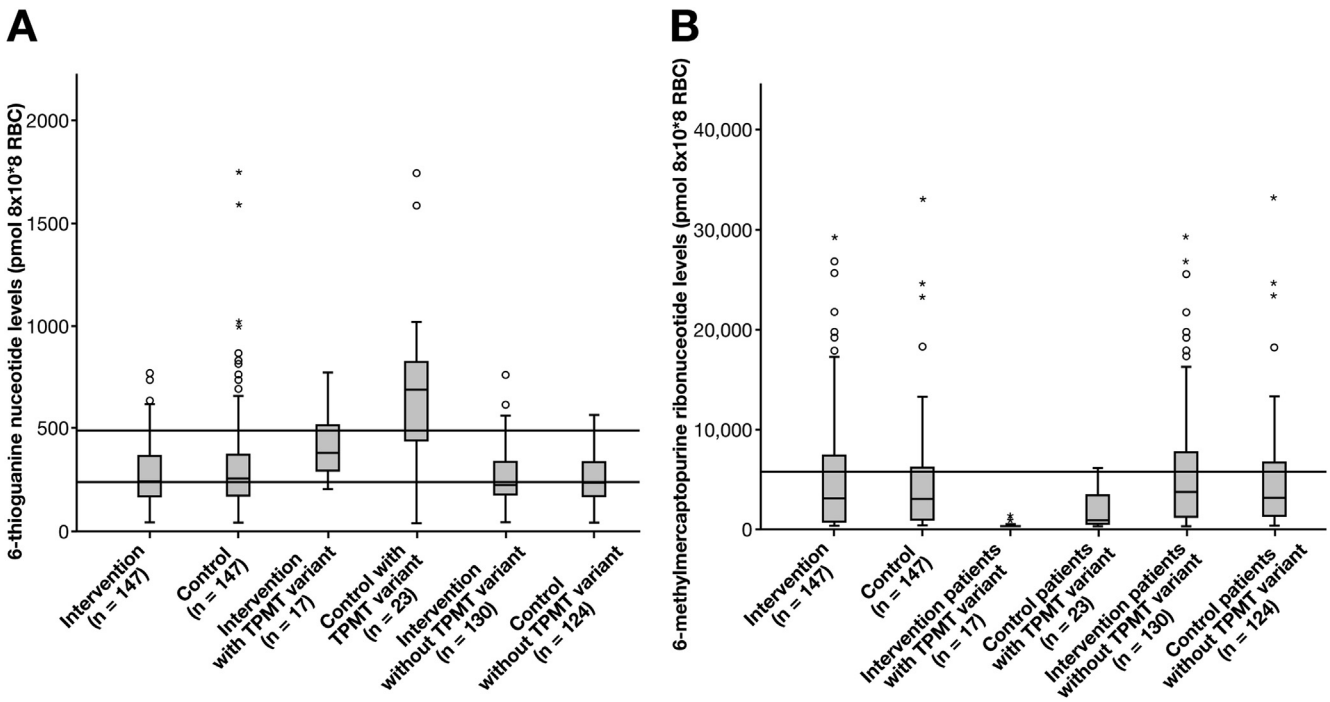

Figure 2. Box-plots for steady-state metabolite levels of (*A*) 6-thioguanine nucleotides and (*B*) 6-methylmercaptopurine ribonucleotides in pmol/8*10^8 red blood cells measured at 8 weeks of treatment. The therapeutic range of 6-thioguanine nucleotide metabolites (235 and 490 pmol/8 $\times$ 10^8 red blood cells) and normal range levels of 6-methylmercaptopurine ribonucleotides (<5700 pmol/8*10^8 red blood cells) are indicated with *horizontal lines* in panels *A* and *B*, respectively. The *boxes* indicate the 25th to 75th percentiles, and the medians are indicated by a *horizontal line* in the box. *Whiskers* indicate the 1.5 interquartile range. *Open circle* indicates outliers (>1.5 interquartile range), and extreme outliers (>3 interquartile range) are indicated by an *asterisk*. The number of patients analyzed are indicated on the x-axis. A statistically significant difference in 6-thioguanine nucleotides ($P = .004$) and 6-methylmercaptopurine ribonucleotides ($P < .001$) was observed between the intervention and control groups for patients carrying a *TPMT* variant. Similar metabolite levels were observed when excluding patients who had a dose change before 8 weeks of thiopurine therapy (Supplementary Figure 3).

hematologic ADR frequency from 22.9% to 2.6%. Our data confirmed the meta-analysis including 47 mainly retrospective studies, which showed an association between decreased TPMT enzyme activity (based on genotype or phenotype) and an increased risk for leukopenia.[3] In agreement with previous reports, the TOPIC trial also showed that pretreatment *TPMT* genotyping cannot prevent all cases of thiopurine-related hematologic ADRs, suggesting that other factors play a role in the development of these ADRs.[21] The results also show that patient *TPMT* enzyme activity measurements would not have identified the other patients with leukopenia (1 patient of 11 with low enzyme activity developed leukopenia). It is possible that patients with leukopenia carry genetic variants in TPMT other than those analyzed.[15] However, sequencing of *TPMT* for 11 patients with the low TPMT enzyme activity who were negative for the 3 common *TPMT* variants did not show other functional mutations, indicating that sequencing the complete TPMT gene would not identify additional patients at risk for leukopenia. Several studies have suggested that genetic variants in other genes in thiopurine metabolism are associated with thiopurine-induced leukopenia.[14,22] A few studies have shown that analyzing variants in 2 or more genes involved in thiopurine metabolism, including *TPMT*, may enhance the prediction of leukopenia,[14,23–26] but further large-scale studies are warranted. Besides genetic factors, viral infections during thiopurine treatment may induce the development of leukopenia.[21] Finally, co-medication might make patients more susceptible to leukopenia.[27] For this reason, we excluded patients receiving allopurinol as a co-medication.[28] However, other commonly used treatments for IBD (eg, sulfasalazine and mesalamine) also are implicated in the development of leukopenia.[29,30] And in this study, we have shown that concurrent biologics' use at treatment initiation is linked to hematological ADR development. These nongenetic factors should be taken into consideration before thiopurine initiation because they might interfere with the genotype-guided dosing. We observed a difference in biologics use at baseline between the intervention and control groups. Therefore, we also performed an analysis in patients who did not receive biologics. In this group we also showed that pretreatment genotyping resulted in a lower occurrence of hematologic ADRs. We concluded that concurrent biologics use does not interfere with the predictive ability of pretreatment *TPMT* genotyping for hematologic ADRs.

Importantly, similar treatment efficacy based on disease activity scores was observed in the intervention and control groups, which indicates that a reduced thiopurine dose does not result in undertreatment. The same was evident at the active metabolite level, in which patients receiving a genotype-guided thiopurine dose-reduction had a median

steady-state 6-thioguanine nucleotide level within the therapeutic range.[31–33]

The results of the TOPIC study indicated that 200 patients would need to be genotyped to avoid 1 episode of a hematologic ADR (7.4% vs 7.9%; ie, 0.5% risk difference). The number needed to treat to avoid one episode of a hematologic ADR would be 5 for at-risk individuals (risk difference in patients with a genetic variant is 20.3; 2.6% vs 22.9%). The huge difference between the number needed to genotype and the number needed to treat can be attributed to the low frequency of the screened genetic variants in *TPMT* (~10%). This nicely illustrates the difficulty in trying to use whole-population randomized studies to investigate the effectiveness of pharmacogenetic testing: the high-risk genotype constitutes a small proportion of the population (here 10%), which makes it extremely hard to show a benefit for all patients; only a portion of the population benefits. Post hoc power analysis indeed showed that the subgroup analysis was powered sufficiently (80% power with 38 patients showing hematologic ADRs), but that a randomized controlled trial with 42,556 participants would be needed to show a benefit for the entire intervention group (power of 80%, based on the incidence of hematologic ADRs observed in our study population).

A limitation of our study was that 12.5% of the patients were not treated according to the advised dose. However, this probably reflected the situation of genotype-guided dosing in the clinical setting. In addition, the study was performed in a nonblinded fashion. Gastroenterologists might have been able to identify patients in the intervention group receiving a reduced thiopurine dose advice (n = 40). However, it is not expected that these patients were treated differently; all patients were monitored regularly and because the study focused on the occurrence of (hematologic) ADRs, it was expected that gastroenterologists would be more alert to ADR development in both the intervention and control groups. Finally, the result of our post hoc analysis should be considered with caution because it was not corrected for multiple comparisons. Thus, in general, large-scale randomized controlled trials should focus their efforts specifically on the group that can be expected to benefit from genotype-guided treatment, in this specific case those patients with a *TPMT* variant. Strong points of our study are its prospective design and the fact that patients were included in general as well as academic hospitals, and that the decision to start thiopurine treatment was at the discretion of the gastroenterologist. This reflects the normal situation in which patients with IBD are treated.

Current guidelines for thiopurine treatment mandate regular hematologic monitoring to detect (severe) leukopenia. However, this is not a complete safeguard because leukopenia can develop suddenly. It has been suggested that pretreatment genotyping is relevant mainly for patients who are homozygous carriers of a genetic variant in *TPMT*.[9] We show that pretreatment *TPMT* genotyping also is relevant for patients heterozygous for a variant in TPMT. Importantly, a recent cost-effectiveness analysis (n = 333), in which also no differences in the ADR rate between the intervention and control groups was observed, indicated that pretreatment TPMT genotyping had a probability of 71% to be cost effective, owing to lower resource use in the intervention group.[34] However, they observed a small negative effect on the quality of life. This latter was not evident from our results because treatment efficacy, as a surrogate for quality of life, was similar between groups. Pretreatment genotyping should not replace current hematologic safety monitoring, but should be considered as a (cost-effective) addition to optimize thiopurine treatment.

The results of the TOPIC trial showed no overall effect of pretreatment *TPMT* screening followed by personalized dosing on hematologic ADRs. However, the study, in combination with the literature, shows that pretreatment *TPMT* screening followed by personalized dosing reduces the risk of leukopenia in patients carrying a genetic variant in *TPMT* and indicates that pharmacogenetic *TPMT* testing should be used as standard care to individualize thiopurine treatment of IBD patients.

CLINICAL AT

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at http://dx.doi.org/10.1053/j.gastro.2015.06.002.

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Conflicts of interest

The authors disclose no conflicts.

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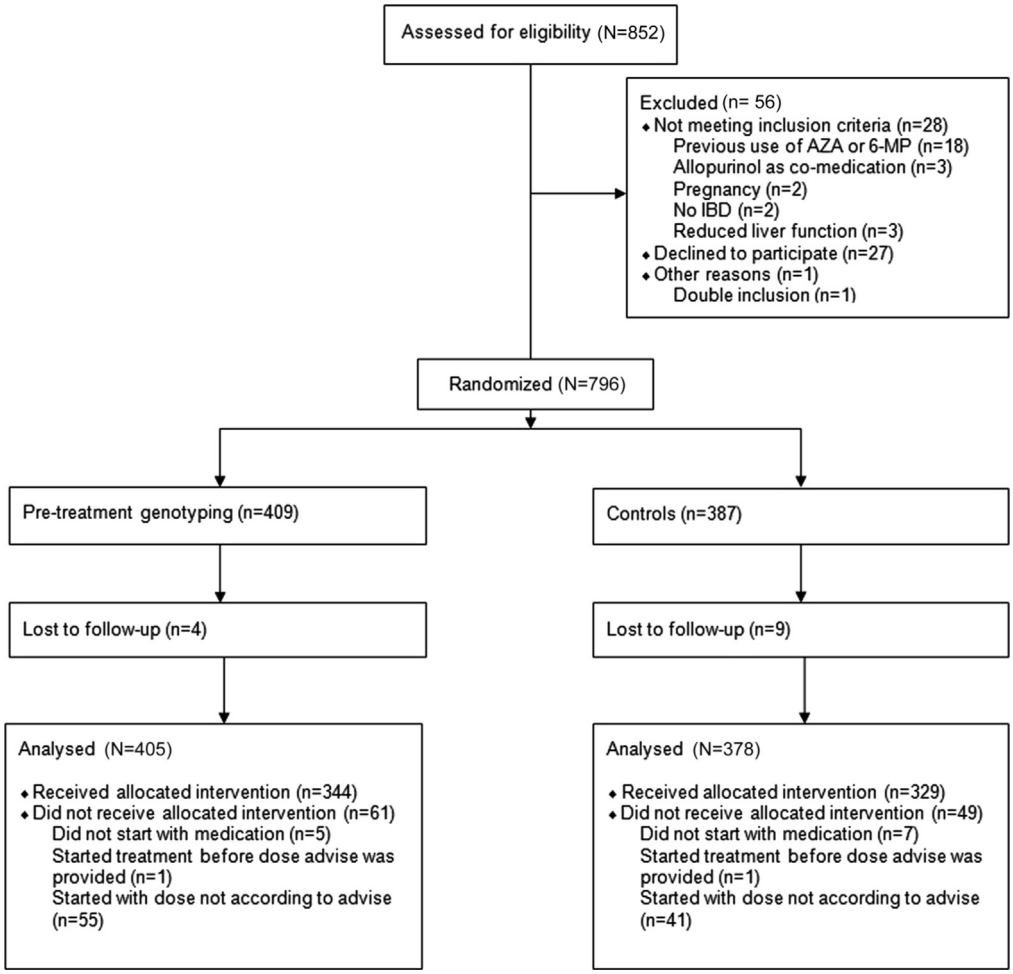
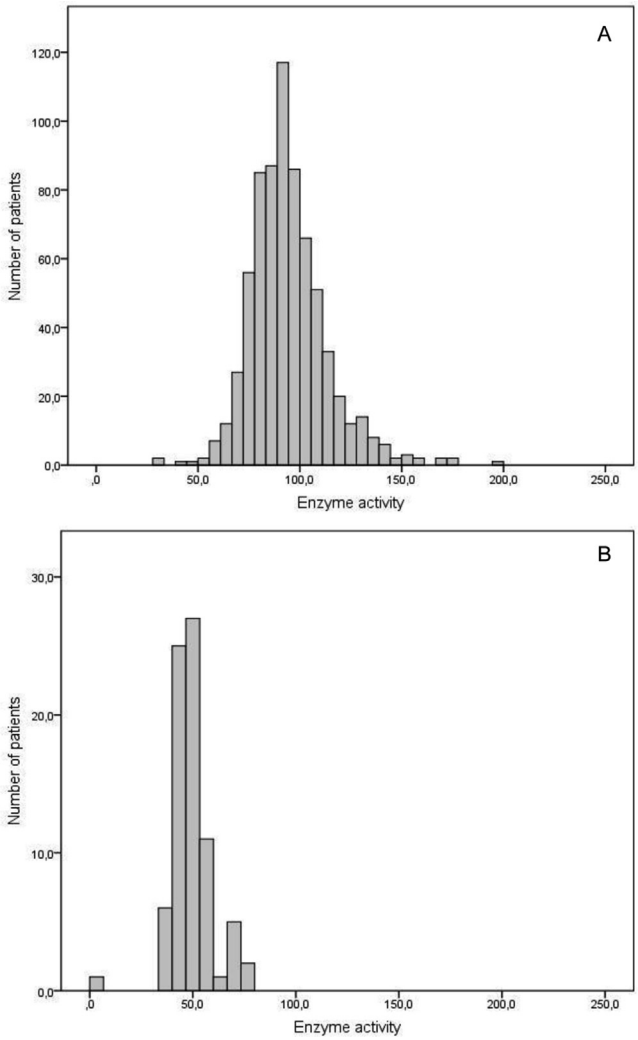
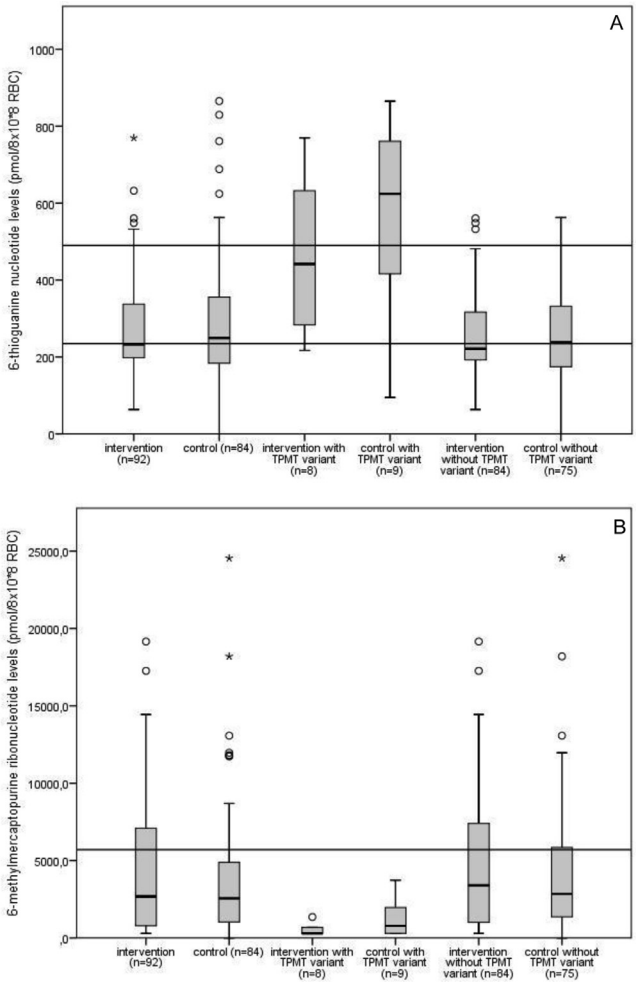


Supplementary Figure 1. Study design of the TOPIC trial. AZA, azathioprine; 6-MP, 6-mercaptopurine.



Supplementary Figure 2. TPMT enzyme activity distribution in the study population. Overview of the enzyme activity (mg/mmol hemoglobin per hour) distribution in patients (*A*) without a genetic variant (n = 705) and (*B*) with a genetic variant (n = 78) in the *TPMT* gene. Each *bar* represents 5 units of the scale (eg, 0–5, >5–10). Patients not carrying a *TPMT* variant had a mean enzyme activity for 6-methylguanine of 94.5 ± 19.0 mg/mmol hemoglobin per hour, and in heterozygous patients the mean TPMT enzyme activity of 6-methylguanine was 49.4 ± 10.7 mg/mmol hemoglobin per hour. The patient homozygous for *TPMT*3A* had an enzyme activity of 0.50 mg 6-methylguanine/mmol hemoglobin per hour before thiopurine therapy.



Supplementary Figure 3. Box-plots for steady-state metabolite levels 6-thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine ribonucleotides (6-MMPR) after exclusion of patients who received a dose change before 8 weeks of treatment start. Box-plots for steady-state metabolite levels of (*A*) 6-TGN and (*B*) 6-MMPR in pmol/8*10^8 red blood cells after exclusion of patients who received a dose change before 8 weeks of treatment start. Metabolite levels were measured at 8 weeks of treatment. The therapeutic range of 6-TGN metabolites (235 and 490 pmol/8 × 10^8 red blood cells) and normal range levels of 6-MMPR (<5700 pmol/8*10^8 red blood cells) are indicated with *horizontal lines* in panels *A* and *B*, respectively. The *boxes* indicate the 25th to 75th percentile and the medians are indicated by a *horizontal line* in the box. *Whiskers* indicate 1.5 interquartile range. *Open circle* indicates outliers (>1.5 interquartile range) and extreme outliers (>3 interquartile range) are indicated by an *asterisk*. The numbers on the x-axis indicate the number of patients analyzed.

Supplementary Table 1. Overview of the Primers Used for Sequencing of the Protein-Coding Part of the *TPMT* Gene

Exon	Forward primer	Reverse primer
3	AGGTTTTCATTTAGTTCATCAAT	TTTTTGATAGAACATTTCTCTATTGT
4	TGAATGAAAAGTGTTCACCTACC	TTTCAAAACTCAATCCAGAAAGA
5	TCTTTGAAACCCTATGAACCTGA	AAAACTTTTGTGGGGATATGGA
6	GCCCTCTTTCCTTGACTATT	GAGGAAGACACCTCCACTCC
7	TGTTGAAGTACCAGCATGCAC	TTCCAAACATAATAACCTATTTCAAAC
8	CGAAAGTAACTTCTGGCTTC	GGCAACTGGTAAAAGAAAAA
9	TGAGAAGAACATGCCACATCA	GCCAGGCCCAAAAGAGTTA
10	CACCCAGCCAATTTTGAGTA	ACAGGTAACACATGCTGATTGG

NOTE. Polymerase chain reaction was performed on 10 ng DNA using AmpliTaq Gold 360 mastermix (Life Technologies). The annealing temperature was 56°C for all exons. Sequencing was performed using Sanger technology.

Supplementary Table 2. Overview of Baseline IBD Classification in the Study Population

	Total population		Intervention group		Control group	
Disease[a]	CD	UC	CD	UC	CD	UC
Total, n (%)	476 (61.0%)	300 (38.5%)	245 (60.5%)	157 (38.8%)	231 (61.1%)	143 (37.8%)
Presence of fistula	43 (9.3%)[b]		19 (7.8%)[c]		24 (10.5%)[d]	
Localization CD known	n = 466		n = 239		n = 227	
Ileum (L1)	159 (34.1%)		88 (36.3%)		71 (31.3%)	
Colon (L2)	113 (24.2%)		52 (21.8%)		61 (26.9%)	
Ileum and colon (L3)	194 (41.6%)		99 (41.4%)		95 (41.9%)	
Localization UC known		n = 293		n = 152		n = 141
Proctitis ulcerosa (E1)		38 (13.0%)		23 (15.1%)		15 (10.6%)
Left-sided colitis (E2)		121 (41.3%)		67 (44.1%)		54 (14.3%)
Pancolitis (E3)		134 (45.7%)		62 (40.8%)		72 (19.0%)

NOTE. L indicates localization of Crohn's disease and E indicates extent of ulcerative colitis, both according to the Montreal classification.
[a]Seven patients in our study population (3 in the intervention group and 4 in the control group) had unclassified inflammatory bowel disease.The total number of patients for whom fistulas were assessed: [b]n = 468, [c]n = 243, and [d]n = 225.

Supplementary Table 3. Corticosteroid Use in the Intervention and Control Groups

	Intervention group (N = 405)	Control group (N = 378)
Steroid use at t = 0, n (%)		
All steroids, systemic and local	328 (81.0)	306 (81.0)
Systemic steroids	321 (79.3)	293 (77.5)
Steroids initiated, n (%)		
All steroids, systemic and local	74 (18.3)	67 (17.7)
Systemic steroids	56 (13.8)	57 (15.1)
Steroids discontinued, n (%)		
All steroids, systemic and local	171 (42.2)	170 (45.0)
Systemic steroids	160 (39.5)	156 (41.3)
Steroids used during the follow-up period, n (%)		
All steroids, systemic and local	348 (85.9)	322 (85.2)
Systemic steroids	340 (84.0)	309 (81.7)
Duration of steroid use,[a] mean (SD)		
All steroids, systemic and local	0.71 (0.39)	0.71 (0.39)
Systemic steroids	0.66 (0.40)	0.66 (0.40)

[a]Duration was calculated as the percentage of the study period. Comparisons in steroid use between the intervention and control groups, using the X^2 test or the Mann–Whitney *U* test for the duration of steroid use, showed no statistically significant differences.

Supplementary Table 4. Thiopurine Use in the Intervention and Control Groups

	Total		Intervention						Control					
	Total[a]	N	Intervention total	N	With a TPMT variant[a]	n	Without a TPMT variant	n	Control total	N	With a TPMT variant	n	Without a TPMT variant	n
Dose start, *mg/kg*, mean (minimum–maximum)														
Azathioprine	2.0 (0–3.1)	506	2.1 (0–2.7)	258	1.1 (0–1.4)	27	2.2 (0–2.7)	231	2.2 (0–3.1)	248	2.1 (0–2.4)	22	2.2 (0–3.1)	226
6-Mercaptopurine	1.1 (0–2.2)	277	1.2 (0–2.2)	147	0.6 (0.5–0.8)	13	1.2 (0–2.2)	134	1.2 (0–2.0)	130	1.2 (0–1.5)	14	1.2 (0.6–2.0)	116
Dose week 20, *mg/kg*, mean (minimum–maximum)														
Azathioprine	2.1 (0.5–3.1)	323	2.1 (0.5–2.7)	162	1.0 (0.5–1.5)	15	2.1 (0.5–2.7)	147	2.2 (0.6–3.1)	161	2.1 (0.7–2.4)	18	2.2 (0.6–3.1)	143
6-Mercaptopurine	1.0 (0.3–1.5)	208	1.1 (0.3–1.5)	104	0.6 (0.3–1.0)	9	1.1 (0.4–1.5)	95	1.1 (0.4–1.5)	104	1.1 (0.8–1.3)	8	1.1 (0.4–1.5)	96
Treatment stop, n (%)	313 (40.0)	783	170 (42.0)	405	19 (47.5)	40	151 (41.4)	365	143 (37.8)	378	9 (25)	36	134 (39.2)	342
Treatment restart, n (%)	117 (14.9)	783	57 (14.1)	405	4 (10.0)	40	53 (14.5)	365	60 (15.9)	378	1 (2.8)	36	59 (17.3)	342
Dose change, n (%)	235 (30.0)	783	118 (29.1)	405	6 (15.0)	40	112 (30.7)	365	117 (31.0)	378	7 (19.4)	36	110 (32.2)	342
Thiopurine treatment week 20, n (%)	531 (67.8)	783	266 (65.7)	405	24 (60)	40	242 (66.3)	365	262 (69.3)	378	26 (72.2)	36	239 (70.0)	342
Days thiopurine use, mean (SD)[b]	0.59 (0.41)	783	0.59 (0.41)	405	0.57 (0.43)	40	0.59 (0.41)	365	0.59 (0.41)	378	0.62 (0.41)	36	0.59 (0.41)	342

[a]Patients not starting treatment were included in the azathioprine or 6-mercaptopurine group depending on the treatment that should be initiated.
[b]Days of thiopurine use were calculated as a percentage of the study period. Treatment stop describes the patients who discontinued treatment during the follow-up period, this includes patients who subsequently restarted treatment. Comparisons between the intervention and control groups, using the X^2 test or the Mann–Whitney *U* test for dose and days of thiopurine use, showed statistically significant differences for 6-mercaptopurine dose at treatment initiation between the intervention and control groups ($P = .045$) and for azathioprine dose at week 20 ($P = .014$). Azathioprine and 6-mercaptopurine doses were both significantly different between the intervention and control groups for the patients with a genetic variant in *TPMT* at treatment start ($P < .004$) and at 20 weeks ($P \leq .001$).

Supplementary Table 5. Patient Enrollment per Center and Number of Patients Starting With a Dose Not According to the Provided Advice

Center number	Total number of patients included	Patients carrying a variant in TPMT[a]	Protocol violations[a]
1	48 (6.1)	5 (10.4)	6 (12.5)
2	10 (1.3)	0 (0)	1 (10)
3	34 (4.3)	4 (11.8)	4 (11.8)
4	14 (1.8)	1 (7.1)	1 (7.1)
5	33 (4.2)	6 (18.2)	8 (24.2)
6	20 (2.6)	1 (5)	1 (1.4)
7	38 (4.9)	4 (10.5)	8 (4.75)
8	19 (2.4)	2 (10.5)	3 (15.8)
9	54 (6.9)	5 (9.3)	17 (31.5)
10	5 (0.6)	1 (20)	1 (20)
11	28 (3.6)	0 (0)	0 (0)
12	59 (7.5)	7 (11.9)	8 (13.6)
13	84 (10.7)	9 (10.7)	7 (8.3)
14	31 (4.0)	5 (16.1)	4 (12.9)
15	51 (6.5)	8 (15.7)	18 (35.3)
16	10 (1.3)	1 (10)	0 (0)
17	26 (3.3)	2 (7.7)	1 (3.8)
18	29 (3.7)	3 (10.3)	3 (10.3)
19	48 (6.1)	2 (4.2)	2 (4.2)
20	2 (0.3)	0 (0)	0 (0)
21	40 (5.1)	2 (5.0)	2 (5.0)
22	19 (2.4)	2 (10.5)	1 (5.3)
23	47 (6.0)	4 (8.5)	5 (10.6)
24	1 (0.1)	0 (0)	0 (0)
25	4 (0.5)	0 (0)	2 (50)
26	1 (0.1)	1 (100)	0 (0)
27	18 (2.3)	2 (11.1)	6 (33.3)
28	4 (0.5)	0 (0)	1 (25)
29	3 (0.4)	0 (0)	0 (0)
30	3 (0.4)	1 (33.3)	0 (0)
Total	783 (100)	78 (10.0)	110 (14.0)

NOTE. Data shown are n (%).
[a]Percentage of the number of patients included at a particular center.

Supplementary Table 6. Treatment Effect Based on Clinical Remission and Inflammatory Markers

	Intervention						Control					
	Total	n total	*TMPT* variant	n total	No variant	n total	Total	n total	*TMPT* variant	n total	No variant	n total
Remission[a]	55 (69.6%)	79	9 (75.0%)	12	46 (68.7%)	67	58 (67.4%)	86	9 (90.0%)	10	49 (64.5%)	76
HBI	19 (61.3%)	31	1 (50.0%)	2	18 (62.1%)	29	31 (75.6%)	41	4 (80.0%)	5	27 (75.0%)	36
Partial Mayo	36 (75.0%)	48	8 (80.0%)	10	28 (73.7%)	38	27 (60.0%)	45	5 (100.0%)	5	22 (55.0%)	40
ESR[b] all patients												
Absolute change	2.0 (-83.0 to 90.0)	208	8.0 (-12.0 to 74.0)[c]	19	2.0 (-83.0 to 90.0)	189	1.0 (-45.0 to 88.0)	190	0.0 (-18 to 19)[c]	17	1.0 (-45.0 to 88.0)	173
Percentage change	22.2 (-1800.0 to 92.3)	208	40.8 (-200 to 92.3)	19	20.7 (-1800.0 to 88.57)	189	5.9 (-900.0 to 94.3)	190	0.0 (-150.0 to 85.7)	17	6.3 (-900.0 to 94.3)	173
High at baseline and normal at 20 weeks	41 (45.1%)	91	9 (64.3%)	14	32 (42.1%)	76	41 (47.1%)	87	3 (37.5%)	8	38 (50.0%)	76
ESR[b] CD patients												
Absolute change	2.0 (-36.0 to 90.0)	122	1.0 (-8.0 to 60.0)	8	2.0 (-36.0 to 90.0)	114	1.0 (-42.0 to 88.0)	114	0.0 (-18.0 to 14.0)	11	1.0 (-42.0 to 88.0)	103
Percentage change	19.6 (-1800 to 92.3)	122	-14.2 (-200.0 to 92.3)	8	19.6 (-1800.0 to 88.6)	114	6.1 (-900.0 to 91.2)	114	0.0 (-90.0 to 85.7)	11	6.3 (-900.0 to 91.2)	103
High at baseline and normal at 20 weeks	23 (43.4%)	53	3 (75.0%)	4	20 (41.7%)	48	25 (46.3%)	54	1 (20.0%)	5	24 (49.0%)	49
ESR[b] UC patients												
Absolute change	2.5 (-83.0 to 74.0)	86	21.0 (-12.0 to 74.0)	11	2.0 (-83.0 to 58.0)	75	0.0 (-45.0 to 65.0)	71	0.0 (-6.0 to 19.0)	6	0.0 (-45.0 to 65.0)	65
Percentage change	25.0 (-700.0 to 88.6)	86	63.6 (-34.3 to 80.9)	11	22.2 (-700.0 to 88.6)	75	0.0 (-383.3 to 94.3)	71	0.0 (-150.0 to 73.1)	6	0.0 (-383.3 to 94.3)	65
High at baseline and normal at 20 weeks	18 (47.4%)	38	6 (60.0%)	10	12 (42.9%)	28	13 (46.4%)	28	2 (66.6%)	3	11 (45.8%)	24
CRP[b] all patients												
Absolute change	1.4 (-264.0 to 209.0)	182	1.0 (-57.0 to 209.0)	19	1.7 (-264.0 to 85.0)	163	1.0 (-108.0 to 178.0)	176	2.5 (-42.0 to 29.1)	14	1.0 (-108.0 to 178.0)	162
Percentage change	26.8 (-11,945.5 to 98.9)	182	50.0 (-3800.0 to 97.7)	19	25.0 (-11,945.5 to 98.8)	163	22.9 (-700.0 to 100.0)	174	52.0 (-210.0 to 85.6)	14	20.0 (-700.0 to 100.0)	160
High at baseline and normal after 20 weeks	60 (51.7%)	116	7 (50%)	14	53 (52.0%)	102	65 (55.1%)	113	4 (44.4%)	9	61 (58.7%)	104
CRP[b] CD patients												
Absolute change	2.0 (-223.0 to 85.0)	122	-1.0 (-57.0 to 45.0)	11	2.0 (-223.0 to 85.0)	111	1.0 (-108.0 to 67.0)	113	3.0 (-42.0 to 29.1)	10	1.0 (-108.0 to 67.0)	103
Percentage change	28.8 (-3800.0 to 98.8)	122	-20.0 (-3800.0 to 94.5)	11	31.0 (-3333.3 to 98.8)	111	20.0 (-700.0 to 98.3)	113	53.3 (-210.0 to 85.6)	10	14.3 (-700.0 to 98.3)	103
High at baseline and normal at 20 weeks	38 (48.7%)	78	2 (33.3%)	6	36 (50.0%)	72	41 (50.6%)	81	3 (42.9%)	7	38 (51.4%)	74
CRP[b] UC patients												
Absolute change	1.0 (-264.0 to 209.0)	59	22.0 (-17.0 to 209.0)	8	0.7 (-264.0 to 84.0)	51	0.5 (-49.0 to 178.0)	58	2.3 (-7.0 to 3.0)	4	0.0 (-49.0 to 178.0)	54
Percentage change	23.3 (-11,945.45 to 98.8)	59	71.7 (-37.8 to 97.7)	8	6.7 (-11,945.5 to 98.8)	51	31.3 (-700.0 to 100.0)	56	50.8 (-31.8 to 75.0)	4	12.5 (-700.0 to 100.0)	52
High at baseline and normal at 20 weeks	22 (57.9%)	38	5 (62.5%)	8	17 (56.7%)	30	22 (78.6%)	28	1 (50.0%)	2	21 (80.8%)	26

NOTE. Values are given as either n (%) or medians (minimum–maximum).

[a]Number (%) of patients that achieved clinical remission at week 20 who were not in remission at treatment initiation. Remission is defined as HBI score less than 5 and partial Mayo score less than 3.

[b]Absolute and percentage change for ESR and CRP is shown as a median and range. Absolute change is calculated as a baseline value minus the value at 20 weeks, thus negative values indicate an increase from baseline to 20 weeks. The percentage change is calculated as follows: ([baseline value - value at 20 weeks]/baseline value)*100%.

[c]The absolute change in ESR was statistically significant between the patients with a genetic variant ($P = .042$), with patients in the intervention group showing better results.