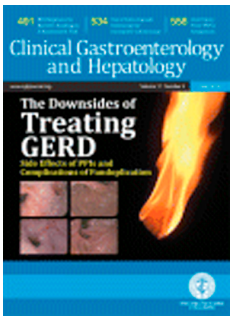

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PII: S1542-3565(19)30909-7
DOI: https://doi.org/10.1016/j.cgh.2019.08.034
Reference: YJCGH 56707

To appear in: *Clinical Gastroenterology and Hepatology*
Accepted Date: 16 August 2019

Please cite this article as: Chang JY, Park SJ, Jung ES, Jung S-A, Moon CM, Chun J, Park JJ, Kim ES, Park Y, Kim T-I, Kim WH, Cheon JH, Genotype-based Treatment With Thiopurine Reduces Incidence of Myelosuppression in Patients With Inflammatory Bowel Diseases, *Clinical Gastroenterology and Hepatology* (2019), doi: https://doi.org/10.1016/j.cgh.2019.08.034.

Genotype-based Treatment With Thiopurine Reduces Incidence of Myelosuppression in Patients With Inflammatory Bowel Diseases

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Short Title: Genotype-based treatment with thiopurine in IBD

Grant Support: This research was supported by a grant 15182MFDS507 from the Ministry of Food and Drug Safety, Korea in 2015.

Abbreviations used in this paper: IBD (inflammatory bowel disease), TNF (anti-tumor necrosis factor), WBC (white blood cell), AZA (azathioprine), 6-MP (6-mercaptopurine), TPMT (thiopurine S-methyltransferase), PLT (platelet), BMI (body mass index), HR (hazard ratio), CI (confidence interval)

Disclosures: All authors declare that there is no conflict of interest regarding the publication of this article.

Writing Assistance: None

Specific author contributions:

JHC: study concept and design, acquisition of data, obtained funding, study supervision

WHK: study concept and design, acquisition of data, study supervision

JYC: acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript for important intellectual content, statistical analysis

SJP: acquisition of data, analysis and interpretation of data, drafting of the manuscript, critical revision of the manuscript, statistical analysis

ESJ, SAJ, CMM, JC, JJP, ESK, YP, TK: acquisition of data, analysis and interpretation of data

All authors commented on drafts of the paper. All authors have approved the final draft of the manuscript.

ABSTRACT

Background & Aims: Thiopurine-related myelosuppression (most frequently leukopenia) interferes with thiopurine therapy for patients with inflammatory bowel diseases (IBD). We investigated whether pretreatment analyses genetic variants associated with thiopurine-induced leukopenia could be used to effectively identify patients who required dose adjustments.

Methods: We performed a multicenter, prospective study of patients with IBD at 5 tertiary medical centers in Korea, from January 2016 through September 2018. Seventy-two patients were randomly assigned to a group that underwent genotype analysis for the NUDT15 variant (rs116855232) and FTO variant (rs79206939) and 3 common TPMT variants (rs1800460, rs1800462, rs1142345) associated with myelosuppression and 92 patients were assigned to a group that did not undergo genotype analysis (non-genotyping group). Patients heterozygous for any variant received 50 mg azathioprine equivalents, whereas those who were homozygous for any variant received alternative drugs. Patients who did not carry any of the genetic variants and patients in the non-genotyping group received 50 mg azathioprine equivalents followed by dose escalation up to 2–2.5 mg/kg. Myelosuppression was defined as white blood cell counts below 3000/μL, levels of hemoglobin 10 g/dL, or platelet counts below 100 K/μL.

Results: Twelve patients (16.7%) in the genotype analysis group and 33 patients (35.9%) in the non-genotyping group developed myelosuppression (P=.005). A multivariate analysis revealed that body mass indices above 21 kg/m^2 (hazard ratio [HR], 0.43; 95% CI, 0.22–0.81; P = .009), pretreatment genotype analysis (HR, 0.37; 95% CI, 0.18–0.77; P = .008), and the maximum dose of thiopurines (HR, 0.34; 95% CI, 0.19–0.59; P < .001) independently

decreased risk of myelosuppression. Pretreatment genotype analysis reduced numbers of outpatient clinic visit and numbers of patients with drug discontinuation or dose reductions.

Conclusions: In a randomized controlled study of patients undergoing thiopurine therapy for IBD, we found that selection of therapy based on genetic variants associated with thiopurine-induced leukopenia significantly reduced the proportion of patients with myelosuppression during treatment. ClinicalTrials.gov no: NCT03719118.

Keywords: immune modulator; adverse drug reaction; genetic risk factor; metabolism

Introduction

Thiopurines are widely used as key drugs to maintain long-term remission in patients with inflammatory bowel disease (IBD).[1, 2] Although these drugs are undoubtedly effective in IBD treatment, drug-related adverse events frequently interfere with their use in daily clinical practice. One of the most common, and potentially serious, side effects is myelosuppression,[3, 4] which might increase the risk of infection and mortality.[1, 3]

Leukopenia is the most common form of thiopurine-induced myelosuppression.[1, 5] Most cases of severe leukopenia develop within the first month of therapy[6]; however, it may occur at any time during the treatment, with reports ranging from 12 days[7] to 27 years.[8] In addition, leukopenia could develop abruptly without any warning symptoms or signs.[3] The incidence of thiopurine-induced leukopenia is estimated as 5% in Caucasian IBD patients,[3, 9, 10] whereas a higher incidence has been reported in East Asian populations, including Koreans (31.2%–39.6%),[11, 12] Chinese (15.6%),[13] and Japanese (20.8%).[14] Ethnic differences in the incidence of thiopurine-induced leukopenia may relate to differences in polymorphisms of genes regulating thiopurine metabolism.

Thiopurines, azathioprine (AZA) and 6-mercaptopurine (6-MP) are prodrugs that undergo intracellular enzymatic modifications leading to the formation of active metabolites.[15] The synthetic pathway of the active metabolites is in competition with inactivation pathways mediated *via* xanthine oxidase or thiopurine S-methyltransferase (TPMT).[15] Thus, patients with the *TPMT* mutation, which decreases enzymatic activity, are at increased risk of thiopurine-induced leukopenia. However, pretreatment assessment of *TPMT* genotype/phenotype has a limited value in East Asian patients, considering the lower frequency of *TPMT* mutations in this population (1-3%)[12, 16-18] compared to that in Caucasians (~10%).[19] However, Asians have a higher incidence of thiopurine-induced

leukopenia. Furthermore, the efficacy of testing for the *TPMT* mutation remains controversial, because only one-quarter of IBD patients who developed thiopurine-induced leukopenia were associated with the *TPMT* mutation.[6, 20]

In an effort to overcome the limitations of TPMT genotyping tests and to identify additional genetic determinants of thiopurine-induced leukopenia, a recent study reported a strong association between *NUDT15* p.R139C and early leukopenia in Korean Crohn's disease patients.[16] However, *NUDT15* p.R139C could not explain late leukopenia.[21] In line with previous studies, we determined that the hypomorphic *FTO* p.A134T variant, which reduced FTO activity by 65%, was associated with thiopurine-induced leukopenia, and this was further validated in two replication cohorts. The frequency of *FTO* p.A134T was higher in Koreans (5.1%) compared to Western populations (0.1%).[5] However, there have been no studies to our knowledge that prospectively evaluate the usefulness of pretreatment genotyping for *NUDT15* and *FTO*.

In this prospective study, we aimed to evaluate the usefulness of pretreatment genotyping for three genes (*NUDT15*, *FTO*, and *TPMT*), followed by adjusted dosing based on the results of genotyping to reduce the incidence of thiopurine-induced myelosuppression in patients with IBD.

Materials and Methods

Patient enrollment

We conducted a multicenter, prospective, randomized controlled study to evaluate the efficacy of pretreatment genotyping testing in preventing thiopurine-related myelosuppression in IBD patients at five tertiary university hospitals from January 2016 to September 2018. Eligible patients were 20–80 years old; diagnosed with IBD based on clinical, endoscopic, radiographic, and histological assessments; and planned to start thiopurines for the first time for treatment of IBD. The following patients were excluded from the study: (1) patients who had previously used thiopurines; (2) those who had abnormal laboratory findings prior to screening, including WBC count < 3,000/μL, platelet (PLT) count < 100 K/μL, or elevation of aminotransferase to more than twice the upper normal limit; (3) those who were diagnosed with other infectious diseases at the time of screening or had received antibiotics within the previous seven days; and (4) those who were pregnant or lactating. We obtained informed written consent from all patients prior to screening. The institutional review boards of all participating hospitals approved this study (IRB number: 4-2015-0812) (ClinicalTrials.gov ID: NCT03719118). All authors had access to the study data and reviewed and approved the final manuscript.

Study design

After assessment for eligibility by gastroenterologists, patients before thiopurine treatment were randomly assigned to pretreatment genotyping (genotyping group) or conventional treatment (non-genotyping group) groups according to computer-generated randomization. Gastroenterologists and patients were not blinded in this study. The patients assigned to the intervention group underwent pretreatment genotyping for three common *TPMT* variants

(rs1800460 [exon 6], rs1800462 [exon 4], and rs1142345 [exon 9]), one *NUDT15* variant (rs116855232 [exon 3]), and one *FTO* variant rs79206939 [exon 3]), which were found to have associations with thiopurine-induced leukopenia in our previous study.[5] Patients who carried any heterozygotic variants received 50 mg AZA or 25 mg 6-MP, while those who had any homozygotic variants were recommended alternative drugs instead of thiopurines. Patients who did not carry any genetic variants in the genotyping group or those who were assigned to the non-genotyping group received standard doses of thiopurines based on the conventional regimen. This conventional regimen starts with 50 mg of AZA, and then the dose is increased to 2.0–2.5 mg/kg by 25 mg every 1–2 weeks along with regular monitoring of general blood tests including WBC counts.[22, 23]

Physicians could freely choose either AZA or 6-MP at the initiation of treatment. The 6-MP dosage in mg/kg bodyweight was converted to an equivalent pharmaceutical dose of AZA by multiplying it by 2.08, a conversion factor, assuming 100% bioavailability.[24] During the thiopurine therapy, dose reduction or discontinuation of drugs was decided based on the results of blood tests and the physician's judgment, as in the standard clinical practice.

Hematological parameters were assessed at baseline, week 1, week 2 or 3, and at two-week intervals within the first three months or in the case of dose elevation. After the first three months of treatment, blood tests were performed every two or three months according to the physicians' decisions.

Among the patients allocated into the non-genotyping group, those who agreed to further genotyping underwent genotyping studies. The physicians and patients were blinded to this pharmacogenetic information, and it was only used to evaluate the influences of individual mutation types on myelosuppression in our further analysis.

Study outcomes and assessment

The primary study endpoint was cumulative incidence of myelosuppression based on genotyping results. The secondary endpoints were the factors associated with myelosuppression; overall drug-related adverse events including pancreatitis, hepatotoxicity, general side effects (dizziness, malaise, general ache, or fever), gastrointestinal side effects (nausea, vomiting, abdominal discomfort, or reduced appetite), dermatologic side effects, and myalgia or arthritis; cumulative dose of thiopurines; and discontinuation of thiopurines.

Myelosuppression was defined as reduction of bone marrow activity leading to leukopenia, anemia, or thrombocytopenia. Leukopenia was defined as WBC < 3,000/μL, thrombocytopenia as PLT < 100 K/μL, and anemia as hemoglobin < 10 g/dL with a decrease of hemoglobin of greater than 2 g/dL compared with the level at screening and not consistent with iron deficiency anemia or hypochromic microcytic anemia.[24]

The demographic and clinical data were collected through interviews or by reviewing the medical records at the time of screening. During the follow-up period, clinical information including drug compliance, complications, and changes of medication was assessed at every visit.

Patients who had stable disease course and continuous thiopurine therapy without adverse drug reactions were followed-up for one year after thiopurine initiation. In patients who discontinued thiopurines because of ineffectiveness or intolerance, the interval between onset and discontinuation of thiopurines was defined as the follow-up period.

Genotyping

Genomic DNA from the patients was isolated from whole blood using commercially available kits (Qiagen, Chatsworth, CA, USA). Genotyping of the five loci, rs1800460

(*TPMT* exon 6), rs1800462 (*TPMT* exon 4), rs1142345 (*TPMT* exon 9), rs116855232 (*NUDT15* exon 3), and rs79206939 (*FTO* exon 3), was performed using Sanger sequencing by an Applied Biosystems 3730 genetic analyzer (Foster City, CA, USA).

Statistical analysis

We assumed a minimum odds ratio of 3.0 to detect a difference, a 1:1 ratio of case and control, a power of 0.80, and a 5% probability of Type I error (Alpha risk) for sample size calculation. The exposed proportion of risk factor in the control group was 13%. The calculation yielded a sample size of 83 per group. A total of 183 subjects should be randomized to allow 10% of patients to be excluded from the study population.

All statistical analyses were carried out using SPSS version 22.0 (SPSS Inc., Chicago, IL, USA). Student's *t*-test was used for continuous variables, and the chi-square test, Fisher's exact test, or linear-by-linear association was used for categorical variables. The Kaplan-Meier method and the log-rank test were performed to evaluate study outcomes between the intervention and control groups or between the heterozygote or homozygote group and the normal group. Moreover, multivariate Cox regression analysis was used to identify independent predictive factors for thiopurine-related adverse events. *P*-values less than 0.05 were considered statistically significant.

Results

Baseline characteristics of enrolled patients

Out of 182 subjects, 87 were randomized to the genotyping and 95 to the non-genotyping group according to a computer-generated four-block permuted randomization table prepared by an independent statistician who was not involved in this study. Because of the four-block scheme, there was an imbalance in the number of patients between the two groups. After excluding 18 subjects (consent withdrawal: 2; homozygotes: 4; no initiation of thiopurines: 11; loss to follow-up before treatment: 1), the treatment population consisted of 72 subjects in the genotyping group and 92 in the non-genotyping group (Figure 1). The baseline characteristics of both groups are summarized in Table 1.

Incidence of thiopurine-induced myelosuppression based on genotyping

Twelve patients (16.7%) in the genotyping group and 33 (35.9%) patients in the non-genotyping group developed thiopurine-induced myelosuppression. The Kaplan-Meier curves showed that patients in the genotyping group had a significantly lower risk of developing thiopurine-induced myelosuppression (P = .005) (Figure 2A) as well as leukopenia (P = .006) (Figure 2B) compared with those in the non-genotyping group. In the first three months, the non-genotyping group also had higher incidence of myelosuppression (26.3% vs. 7.7%, P =.008) and leukopenia (23.7% vs. 7.7%, P =.019) compared with the genotyping group. The per protocol analysis excluding those who discontinued thiopurines due to drug-associated side effects other than myelosuppression is shown in Supplementary Figure 1. The clinical characteristics at development of thiopurine-induced myelosuppression did not differ between the two groups (Supplementary Table 1). One case of severe myelosuppression defined as WBC count less than 1500/mm^3 was found in each group.

Other clinical outcomes based on genotyping

Although follow-up duration or cumulative drug dosage did not show statistical difference according to genotyping, the patients in the genotyping group had a smaller number of outpatient clinic visits (7.8±3.2 vs. 9.0±3.9; P = .052) and required fewer thiopurine discontinuations or dose reductions (15.3% vs. 33.7%; P = .007) during the follow-up period (Table 2).

Predictive factors associated with thiopurine-induced myelosuppression

In the univariate Cox regression analysis, Crohn's disease (P = .020) and intestinal Behçet's disease (P = .004) increased the risk of thiopurine-induced myelosuppression, whereas BMI > 21 kg/m^2 (P = .053), higher maximum dosage of thiopurines (P = .001), and preassessment genotyping (P = .006) significantly decreased its risk. In the multivariate Cox analysis, BMI > 21 kg/m^2 (hazard ratio [HR], 0.43; 95% confidence interval [CI], 0.22–0.81; P = .009), preassessment genotyping (HR, 0.37; 95% CI, 0.18–0.77; P = .008), and higher maximum dosage of thiopurines (HR, 0.34; 95% CI, 0.19–0.59; P < .001) were independent predictors for thiopurine-induced myelosuppression (Table 3).

Relative risk of individual genetic mutations on myelosuppression

Because 5 patients in the non-genotyping group did not agree to further gene analysis, a total of 159 patients was genotyped for further analysis (Supplementary Table 2). The relative risks of any mutation, *NUDT15* mutation, and *FTO* mutation during thiopurine treatment are shown in Supplementary Figure 2. Patients with any genetic mutation (HR, 2.77; 95% CI, 1.47–5.21; P = .002) and those with a *NUDT15* mutation (HR, 3.63; 95% CI, 1.87–7.05; P < .001) had a significantly higher probability of myelosuppression compared with those

without any mutations. Although patients with *FTO* mutation did not exhibit a statistically significant difference, the curve suggested that *FTO* mutation is related to a numerically higher risk of late-onset myelosuppression (HR, .92; 95% CI, 0.22–3.98; P = .921).

Other thiopurine-associated complications according to preassessment genotyping

Gastrointestinal complications, including nausea or vomiting, were the most common side effects and were reported by 18 (25.0%) patients in the genotyping group and 24 (26.1%) patients in the non-genotyping group. There was no statistical difference between the two groups (P = .874). The incidences of hepatotoxicity, pancreatitis, or alopecia also showed no statistical differences according to genotyping status.

Discussion

In this study, we for the first time found that a tailored therapy based on pretreatment genotyping of one *NUDT15* variant (rs116855232), one *FTO* variant (rs79206939), and three common *TPMT* variants (rs1800460, rs1800462, rs1142345) was effective to reduce thiopurine-related myelosuppression. Pretreatment genotyping also contributed to reduce the numbers of outpatient clinic visits and patient with thiopurine discontinuation or dose reduction during thiopurine administration. Regarding the influence of each individual genotype mutation on myelosuppression risk, patients with a *NUDT15* mutation had a higher probability of myelosuppression, but those with *FTO* or *TPMT* mutations did not.

Despite the proven efficacy of thiopurines, a high incidence of myelosuppression in East Asian patients, including Koreans, has led to limited clinical use. As part of an effort to find a genetic polymorphism of enzymes involved in thiopurine metabolism, a Korean study group found that *NUDT15* p.R139C increases the risk of thiopurine-induced early leukopenia, with an odds ratio of 35.6 and high sensitivity (89.4%) and specificity (93.2%) in Korean Crohn's disease patients.[16] Also, our group recently identified a novel genetic variant, *FTO* p.A134T, associated with increased susceptibility to thiopurine-induced leukopenia, using high-throughput sequencing.[5] Based on these results, this study was planned to validate the value of the pretreatment genotyping test and provide the ideal protocol for thiopurine administration to patients with IBD.

In this study, we demonstrated that pretreatment genotyping could significantly reduce thiopurine-induced myelosuppression or leukopenia, and this effectiveness was confirmed when other variables were adjusted. In the multivariate Cox regression analysis, lower BMI, lower maximum dosage of thiopurines, and absence of genotyping were associated with a higher risk of myelosuppression. Obesity affects the distribution, clearance, and half-life of

elimination of many types of medications.[25] Obese patients had a higher incidence of flares in ulcerative colitis while receiving azathioprine,[26] poor clinical outcomes with infliximab,[27] and a greater need for adalimumab dose escalation.[28] Furthermore, inverse relationship between BMI or body fat index and 6-TGN level has been reported.[28] These findings indicate that adipose tissue distribution of thiopurines can affect drug efficacy or side effects.

Myelosuppression is a dose-dependent adverse reaction, and the median dosage of azathioprine at the time of myelosuppression was reported as 1.3–1.7 mg/kg/day[16, 21] in Asian populations, which is similar to that of our study (1.2 mg/kg in the genotyping group and 1.6 mg/kg in the non-genotyping group). However, in our Cox regression analysis, the incidence of myelosuppression was negatively correlated with the maximum dosage of thiopurines. This inverse correlation could be explained by our inherent study design. In this study, the patients with a tendency toward myelosuppression were not allowed to increase their thiopurine dosage even before development of actual myelosuppression, based on the physicians' decisions. On the other hand, those who were thought to be less likely to develop myelosuppression could increase thiopurines up to the maximum dose (Supplementary Table 3).

Pretreatment genotyping was also found to decrease the number of thiopurine discontinuations or dose reductions. Considering that approximately 20% of patients had to discontinue thiopurines due to side effects,[29] pretreatment genotyping could also contribute to reduce medical budget and subsequent laboratory tests, which are generally required to monitor serious adverse events and ensure continuity of thiopurine treatment. Although we did not compare the actual medical costs of treating myelosuppression, such as admission, use of antibiotics, or transfusions, between the two groups, considering that

approximately 49% of myelosuppression was associated with one of the three mutations in our study, the pharmaco-economic value of prevention of myelosuppression based on pretreatment genotyping would be even more significant compared with that of *TPMT* polymorphism screening.

In our previous study, we provided a potential role of *FTO* as a predictor of thiopurine-induced myelosuppression in replication cohorts as well as *in vivo* experiments. However, we could not find a statistically significant association between myelosuppression risk and *FTO* mutation, whereas *NUDT15* mutation significantly increased the risk of myelosuppression. The small number of patients with *FTO* mutations and the relatively short follow-up period were thought to be possible causes of this lack of significance and are limitations of this study. Thus, further evaluation with a larger number of patients is needed.

This study is valuable as the first to confirm the usefulness of pretreatment genotype testing through a prospective multicenter trial. Our data could contribute to establishing a systemic database of functional mutations associated with thiopurine-induced adverse reactions that sequentially leads to optimization of thiopurine therapy.

Based on our results, we propose the following protocol for the dosing of thiopurines in IBD patients (Figure 3). It would be beneficial to avoid the use of thiopurines in patients with any homozygotic mutations of these three genes. In particular, patients with *NUDT15* homomutations should not receive thiopurine therapy. For patients with any heterozygotic mutations of the three genes, the initial dosage of 50 mg of azathioprine equivalents with maintenance of any stable dose is recommended. For patients without any variants, the conventional regimen (initial dose of 50 mg azathioprine equivalents followed by dose escalation up to 2–2.5 mg/kg at two-week intervals) would be appropriate.

In conclusion, our prospective multicenter study identified a tailored therapy based on pretreatment genotyping as useful to reduce the risk of thiopurine-induced myelosuppression in patients with IBD. Among the three genetic polymorphisms, *NUDT15* had the best predictive power for thiopurine-induced myelosuppression compared with *FTO* or *TPMT*. Personalized therapy based on individual pharmacogenomic information would be beneficial for safe use of thiopurines by minimizing thiopurine-induced myelosuppression in IBD patients. Further multi-ethnic and long-term prospective studies enrolling larger numbers of patients are required to delineate the myelosuppression risks associated with the genetic mutations.

Figure Legends

Figure 1. Flow chart of study subject enrollment.

Figure 2. ITT analysis: cumulative risks of myelosuppression according to genotyping. Patients in the genotyping group had significantly lower risk for developing thiopurine-induced myelosuppression (A) as well as leukopenia (B).

Figure 3. The proposed guidelines for optimal dosage of thiopurines.

Supplementary Figure Legends

Supplementary Figure 1. PP analysis: cumulative risks of myelosuppression (A) and leukopenia (B) according to genotyping.

Supplementary Figure 2. Cumulative risks of myelosuppression according to individual pharmacogenomic information. Patients with any genetic mutation (A) and *NUDT15* mutation (B) had a significantly higher probability of myelosuppression compared with those without any mutation, whereas patients with *FTO* mutation (C) had no statistical difference.

References

1. Gisbert JP, Gomollón F. Thiopurine-Induced Myelotoxicity in Patients With Inflammatory Bowel Disease: A Review. Am J Gastroenterol. 2008;103:1783.
2. Khan KJ, Dubinsky MC, Ford AC, et al. Efficacy of immunosuppressive therapy for inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol. 2011;106(4):630-42. Epub 2011/03/17.
3. Connell WR, Kamm MA, Ritchie JK, et al. Bone marrow toxicity caused by azathioprine in inflammatory bowel disease: 27 years of experience. Gut. 1993;34(8):1081-5.
4. Kim JH, Cheon JH, Kim WH. The frequency and the course of the adverse effects of azathioprine/6-mercaptopurine treatment in patients with inflammatory bowel disease. Korean J Gastroenterol. 2008;51(5):291-7. Epub 2008/06/03.
5. Kim HS, Cheon JH, Jung ES, et al. A coding variant in FTO confers susceptibility to thiopurine-induced leukopenia in East Asian patients with IBD. Gut. 2017;66(11):1926-35.
6. Dewit O, Moreels T, Baert F, et al. Limitations of extensive TPMT genotyping in the management of azathioprine-induced myelosuppression in IBD patients. Clin Biochem. 2011;44(13):1062-6.
7. Gearry RB, Barclay ML, Burt MJ, et al. Thiopurine drug adverse effects in a population of New Zealand patients with inflammatory bowel disease. Pharmacoepidemiol Drug Saf. 2004;13(8):563-7.
8. Warman JI, Korelitz BI, Fleisher MR, et al. Cumulative Experience With Short- and Long-Term Toxicity to 6-Mercaptopurine in the Treatment of Crohn's Disease and Ulcerative Colitis. J Clin Gastroenterol. 2003;37(3):220-5.

9. Present DH, Meltzer SJ, Krumholz MP, et al. 6-Mercaptopurine in the management of inflammatory bowel disease: short- and long-term toxicity. Ann Intern Med. 1989;111(8):641-9. Epub 1989/10/15.

10. Fraser AG, Orchard TR, Jewell DP. The efficacy of azathioprine for the treatment of inflammatory bowel disease: a 30 year review. Gut. 2002;50(4):485-9.

11. Hyun Ju L, M.D.*, Suk-Kyun Y, et al. The Safety and Efficacy of Azathioprine and 6-Mercaptopurine in the Treatment of Korean Patients with Crohn's Disease. Intest Res. 2009;7(1):22-31.

12. Kim JH, Cheon JH, Hong SS, et al. Influences of Thiopurine Methyltransferase Genotype and Activity on Thiopurine-induced Leukopenia in Korean Patients With Inflammatory Bowel Disease: A Retrospective Cohort Study. Journal of Clinical Gastroenterology. 2010;44(10):e242-e8.

13. Qiu Y, Mao R, Zhang SH, et al. Safety Profile of Thiopurines in Crohn Disease: Analysis of 893 Patient-Years Follow-Up in a Southern China Cohort. Medicine. 2015;94(41):e1513. Epub 2015/10/16.

14. Odahara S, Uchiyama K, Kubota T, et al. A Prospective Study Evaluating Metabolic Capacity of Thiopurine and Associated Adverse Reactions in Japanese Patients with Inflammatory Bowel Disease (IBD). PLOS ONE. 2015;10(9):e0137798.

15. Zaza G, Cheok M, Krynetskaia N, et al. Thiopurine pathway. Pharmacogenet Genomics. 2010;20(9):573-4. Epub 2009/12/03.

16. Yang S-K, Hong M, Baek J, et al. A common missense variant in NUDT15 confers susceptibility to thiopurine-induced leukopenia. Nat Genet. 2014;46(9):1017-20.

17. Takatsu N, Matsui T, Murakami Y, et al. Adverse reactions to azathioprine cannot be predicted by thiopurine S-methyltransferase genotype in Japanese patients with inflammatory bowel disease. J Gastroenterol Hepatol. 2009;24(7):1258-64. Epub 2009/08/18.

18. Fangbin Z, Xiang G, Minhu C, et al. Should thiopurine methyltransferase genotypes and phenotypes be measured before thiopurine therapy in patients with inflammatory bowel disease? Ther Drug Monit. 2012;34(6):695-701. Epub 2012/11/15.

19. Collie-Duguid ES, Pritchard SC, Powrie RH, et al. The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations. Pharmacogenetics. 1999;9(1):37-42. Epub 1999/04/20.

20. Booth RA, Ansari MT, Loit E, et al. Assessment of thiopurine S-methyltransferase activity in patients prescribed thiopurines: a systematic review. Ann Intern Med. 2011;154(12):814-23, w-295-8. Epub 2011/06/22.

21. Kakuta Y, Naito T, Onodera M, et al. NUDT15 R139C causes thiopurine-induced early severe hair loss and leukopenia in Japanese patients with IBD. Pharmacogenomics J 2015;16:280.

22. Lichtenstein GR, Abreu MT, Cohen R, et al. American Gastroenterological Association Institute technical review on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. Gastroenterology. 2006;130(3):940-87. Epub 2006/03/15.

23. Lee KM, Kim YS, Seo GS, et al. Use of Thiopurines in Inflammatory Bowel Disease: A Consensus Statement by the Korean Association for the Study of Intestinal Diseases (KASID). Intest Res. 2015;13(3):193-207. Epub 2015/07/02.

24. Broekman M, Coenen MJH, van Marrewijk CJ, et al. More Dose-dependent Side Effects with Mercaptopurine over Azathioprine in IBD Treatment Due to Relatively Higher Dosing. Inflamm Bowel Dis. 2017;23(10):1873-81. Epub 2017/06/24.

25. Hanley MJ, Abernethy DR, Greenblatt DJ. Effect of obesity on the pharmacokinetics of drugs in humans. Clin Pharmacokinet. 2010;49(2):71-87. Epub 2010/01/14.

26. Holtmann MH, Krummenauer F, Claas C, et al. Significant differences between Crohn's disease and ulcerative colitis regarding the impact of body mass index and initial disease activity on responsiveness to azathioprine: results from a European multicenter study in 1,176 patients. Dig Dis Sci. 2010;55(4):1066-78. Epub 2009/06/11.

27. Harper JW, Sinanan MN, Zisman TL. Increased body mass index is associated with earlier time to loss of response to infliximab in patients with inflammatory bowel disease. Inflamm Bowel Dis. 2013;19(10):2118-24. Epub 2013/07/19.

28. Poon SS, Asher R, Jackson R, et al. Body Mass Index and Smoking Affect Thioguanine Nucleotide Levels in Inflammatory Bowel Disease. J Crohns Colitis. 2015;9(8):640-6.

29. Chaparro M, Ordas I, Cabre E, et al. Safety of thiopurine therapy in inflammatory bowel disease: long-term follow-up study of 3931 patients. Inflamm Bowel Dis. 2013;19(7):1404-10. Epub 2013/05/15.

Table 1. Baseline characteristics of enrolled patients

	Genotyping (+) (n = 72)	Genotyping (-) (n = 92)	*P* value
Age (years)	39.7±15.9	37.7±17.1	.441
Male sex	49 (68.1)	49 (53.5)	.055
BMI (Kg/m^2)	21.7±3.2	20.9±3.4	.114
Family history of IBD	5 (6.9)	3 (3.3)	.301
Smoking			.275
Current	9 (12.5)	5 (5.4)	
Ex-smoker	16 (22.2)	22 (23.9)	
Never	47 (65.3)	65 (70.7)	
Alcohol			.073
Heavy	3 (4.2)	0 (0)	
Social	29 (40.3)	31 (33.7)	
None	40 (55.6)	61 (66.3)	
Disease type			.606
Ulcerative colitis	29 (40.3)	39 (42.4)	
Crohn's disease	41 (56.9)	44 (47.8)	
Intestinal Behçet's disease	2 (2.8)	9 (9.8)	
Disease activity index			.201
Remission to mild	48 (66.7)	50 (53.8)	
Moderate	17 (23.6)	33 (36.3)	
Severe	7 (9.7)	9 (9.9)	
Initial lab findings			
White blood cell (/μL)	8153±3083	8252±2696	.829
Hemoglobin (g/dL)	12.9±2.0	12.7±1.7	.425
Platelet (K/μL)	300±77	347±116	.002
AZA initial dose (mg/Kg)[a]	0.8±0.2	0.8±0.2	.901
Concomitant medications			
5-ASA	67 (93.1)	86 (93.5)	> .999
Biologics	10 (14.3)	5 (5.5)	.057
Follow-up duration (days)	241.0±126.4	237.8±140.7	.878

Variables are presented as n (%), or mean±SD.

BMI, body mass index; IBD, inflammatory bowel disease; AZA, azathiopurine; 5-ASA, 5-aminosalicylic acids

[a]6-MP dose was converted into an equivalent pharmaceutical dose of AZA by multiplying 2.08.

Table 2. Other clinical outcomes based on genotyping

	Genotyping (+)	Genotyping (-)	*P* value
Number of OPC visits	7.8±3.2	9.0±3.9	.052
Maximum dosage of AZA (mg/Kg)[a]	1.5±0.6	1.7±0.6	.111
Cumulative drug dosage (mg/Kg)	22553.7±13498.7	23512.3±13700.2	.655
Drug discontinuation or dose reduction, n (%)	11 (15.3)	31 (33.7)	.007

OPC, outpatient clinic; AZA, azathioprine

[a]6-MP dose was converted into an equivalent pharmaceutical dose of AZA by multiplying 2.08.

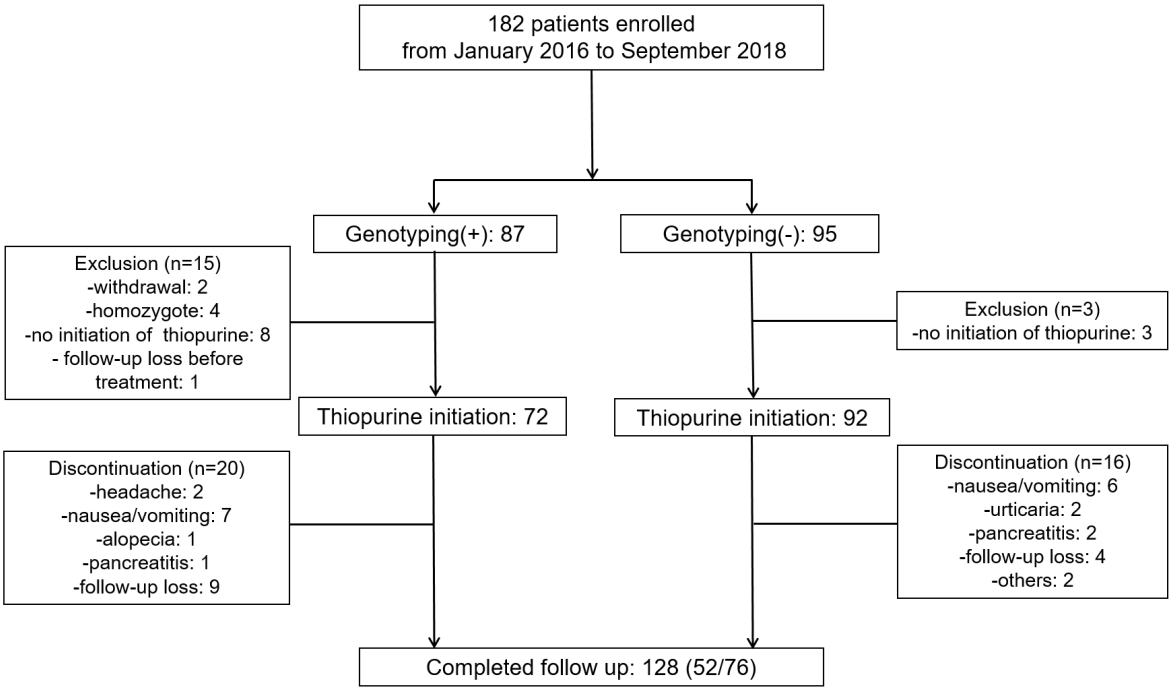
Table 3. Predictive factors associated with thiopurine -induced myelosuppression

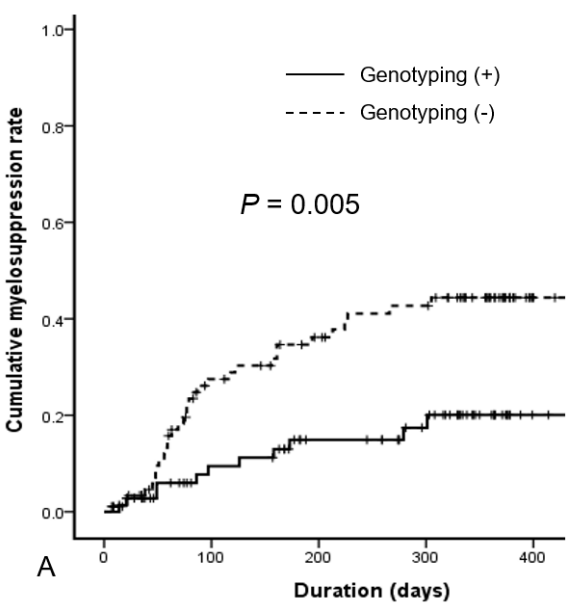
	Univariate			Multivariate		
Variable	HR	95%CI	*P*	HR	95%CI	*P*
Female	1.75	0.97-3.14	.062	1.37	0.71-2.63	.345
Age > 34 years[a]	0.94	0.55-1.76	.942	-	-	-
BMI > 21 (Kg/m^2)	0.53	0.31-1.01	.053	0.43	0.22-0.81	.009
Family history of IBD	2.37	0.33-17.25	.393	-	-	-
Smoking						
Never	1 (ref)			-	-	-
Current	1.05	0.37-3.00	.931	-	-	-
Alcohol						
None	1 (ref)			-	-	-
Current	0.76	0.41-1.42	.395	-	-	-
Disease type						
Ulcerative colitis	1 (ref)			1 (ref)		
Crohn's disease	2.42	1.15-5.12	.020	1.72	0.79-3.74	.409
Intestinal Behçet's disease	4.65	1.65-13.07	.004	2.72	0.93-8.00	.069
Disease activity						
Remission to mild	1 (ref)			-	-	-
Moderate	0.94	0.47-1.91	.787	-	-	-
Severe	1.76	0.72-4.28	.212	-	-	-
Genotyping (+)	0.40	0.21-0.77	.006	0.37	0.18-0.77	.008
Maximun dosage of AZA[b]	0.40	0.23-0.69	.001	0.34	0.19-0.59	< .001
Concomitant use of 5-ASA	1.49	0.36-6.17	.580	1.39	0.33-5.87	.651
Concomitant use of biologics	0.34	0.08-1.45	.146	0.39	0.08-1.96	.252

HR, hazard ratio; CI, confidence interval; BMI, body mass index; IBD, inflammatory bowel disease; AZA, azathioprine; 5-ASA, 5-aminosalicylic acids

[a]The median age of the patients was 34 years.

[b]6-MP dose was converted into an equivalent pharmaceutical dose of AZA by multiplying 2.08.





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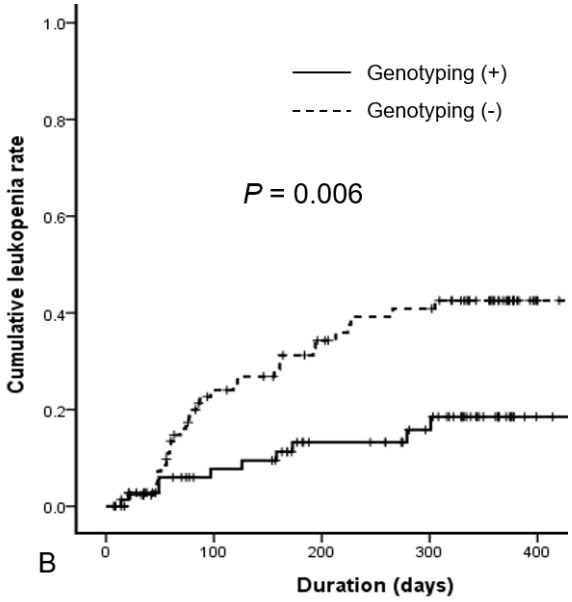


B

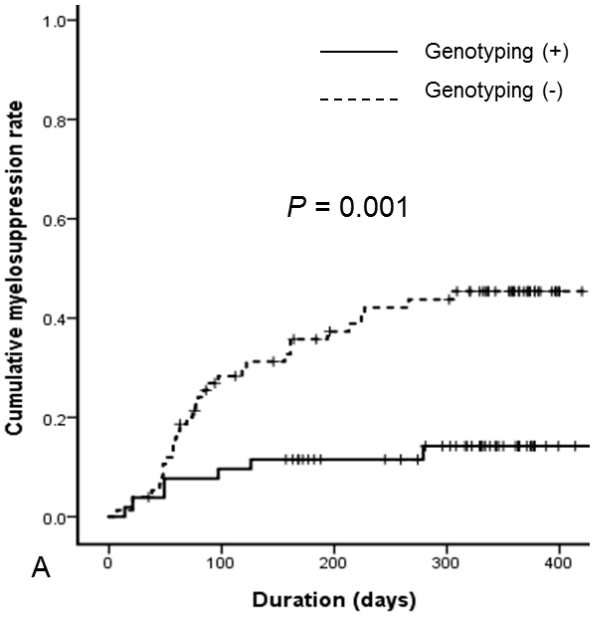
- **Genotyping (-)**: Conventional regimen
  (50 mg of azathioprine equivalents → dose escalation until 2-2.5 mg/Kg with 1-2 weeks intervals)
- **Genotyping (+)**
  - Variant (-): Conventional regimen
  - Variant (+): Heterozygote: 50 mg of azathioprine equivalents
    → maintenance of any stable dose
    (→ dose adjustment if myelosuppression occurs)
    Homozygote: exclusion of thiopurines

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	4
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	6-7
	2b	Specific objectives or hypotheses	7
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	8
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	-
Participants	4a	Eligibility criteria for participants	8
	4b	Settings and locations where the data were collected	8
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8-9
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10
	6b	Any changes to trial outcomes after the trial commenced, with reasons	-
Sample size	7a	How sample size was determined	11
	7b	When applicable, explanation of any interim analyses and stopping guidelines	9
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	8
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	-
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	8
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	8
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	-

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	-
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	11
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	11
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	12
	13b	For each group, losses and exclusions after randomisation, together with reasons	12
Recruitment	14a	Dates defining the periods of recruitment and follow-up	12
	14b	Why the trial ended or was stopped	-
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	12
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	12
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	12-13
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	-
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	14
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	14
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	18
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	15-18
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	18-19
Other information			
Registration	23	Registration number and name of trial registry	8
Protocol	24	Where the full trial protocol can be accessed, if available	-
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	2

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.



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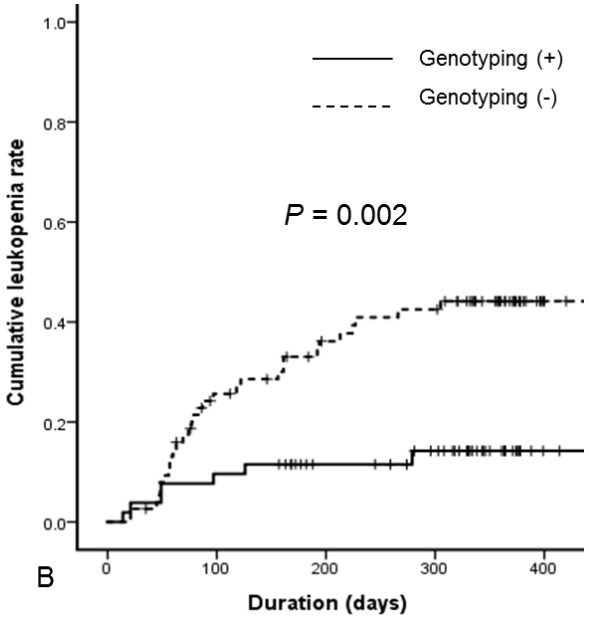

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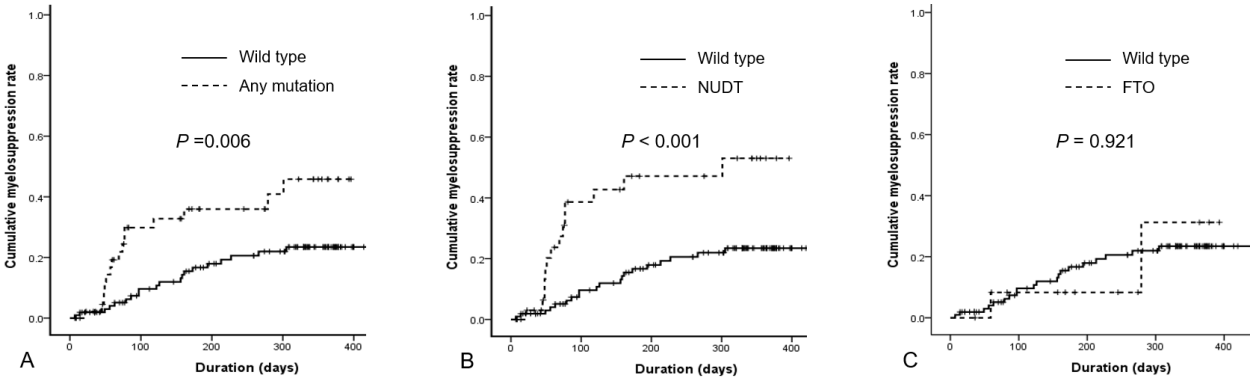


Supplementary Table 1. Clinical characteristics at the time of myelosuppression

	Genotyping (+) (n = 12)	Genotyping (-) (n = 33)	*P* value
Interval (days)	227.4±132.4	194.4±144.1	.133
Accompanied symptoms			
Nausea or vomiting	1 (8.3)	4 (12.1)	> .999
Chilling	0 (0)	5 (15.2)	.303
Disease activity index			.167
Remission to mild	7 (58.3)	26 (78.8)	
Moderate	5 (41.7)	5 (15.1)	
Severe	0 (0)	2 (6.1)	
Laboratory findings			
White blood cell (/μL)	2661±884	2798±1339	.744
ANC (/μL)	1386±532	1503±1333	.770
Hemoglobin (g/dL)	10.9±2.2	10.1±2.2	.319
Platelet (K/μL)	251±118	258±98	.846
CRP (mg/L)	6.4±11.4	13.4±35.8	.570
ESR (mm/hr)	23.0±27.4	32.2±28.3	.944
AZA dosage at the time of MS (mg/Kg)[a]	1.4±0.7	1.6±0.5	.405
Drug discontinuation at the time of MS	3 (25.0)	11 (33.3)	.086

ANC, absolute neutrophil count; CRP, C-reactive protein; ESR, erythrocyte sediment rate; AZA, azathioprine; MS, myelosuppression

[a]6-MP dose was converted into an equivalent pharmaceutical dose of AZA by multiplying 2.08.

Supplementary Table 2. Results of genotyping in all patients

	Genotyping (+) (n=72)	Genotyping (-) (n=92)	Total
Wild type	47 (65.3)	58 (63.0)	105 (64.0)
Heterozygous	25 (34.7)	27 (29.3)	52 (31.7)
NUDT	15 (20.8)	22 (23.9)	37 (22.6)
FTO	8 (11.1)	4 (4.3)	12 (7.3)
NUDT & *FTO*	1 (1.4)	0 (0)	1 (0.6)
TPMT	1 (1.4)	1 (1.1)	2 (1.2)
Homozygous[a]	-	2 (2.2)	2 (1.2)
Missing	-	5 (5.4)	5 (3.0)

[a]Two patients in the non-genotyping group had homozygotic mutation in *NUDT15*.

Supplementary Table 3. Drug-related information based on myelosuppression

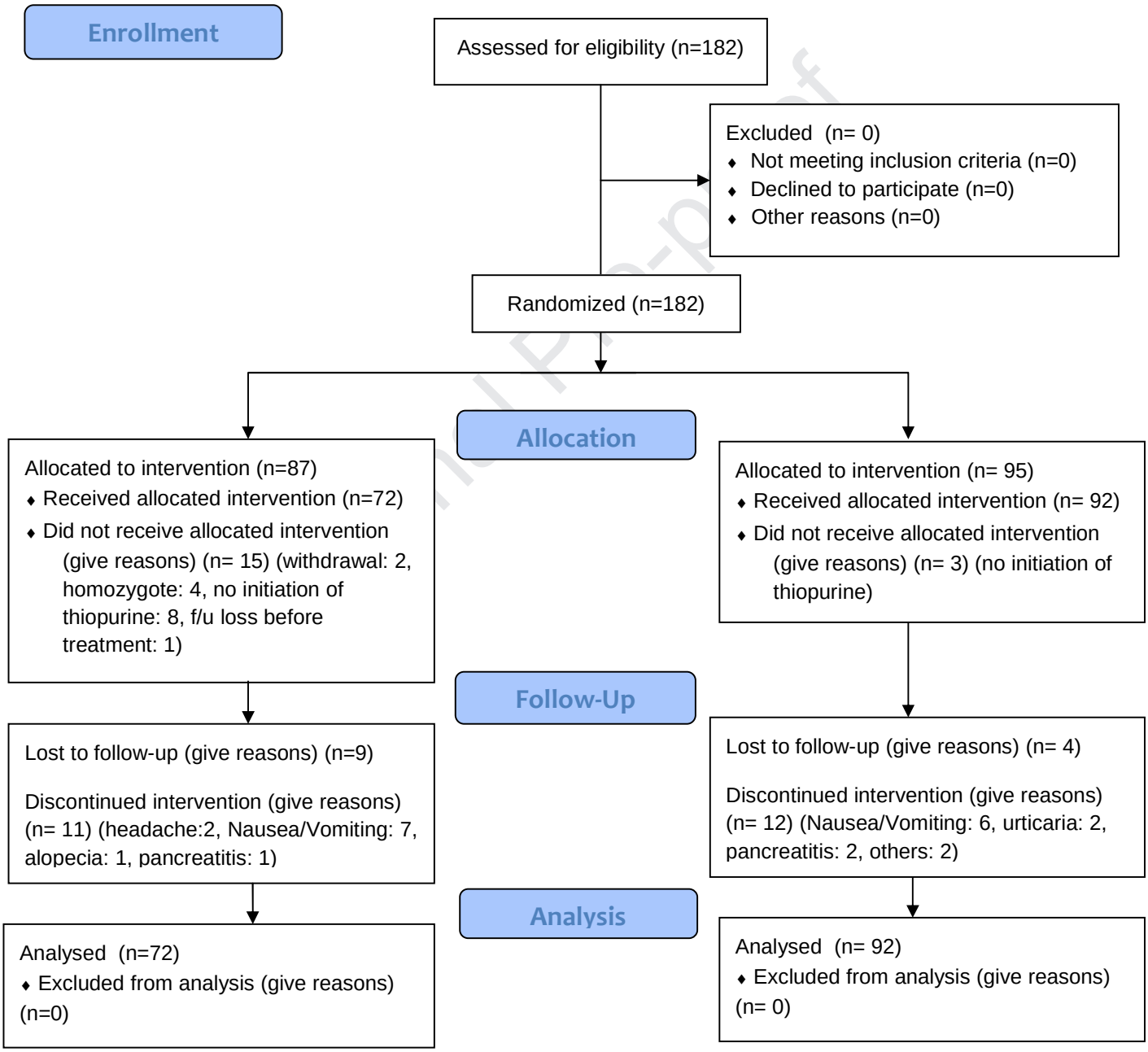
	Myelosuppression (+)	Myelosuppression (-)	*P*	Leukopenia (+)	Leukopenia (-)	*P*
Drug discontinuation or dose reduction, n (%)	25 (55.6)	17 (14.3)	< 0.01	24 (57.1)	18 (14.8)	< 0.01
Achievement of ideal AZA dosage, n (%)	18 (40.0)	65 (54.6)	0.095	17 (40.5)	66 (54.1)	0.128
Maximun dosage of AZA (mg/Kg)[a]	1.49 ± 0.63	1.63 ± 0.57	0.200	1.49 ± 0.63	1.63 ± 0.57	0.200

AZA, azathioprine

[a]6-MP dose was converted into an equivalent pharmaceutical dose of AZA by multiplying 2.08.

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram



What You Need to Know

Background

Myelosuppression, one of the most common and potentially serious side effects has been found more frequently in East Asian populations, including Koreans. We investigated whether pre-treatment analysis of patients for genetic variants associated with thiopurine-induced leukopenia could be used to select doses for patients with inflammatory bowel diseases and reduce the incidence of myelosuppression.

Findings

Selection of therapy based on variants in NUDT15, FTO, and TPMT reduced the proportions of patients with thiopurine-related myelosuppression. Lower proportions of patients who received pretreatment genotype analysis had thiopurine discontinuations or dose reductions.

Implications for patient care

Genotype analysis before treatment can reduce risk of thiopurine-induced myelosuppression in patients with inflammatory bowel diseases.