Re: Revision request for " Comparison of TPMT and NUDT15 polymorphisms in Chinese patients with inflammatory bowel disease" (NO:37638).

Dear Sir,

Here is our revised manuscript, which entitled " **Comparison of TPMT and NUDT15 polymorphisms in Chinese patients with inflammatory bowel disease** " (NO:37638), after a conscientious revision according to the editor and reviewers' kind and professional suggestions.

A detailed point-by-point response describing how we have responded to the specific points raised by the reviewers and editors is attached by the end of this cover letter. To save every possible space to limit the words of title, the original title "Comparision of the effects of TPMT and NUDT15 polymorphisms on azathioprine-induced leukopenia in Chinese patients with inflammatory bowel disease" has been changed to "Comparison of TPMT and NUDT15 polymorphisms in Chinese patients with inflammatory bowel disease". We have revised the whole manuscript and sought the language editing by MedE Editing Group- a professional English language editing company as you suggest so that there are so many red-colored adjustments and revision in the text.

We gratefully acknowledge the nice editors and reviewers, who had spent a lot of time and made great efforts to significantly improve our manuscript. We have done our best in the revision to respond positively and accordingly to all concerns. Thank you very much for your favorable decision on our manuscript.

Best wishes,

Hui-Xiang Yang, Ph.D.

January 15, 2018

Reviewer 00004011:

1 The authors should better described the genotyping section at material and methods, giving more details.

Response: Thanks for your good suggestion. In the revised manuscript, we have added some details to describe the genotyping section at *material and methods* on page 9.

The variants in the NUDT15 gene of R139C (c.415C>T, rs116855232) and the TPMT variants of TPMT*3C (p.Tyr240Cys, c.719A>G, rs1142345) were genotyped by pyrosequencing and results were validated by Sanger sequencing. Pyrosequencing and Sanger sequencing primers were designed with PyroMark® Assay Design software 2.0 (Qiagen) and PrimerQuest® Tool (IDT), respectively. The sequences of the forward, reverse and sequencing primers for rs116855232 were 5'-GTGGGTTCCTTGGGAAGAACTA-3', 5'-ATCCCACCAGATGGTTCAGATCTT-3' and 5'-GCTTTTCTGGGGACTG-3', respectively. The sequences of the forward, reverse and sequencing primers for rs1142345 were 5'-TGGGGAATTGACTGTCTTTTTGA-3', 5'-TCCATTACATTTTCAGGCTTTAGC-3' and 5'-GACTGTCTTTTTGAAAAGTT-3', respectively. Conditions for polymerase chain reaction was 35 cycles of 30 s at 95 ℃ for denaturation, 30 s at 57 ℃ for annealing and 30 s at 72 ℃ for extension.

2 References on different populations should be included: ie Ann Gastroenterol. 2012;25(3):249-253., J Clin Pharm Ther. 2010 Feb;35(1):93-7.

Response: After reading these two papers, we have found they are all valuable and very helpful for understanding the association between TPMT polymorphisms and

thiopurines-related adverse events in Greek populations. In the first paper, researchers observed the effect of TPMT polymorphism on TPMT enzymatic activity and the association of thiopurine toxicity with TPMT status. They suggested that carriers of at least one variant allele and both intermediate and absent TPMT activity had an increased risk of developing thiopurine-induced myelotoxicity. In the second paper, 97 patients were enrolled and ten of them developed adverse events while four of them had one of the variant alleles. Researchers have not found the relationship between TPMT and thiopurines-induced adverse events. And we have got similar conclusions in Chinese populations. We cite these two articles as evidence of our study(ref 6 and ref 17, respectively).

Reviewer 00159305:

1 There are many grammatical/syntax/spelling errors throughout the manuscript which should be corrected. Several paragraphs for all sections of the manuscript are rather confusing and should be rewritten. You should seek a copyediting service provided by professional English language editing company.

Response: We have carefully rechecked the manuscript to correct grammatical/syntax/spelling errors and have rewritten the confusing paragraphs. Meanwhile, we have sought the language editing service provided by MedE Editing Group and got the language editing certificate in accordance with your suggestions.

2 There are a huge number of abbreviations which makes difficult to read and follow the text by a clinicians (majority of readers of WJG). Moreover, all abbreviations should be explained and spelled out at the site where are first presented.

Response: Thanks for your good suggestion. We have reduced the use of abbreviations as much as possible. Also, we have explained and spelled out all abbreviations at the site where are first presented.

3 Abstract: is too long; should be rewritten and drawn more precisely and clear. There are too many repetitions ("meanwhile"…"appeared leukopenia", "higher reached"..etc.); (please, see page 3).

Response: Done in accordance with your professional comment. We have rewritten the abstract and reduced the repeated words (page 4, 5).

4 Methods: is your study prospective or retrospective? "Inclusion criteria included"; "exclusion criteria excluded"-please reformulate! You should include here the performance of logistic regression analysis.

Response: Our study is a prospective study. First, we detected the genotypes of TPMT and NUDT15. Then we provided azathioprine treatment to proper patients and followed up the blood tests of these populations to find the association between gene polymorphisms and azathioprine-induced leukopenia. We regret to misuse "included" in the original version. Done in accordance with your professional comment. "Inclusion criteria included" and "exclusion criteria included" have been changed to "Inclusion criteria were" and "exclusion criteria were", respectively (page 8, 9). The performance of logistic regression analysis has been mentioned in the materials and methods section on page 11.

5 Results section: too long, several paragraphs are confusing (page 9:"females//were

less than males"!); please, rewrite and make them clearer.

Response: Results section has been rewritten more precisely and clearly according to your professional comment. "females//were less than males" has been changed to "Fewer women than men developed leukopenia after receiving AZA (P = 0.039, OR 0.146, 95% CI 0.023–0.909, RR 0.527, PF 0.124)" on page 13.

6 Discussion: First paragraph is too long and included too many abbreviations on the AZA's metabolism! Second paragraph, first sentence is confusing: "…. life eventually terminate the therapy"-should be rewritten. Page 10, last two sentences are confusing; please rewrite it.

Response: Done in accordance with your professional comment.
We have rewritten the first paragraph of discussion and reduced using abbreviations on the AZA's metabolism (page 13). The sentence "…. life eventually terminate the therapy" has changed to "Although AZA is cost-effective, adverse reactions such as leukopenia may lead to severe and life-threatening infections that result in treatment discontinuation" (page 14) and the last two sentences on page 10 in original manuscript has been rewritten in the limitation section (page 16).

7 Did your study add any new information to those already known?

Response: We add some new information as follows: (1) gene polymorphism frequency distributions of TPMT and NUDT15 in Chinese population which hadn't been studied in previous work; (2) combined use of corticosteroid was a potential method to reduce the risk of azathioprine-induced leukopenia; and (3) we found that men had a higher risk of developing leukopenia after azathioprine therapy than women and need to be more closely monitored.

8 Please, mention the strength and limitations of your study.

Response: We have mentioned the strength and limitations of our study on page 15, 16.

Strength: In the present work, we have not only observed TPMT and NUDT15 polymorphisms but also estimated their values in predicting AZA-induced leukopenia in Chinese IBD patients. This is a prospective study that has a lot of clinical value. The results of our study are significant to optimize AZA therapy for Chinese patients with IBD and helpful to provide more data in Chinese populations and to further multicenter large-scale research. In addition, our study is the first to describe that combined use of corticosteroids and female gender are negatively associated with developing AZA-induced leukopenia.

Limitations: No patients with *NUDT15*(T/T) were enrolled in our study. Recent studies have detected additional variants of *NUDT15* except *R139C* including Arg139His, Val18Ile, and p.Val18_Val19insGlyVal and defined six haplotype (*1 to *6) combinations of these variants, but we have not investigated these in the present study. So, further multicenter studies with larger sample size are warranted in this area.

9 Conclusion: please, mention which gender?

Response: “Female gender” has been mentioned in the conclusion section in accordance with your professional comment (page 16).

Reviewer 02537773:

1 The language is of insufficient quality. The paper needs to be checked for typos: NDUT15. Paper is not adjusted to the guidelines of the WJG (for instance abstract is too long, formatting).

Response: Thanks for your kind suggestions. We have carefully revised the manuscript and adjusted to the guidelines of the WJG. MedE Editing Group, a professional English language editing company, has provided the editing service and the certificate guaranteeing the language of revised version has reached grade A. "NDUT15" has been corrected as "NUDT15".

2 Why did the authors decided to evaluate only one TPMT (3). There several other that are frequently used in addition (*2, *3A) in TPMT.

Response: We do agree to your comment that several other variant TPMT alleles have been studied frequently. Based on studies in Asian populations (J Gastroen Hepatol 2009; 24: 1258-1264 [PMID: 19682195] & Pharmacogenet Genom 2015; 25: 143-146 [PMID: 25564374]), TPMT *3C has been found the most frequent TPMT variant. Also, a study in a Chinese cohort has showed the most frequent mutant allele was TPMT *3C while TPMT *2, TPMT *3A and TPMT *3B were not detected (Clin Chim Acta 2007; 376: 45-51 [PMID: 16952345]).

3 The study design is not sufficiently explained: AZA-treatments subsection.

Response: we have provided more information in the revision to explain the AZA treatment on page 10. Initial dose of AZA was 0.5–1.5 mg/kg daily and patients who used AZA underwent routine blood tests every week during the first month, then

every 2 wk for 2 mo, followed by every month. If patients had no adverse reactions, then AZA dose was increased by 0.5 mg/kg daily every month to 1.0–2.0 mg/kg/d. If AZA was effective without any adverse reactions, patients should take it for life. If patients developed leukopenia (white blood cell count < 3.5 $\times 10^9$/L or neutrophils < 1.5 $\times 10^9$/L) and/or other severe adverse effects, the treatment was discontinued.

4 What is the explanation that initial dosage of AZA in C/T NUDT15 was higher as in C/C cohort?

Response: Thanks for your kind comment. We have compared the initial dosage of AZA in NUDT15 C/T and NUDT15 C/C cohort and found no statistical difference (P=0.596, Table 2).

5 The authors may consider complete presentation of the data in regard of IBD disease stage.

Response: Thank you for your suggestion to make us understand the inadequacies of the present work. We have count the IBD disease stage of patients and analyzed the relationship between IBD disease stage and AZA-induced leukopenia, finding no effect of IBD disease stage on AZA-induced leukopenia (Table 2, 3).

6 The authors should consider a chart plot presentation to follow the description: inclusions, AZA, TPMT/NUDT15 genotypes etc.

Response: Thanks for your professional point. We have added the following chart plot to describe these data more clearly, see figure 2, 3 and 4.

7 The authors may consider providing the protocol how the AZA was initiated and how the management was unified.

Response: This is such an important point. We have added the protocol in accordance with your professional comment (Figure 1).

8 What was the duration of the treatment?

Response: Thank you very much for pointing this out. The average (±SD) duration of AZA treatment in patients with NUDT15 C/C and NUDT15 C/T were 8.1 (±11.19) months and 9.3 (±9.95) months, respectively. The association between duration of the treatment and AZA-induced leukopenia analyzed by $\chi 2$ tests found no difference (P= 0.839, Table 2).

9 Data to odds-ration should be provided together with 95% confidence interval.

Response: Appreciations to your kind suggestion. We have added the 95% confidence interval of odds-ration in the revision, see on page 13 and Table 3.

NUDT15 polymorphism was significantly associated with AZA-induced leukopenia [P = 0.004, OR 7.663, 95% confidence interval (CI) 1.893–31.023]. Fewer women than men developed leukopenia after receiving AZA (P = 0.039, OR 0.146, 95% CI 0.023–0.909). And combination with glucocorticoids reduced the risk of AZA-induced leukopenia (P = 0.023, OR 0.201, 95% CI 0.050–0.798).

Response to other comments:

1 Comments to EDITORS: This manuscript is inadequate for publication in WJG as it stands now. It is poorly written, results are given in a confusing manner and conclusions are not clearly supported by them. It has to be thoroughly revised.

Response: Thanks for your kind suggestions. We have carefully revised the manuscript and MedE Editing Group has provided professional language editing service to improve our language.

2 Comments to AUTHORS: English usage is not very adequate throughout the text, and it makes difficult to readers to focus on the text. The text should be thoroughly revised. To mention but a few some samples follow:

P3 "…11 cases…appeared leukopenia…" change to "…11 cases…developed leukopenia" "The remain 216 patients…were found to be with the wide genotype…" change to "The remaining 216….were found to bear the genotype"

P4 Conclusion "NUDT15 polymorphism…compared that with TPMT" change to "NUDT polymorphism…..leukopenia than TPMT polymorphism.

P5 "IBD has became…" change to "IBD has become…"

P9 "It can be used to patients…" change to "In can be used in patients…"

P10 "TPMT*3C is the most popular variant…." Meaning?

P11 "We are the first found…." I guess what authors suggest is that "We are the first to describe that combined use…"

And some more allover the text. It requires extensive re-writing.

Response: Thanks for your kind suggestions.

"…11 cases…appeared leukopenia…" (P3, original version) has been changed to "…11…developed leukopenia"(P5, revision). "The remain 216 patients…were found

to be with the wide genotype…" has been changed to "The remaining 216 patients (98.6%) were found to bear the wild genotype of TPMT (A/A)".

Conclusion "NUDT15 polymorphism…compared that with TPMT" (P3, original version) has been changed to "NUDT15 polymophism is a better predictor for AZA-induced leukopenia than TPMT polymorphism" (P5, revision).

"IBD has became…" (P3, original version) has been changed to "IBD has become…"(P7, revision).

"It can be used to patients…" (P10, original version) has been changed to "It can be used in patients…" (P13, revision).

The sentence "TPMT*3C is the most popular variant…." (P10, original version) means that although more than forty different kinds of TPMT alleles (TPMT*2–*41) have been studied, TPMT*3C is the most popular variant in Asians among them. We have rewritten this sentence more clearly (P14, revision).

"We are the first found…." (P11, original version) has been changed to "We are the first to describe that combined use…"(P15, revision)

We have carefully revised the whole manuscript and MedE Editing Group has provided professional language editing service to improve our language.

3 In addition, the text is confusing regarding the number of patients involved. Although 219 patients are initially mentioned, only 80 were treated with AZA, and only those were used for comparisons. The 219 initially mentioned are irrelevant and the sentence in the RESULTS section "In total 219 patients participated…" is misleading. Table 1 should be modified accordingly.

Response: Appreciations to your professional suggestion. One of our purposes is to observe the gene polymorphism frequency distributions of TPMT and NUDT15, so we have detected TPMT and NUDT15 genotypes of all enrolled 219 patients even though some of them do not receive AZA treatment. In addition, we have analyzed the

difference between variation of TPMT and that of NUDT15, concluding that mutation rate of TPMT (1.4%) was significantly lower than that of NUDT15 (20.1%) (P = 0.000, Table 1). We have rewritten the Results section more clearly in the revision (page 11).

4 The number of patents tested is thus low to reach firm conclusions.

Response: Appreciations to your professional suggestions making us understand the limitation of the present work. We'll try to improve the scale of patients and obtain more results in accordance with your suggestions in the future.

5 Moreover, results are presented in a confusing manner and the reader cannot reach the conclusions given. For instance, how is the protective factor of corticosteroid usage and female gender granted?

Response: Thanks for your suggestion. We have rewritten the results section. In the present work, logistic regression analyses have shown that combined use of corticosteroids reduced the risk of AZA-induced leukopenia (P = 0.023, OR 0.201, 95% CI 0.050–0.798, RR 0.437, PF 0.253) and women were less likely to develop leukopenia after receiving AZA than men(P = 0.039, OR 0.146, 95% CI 0.023–0.909, RR 0.527, PF 0.124). We conclude that the incidence of AZA-induced leukopenia negatively correlates with corticosteroid usage and female gender. The exact mechanism remains unclear. We supposed that might be related to the functions of corticosteroid and gender differences in pharmacokinetics. More specifically, corticosteroids have the effects of enhancing bone marrow hematopoiesis and promoting neutrophil release and increasing the number of circulating neutrophils, as well as reducing the infiltration and consumption of neutrophils in the inflammatory regions which may help reduce the risk of AZA-induced leukopenia. Females may

have a lower apparent volume of distributions than males because of the solubility of the drug so that AZA might be removed more quickly in females, which reduces the risk of AZA-induced leukopenia. However, since more men received AZA treatment than women in our study, further research is necessary to verify this relationship and find out the precise mechanism.

5 Additionally, I suggest authors that in addition to p values, Relative Risk (RR) and etiological fraction (δ) values should be calculated, much like what has been done in HLA and disease susceptibility studies, to study the implication of different (NUDT and TPMT) genes in AZA-mediate leukopenia.

Response: Thanks for your professional and helpful suggestions. We've calculated the Relative Risk (RR) and etiological fraction (δ) values and added these data in the revision (both of page 13 and Table 3).

7 Finally, it is unclear why this work is relevant. Authors mention a previous work by Zhu X et al (ref 17) that seems to address the same question and with similar results (apparently, more statistically sound) that the authors address in the current manuscript. Hence, authors should stress the novelty of their work.

Response: Thank you for pointing this out. Zhu X *et al* have done the similar work to observe the effect of NUDT15 polymorphism on thiopurine-induced leukopenia in Chinese patients with Crohn's disease. They recruited patients who had already received AZA treatment to detect the genotype. Different from their work, genotypes of TPMT and NUDT15 were detected before patients received AZA therapy in our study. This is a prospective study that has a lot of clinical value. In addition, we compared the gene polymorphism frequency distributions of TPMT and NUDT15. And our study was the first to describe other factors- such as corticosteroid usage and

female gender- were related to the risk of developing AZA-induced leukopenia. The results of our study are significant to optimize AZA therapy for Chinese patients with IBD and helpful to provide more data in Chinese populations and to further multicenter studies with larger sample size. We have added explanation of the novelty of our work in the revision (page 15).

Great appreciations to the editors and the professional reviewers for all your valuable time and expert opinions, which help us to significantly improve our manuscript.