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Body Mass Index and Smoking Affect Thioguanine Nucleotide Levels in Inflammatory Bowel Disease

Shi Sum Poon.[a] Rebecca Asher,[b] Richard Jackson,[b] Andrew Kneebone,[a] Paul Collins,[a] Chris Probert,[a] Martyn Dibb,[a] Sreedhar Subramanian[a]

[a]Department of Gastroenterology, Royal Liverpool University Hospital, Liverpool, UK [b]Cancer Research UK Liverpool Cancer Trials Unit, Waterhouse Building, Liverpool, UK

Corresponding author: Sreedhar Subramanian, MD, MRCP, Department of Gastroenterology, Royal Liverpool University Hospital, Prescot Street, Liverpool L7 8XP, UK. Tel.: +44-151-706 3414; fax: +44-151-706 5832; email: sreedhar.subramanian@rlbuht.nhs.uk

Abstract

Introduction: Optimal levels of the thiopurine metabolite, 6-thioguanine nucleotides [6-TGN] correlate with remission of inflammatory bowel disease [IBD]. Apart from variations in the thiopurine methyl transferase [TPMT] gene, little is known about other predictors of 6-TGN levels. Obesity adversely affects response to infliximab and adalimumab and clinical course in IBD, but little is known about the interaction of thiopurines and obesity. We investigated the relationship between body mass index [BMI] and 6-TGN levels and sought to examine other predictors of 6-TGN levels.

Methods: This retrospective cohort study included patients with concurrent measurements of 6-TGN and BMI. The association between 6-TGN and clinical variables including BMI was estimated using a multivariable linear regression model.

Results: Of 132 observations, 77 [58%] had Crohn's disease and 55 [42%] ulcerative colitis. BMI, smoking, and TPMT levels were associated with 6-TGN levels in multivariable analysis. Every 5 kg/m^2 increase in BMI was associated with an 8% decrease in 6-TGN (0.92; 95% confidence interval [CI] 0.87–0.98; $p = 0.009$). Smokers had higher 6-TGN levels in comparison with non-/ex-smokers [1.43; 95% CI 1.02–2.02; $p = 0.041$]. Patients with intermediate TPMT had higher 6-TGN compared to those with normal levels [2.13; 95% CI 1.62–2.80; $p < 0.001$]. Obese patients were more likely to have sub-therapeutic 6-TGN levels and a higher methyl mercaptopurine nucleotide [MMPN/TGN] ratio despite a similar dose of thiopurines.

Conclusions: Active smoking and intermediate TPMT values were associated with higher 6-TGN levels but increasing BMI resulted in lower 6-TGN and higher MMPN levels. This may explain the worse outcome that has been reported previously in obese IBD subjects.

Keywords: Inflammatory bowel disease; Crohn's disease; ulcerative colitis; thiopurines; thioguanine nucleotides; body mass index; smoking

1. Introduction

Inflammatory bowel disease [IBD] is a lifelong disease and often requires long-term immunosuppressive treatment. The thiopurines, mercaptopurine [MP] and azathioprine [AZA], are commonly prescribed for maintenance of remission in Crohn's disease [CD] and ulcerative colitis [UC].[1,2] Previous meta-analyses have established the efficacy of thiopurines in both CD [3] and UC.[4] However, two

recent prospective clinical trials suggested that early administration of azathioprine was no more effective than placebo to achieve corticosteroid-free remission in CD.[5,6] Several factors could explain this apparent discrepancy in efficacy, including heterogeneity among study subjects and lack of monitoring and thiopurine optimisation based on measurement of the thiopurine metabolite profile. Previous studies have established the improved efficacy of thiopurines with optimal 6-TGN levels. Two separate meta-analyses have shown an association between optimal 6-TGN levels and clinical remission in patients with IBD, suggesting that therapeutic monitoring of thiopurine metabolites improves disease outcome.[7,8] This is in keeping with emerging evidence of poor correlation between weight-based dosing regimens of thiopurines and 6-TGN levels.[9,10,11,12,13]

Despite the variable clinical response to thiopurines, few studies have addressed clinical predictors of response to thiopurines in IBD. A previous study reported that body mass index [BMI] influenced response to AZA,[14] with a better outcome in ulcerative colitis [UC] patients with a BMI < 25 and Crohn's disease [CD] patients with a BMI > 25. However, this study did not examine the relationship between obesity and 6-TGN levels as a possible explanation for the differential responsiveness. Obesity has also been reported to negatively affect response to the anti-tumour necrosis factor [TNF] agents, infliximab and adalimumab.[15,16,17] In keeping with this, obesity adversely affects disease outcomes as obese IBD subjects are more likely to be hospitalised and develop active disease as compared with non-obese IBD subjects.[18] In light of this, we systematically examined the relationship between multiple clinical variables including body weight, BMI, body fat index [BFI], and 6-TGN levels.

2. Methods

2.1. Subjects and data collection

We conducted a retrospective cohort study of patients with IBD treated with AZA and MP in our institute between January 2011 and September 2013. All patients who had concurrent measurements of 6-TGN, height, and weight were included in this analysis. Patients with incomplete measurements were excluded from the analysis. Case notes of eligible subjects were abstracted for the following information: patient demographics, dose of AZA or MP, disease type [UC or CD], smoking status, thiopurine methyltransferase [TPMT] level, height, weight, BMI, concurrent steroid use, and other concomitant medications at the time of 6-TGN measurement. Patients had to be on a stable dose of thiopurines for at least 3 months before inclusion in the study. In order to examine the association of weight-based dosage to 6-TGN concentrations, the dose of 6-MP was adjusted by a factor of 2.07.[19] Thiopurine metabolite testing was undertaken at the discretion of the clinician. Patients with normal TPMT were started at an initial dose of 50 mg azathioprine and escalated to a target dose of 2.5 mg/kg for azathioprine and 1.5 mg/kg mercaptopurine after 2 weeks, failing which patients were escalated to a maximum tolerated dose. For patients with intermediate TPMT, the target dose was 50% of that for individuals with normal TPMT levels. Patients were allowed to contribute more than one measurement of 6-TGN as there could have been a change in BMI, thiopurine dose, smoking status, or disease activity. Patients were classed as being of normal weight [BMI< 25] or overweight [BMI > 25]. Body fat index [BFI] was calculated as described previously using BMI, age and sex.[20] This method was shown to correlate accurately with body fat measurement using air displacement plethysmography.[20] The study was approved by the audit department of the Royal Liverpool University Hospital.

2.2. Metabolite and TPMT assay

Red blood cell TPMT activity was determined when patients had not been transfused for the past 2 months, using the previously described method.[21] TPMT levels were defined as normal [26–50 pmol/h/mgHb], intermediate [10–25 pmol/h/mgHb] or deficient [< 10 pmol/h/mgHb]. Patients on concomitant medications that might affect TPMT activity, eg allopurinol, diuretics, angiotensin converting enzyme inhibitors [ACEI], were excluded.

Metabolite measurements were performed on whole blood with ethylenediamine tetraacetic acid [EDTA] by the Purine Research Laboratory, Viapath, St Thomas' Hospital, London, using a modification of the method of Dervieux *et al*.[22] Briefly, thioguanine nucleotides were reduced to the thiopurine base by heating with perchloric acid followed by separation using reverse phase liquid chromatography on a Waters UPLC system with ultraviolet detection at 340 nm for 6-TGN and 303 nm for 6-methyl mercaptopurine nucleotides [6-MMPN]. Calibrators for 6-TGN and 6-MMPN were obtained from Sigma [Poole, UK]; 6-TGN levels were measured as pmol/8 x 10^8 red blood cells [RBCs] and classed as sub-therapeutic [< 230 pmol/8 x 10^8 RBCs], therapeutic [230–450 pmol/8 x 10^8 RBCs] and supra-therapeutic [> 450 pmol/8 x 10^8 RBCs] based on previously published thresholds.[8]

2.3 Statistical analysis

Categorical variables have been summarised as frequency [%] and continuous variables as mean [SD]; 6-TGN was log-transformed before analysis on account of its non-normal distribution. The univariate association of each variable with 6-TGN was investigated using regression analysis.

Multivariable linear regression was used to model 6-TGN. Univariate associations which gave a *p*-value less than 0.25 were included as candidate variables to be modelled alongside BMI and dose [per kg]. A stepwise model selection procedure was performed which employed a significance level of 0.05 for addition into the model and 0.1 for removal. To investigate the association of BMI with 6-TGN and 6-MMPN, univariate regression analyses were conducted.

All regression analysis was implemented using robust standard errors which allowed for the intragroup correlation to take into account the repeated measures nature of the data. Analysis was performed using Stata v13 software [Stata Statistical Software: Release 13. College Station, TX: StataCorp LP].

3. Results

3.1. Baseline characteristics

A total of 105 patients with concurrent measurements of 6-TGN, height, and weight were identified and contributed a total of 134 observations. Two patients had undetectable 6-TGN levels [documented non-adherence] and were excluded from further analyses. A total of 132 observations [62 male, 70 female] met our pre-specified inclusion criteria and were included in the study. The characteristics of the included subjects are summarised in Table 1; 60 patients [45%] had sub-therapeutic levels of 6-TGN, 48 [36%] had levels within the therapeutic range, and 24 [18%] had levels above the therapeutic range. A total of 14 patients [11%] were treated with MP and the remainder were on azathioprine. Neither diagnosis [UC or CD] nor concomitant prescription of 5-aminosalicylic acid [5-ASA] or anti-TNF agents influenced 6-TGN levels in a univariate analysis [Table 1].

Table 1. Baseline characteristics of study subjects. 6-TGN levels are presented according to the variable of interest.

Variable	Frequency	6-TGN [pmol/8 x 10^8 RBCs]	Univariate analysis	*p*-Value
Sex				
Male	62 [47%]	288.9 [146.3]	0.96 [0.75, 1.24]	0.766
Female	70 [53%]	316.7 [253.0]		
Mean age [SD], years	35.8 [17.2]	-	1.00 [1.00, 1.01]	0.520
Mean weight [SD], kg	71.9 [22.0]	-	0.99 [0.99, 1.00]	0.001
Mean BMI [SD], kg/m^2	25.0 [7.3]	-	0.98 [0.97, 0.99]	0.002
Diagnosis				
Crohn's disease	77 [58%]	282.9 [192.3]	1.16 [0.91, 1.50]	0.231
Ulcerative colitis	55 [42%]	337.1 [230.6]		
Smoking status:				
Non- or ex-smoker	101 [77%]	277.9 [162.9]	1.50 [1.07, 2.12]	0.021
Current smoker	22 [16%]	446.1 [335.0]		
Unknown	9 [7%]	-		
TPMT levels				
Intermediate	10 [8%]	521.4 [313.6]	1.78 [1.27, 2.49]	<0.001
Normal	83 [63%]	287.1 [207.4]		
Unknown	39 [29%]			
Mean dose [SD] of thiopurine [mg/kg]	1.7 [0.7]	-	1.01 [0.85, 1.21]	0.879
BMI				
Normal [< 25]	80 [61%]	328.7 [196.8]	0.78 [0.62, 0.97]	0.029
Overweight or Obese [> 25]	52 [39%]	269.6 [226.0]		
Concomitant medications				
Steroids				
Yes	33 [25%]	256.0 [154.3]	0.82 [0.60, 1.10]	0.182
No	97 [73%]	319.5 [225.0]		
Missing	2 [2%]			
5-ASA				
Yes	67 [51%]	332.6 [215.3]	1.21 [0.94, 1.55]	0.144
No	65 [49%]	277.5 [202.1]		
Anti-TNF therapy				
Yes	26 [20%]	361.5 [264.5]	1.22 [0.91, 1.63]	0.190
No	106 [80%]	291.7 [193.3]		

RBCs, red blood cells; TPMT, thiopurine methyl transferase; 5-ASA, 5-aminosalicylic acid; TNF, tumour necrosis factor.

3.2. Weight-based dosing and 6-TGN nucleotides

There was no significant association between weight-based dosing and 6-TGN nucleotides [Figure 1, $p = 0.879$]. More specifically, the mean [SD] dose per kg of thiopurine was 1.70 mg/kg [0.66], 1.63 mg/kg [0.64], and 1.81 mg/kg [0.68] in the sub-therapeutic, therapeutic, and supra-therapeutic groups respectively. There was a significant association between TPMT and 6-TGN levels [$p < 0.001$]; TPMT carriers had higher 6-TGN levels compared with patients with normal TPMT levels [521.5 ± 313.6 vs 287.1 ± 207.4 pmol/8 x 10^8 RBCs].

3.3 Predictors of 6-TGN nucleotides

A multivariable model was constructed to examine factors associated with 6-TGN levels. Concurrent 5-ASA use, concurrent corticosteroid use, smoking status, BMI, and TPMT levels were significantly associated with 6-TGN levels [Table 2]. An increase in BMI resulted in a decrease in 6-TGN; every 5 kg/m^2 in BMI was associated with a 8% decrease in 6-TGN levels [0.92; 95% CI 0.87–0.98; $p = 0.009$]. Current smokers had higher levels of 6-TGN in comparison with non- or ex- smokers [1.43, 95% CI 1.02–2.02; p 0.041]. Patients with intermediate TPMT levels had higher 6-TGN in comparison with those with normal TPMT levels [2.13, 95% CI 1.62–2.80; $p < 0.001$]. Furthermore, lower 6-TGN levels were found in those who were using concurrent steroids [0.70; 95% CI 0.53–0.93; $p = 0.013$] and higher levels found in those using concurrent 5-ASA [1.30; 95% CI 1.02–1.65; $p = 0.013$].

3.4. Relationship between weight, BMI, BFI, and 6-TGN

We next investigated the relationship between weight, BMI, BFI, and 6-TGN levels. Weight, BMI, and BFI showed an inverse relationship with 6-TGN levels [Figure 2a–c]. Patients with sub-therapeutic levels had higher weight, BMI, and BFI [Table 3]. There was a significant association between categorical 6-TGN and categorical BMI [$p = 0.029$, Table 4] with a higher proportion of sub-therapeutic TGN levels found in patients with a BMI > 25. Patients with BMI > 30 had a higher MMPN:TGN ratio and were also more likely to have a skewed thiopurine metabolism with an MMPN:TGN ratio > 11. There were no differences in TPMT values or dose per kg that could have accounted for the differences in TGN or MMPN:TGN ratio [Table 4]. The frequency of 5-ASA co-prescription was similar in patients with BMI < 25 and those with BMI > 25 [49% vs 50%, $p = 0.842$]

4. Discussion

In this study, we report some novel predictors of TGN nucleotide levels. BMI was inversely related to 6-TGN levels while holding the dose per kg constant. This difference was not accounted for by variations in TPMT levels between overweight and normal individuals. It is well known that individuals with intermediate TPMT activity need a lower dose of thiopurines to achieve a similar 6-TGN level.[23] We also did not note a significant difference in the concomitant prescription

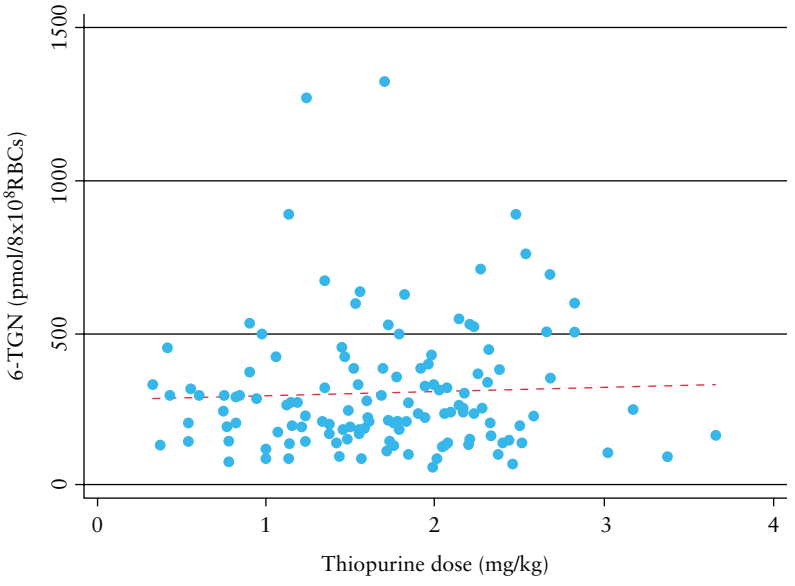

Figure 1. Relationship between weight-based dosing of thiopurine and 6-thioguanine nucleotide levels.

Table 2. Multivariable model of factors associated with 6-TGN levels.

	Estimate	Exp	95% CI	*p*-Value
BMI [5 kg/m^2]:	-0.08	0.92	[0.87, 0.98]	0.009
Dose per kg [0.5 mg/kg]:	0.06	1.06	[0.98, 1.15]	0.130
Concomitant 5-ASA use:	0.26	1.30	[1.02, 1.65]	0.034
Smoking:	0.36	1.43	[1.02, 2.02]	0.041
Concomitant steroid use:	-0.35	0.70	[0.53, 0.93]	0.013
TPMT range:				
Carrier	0.75	2.13	[1.62, 2.80]	<0.001
Unknown	0.02	1.02	[0.81, 1.28]	0.891

TPMT, thiopurine methyl transferase; 5-ASA, 5-aminosalicylic acid.

of 5-aminosalicylates between overweight and normal individuals. It has been previously reported that 5-ASA compounds inhibit the activity of TPMT and increase the production of 6-TGN.[24] Thus, the differences may be related to bioavailability, drug distribution, metabolism, or excretion. The oral bioavailability of AZA ranges from 27% to 83% and that of MP from 5% to 37%,[25,26] but little is known about the distribution of thiopurines in various compartments. Obesity is known to affect the distribution, clearance, and elimination half-life of a variety of drugs such as antibacterials and anticonvulsants,[27] but the impact of obesity on thiopurine distribution and clearance is unknown. The inverse relationship of 6-TGN and BFI observed in our study indicates that adipose tissue distribution of thiopurines may be an important determinant of inter-individual variability of thiopurine efficacy. Besides distribution, adipose tissue may also modulate thiopurine metabolism due to an increased expression of Kruppel-like factor [KLF14]. KLF14 transregulates expression of several genes in adipose tissue, including TPMT.[28] This may explain the skewed metabolism towards methylation observed in heavier individuals. Our observation supports the use of allopurinol and low-dose thiopurine in individuals with a BMI > 30 as this strategy has been reported to correct the skewed metabolism towards methylation.[29]

Our findings are consistent with limited observations by others that obesity adversely affects outcomes of IBD therapy. A previous study by Holtmann *et al.* reported that with AZA therapy UC patients with a BMI > 25 had a higher incidence of flares as compared with patients with BMI < 25, whereas this effect was not observed in CD.[14] This study did not look at the effect of BMI on 6-TGN levels, but our observation provides a possible mechanistic explanation for the adverse outcomes observed by Holtmann. Somewhat intriguingly, a similar interaction between increased BMI and adverse clinical outcome was reported for infliximab, the only anti-TNF adjusted for body weight, by Harper and colleagues.[17] However, the authors were unable to verify if the observed phenomenon was due to low circulating levels of infliximab. BMI has also been noted to independently predict the need for adalimumab dose escalation.[15,16] These observations together support the notion that obesity perhaps negatively impacts on drug efficacy in IBD, perhaps due to the involvement of visceral fat in the pathogenesis of IBD, particularly in CD. It has been observed that despite being underweight, the ratio of intra-abdominal fat to total fat is greater in CD patients as compared with controls, when assessed by magnetic resonance scanning.[30] The same authors reported that mesenteric adipocytes were a major source of the proinflammatory mediator, TNF alpha. Other possible mechanisms for adipose tissue in IBD pathogenesis include a proinflammatory role for the adipokine leptin in animal models of colitis,[31] and a potential role for adiponectin in down-regulating inflammatory responses.[32] Despite these plausible mechanisms, no association was found between BMI and the risk for CD or UC in a European prospective cohort study.[33] Thus, obesity appears to be involved in affecting response to drugs in IBD but does not seem to have a role in the aetiology of IBD.

Our observations are relevant also as obesity is an increasing problem confronted by clinicians managing IBD patients. For instance, Steed *et al.* report a similar proportion of obese [BMI > 30 kg/m^2] subjects in their IBD population compared with the general population.[34,35] In the paediatric setting, the prevalence of overweight or obesity in IBD patients was 23.6%.[35] Obese IBD patients have worse outcomes, with shorter time to first surgery in CD, more frequent anoperineal[36] and penetrating[37] complications in CD, and increased rate of surgery in paediatric UC patients.[35] Data from us and others suggest that careful attention to optimising therapy by close therapeutic drug monitoring may be helpful in obese IBD subjects.

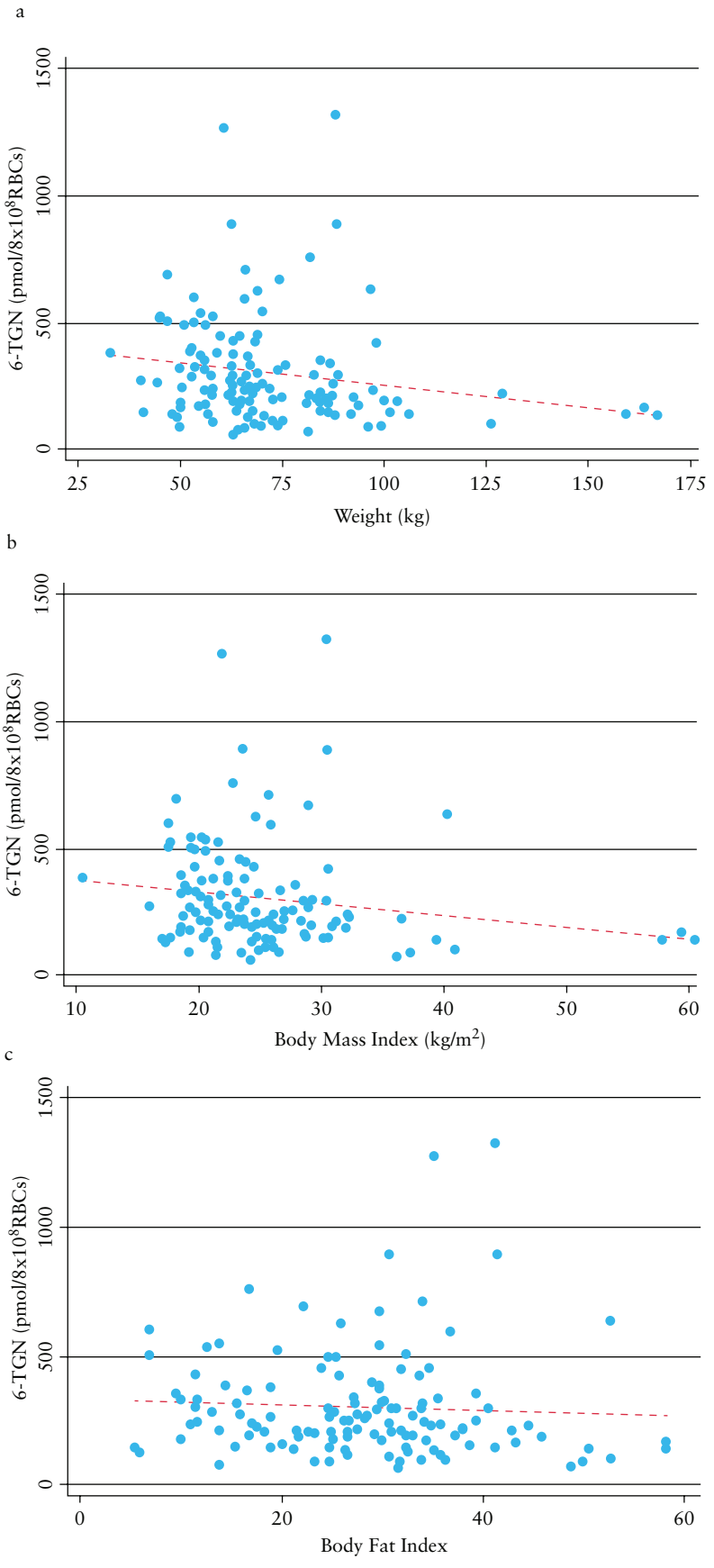

Figure 2a–c. Relationship between 6-thioguanine nucleotides [6-TGN] levels and weight [2a], BMI [2b] and body fat index [BFI] [2c].

Table 3. Relationship between BMI, BFI, weight and categorical 6-TGN levels; mean [SD].

	TGN Level			p-Value
	Sub- $n = 60$	Therapeutic $n = 48$	Supra- $n = 24$	
Weight	79.8 [26.6]	66.5 [14.4]	63.2 [14.4]	**0.001**
Body mass index	27.3 [9.0]	23.2 [4.3]	22.9 [5.4]	**0.009**
Body fat index	30.6 [12.2]	25.0 [10.1]	26.8 [11.4]	0.075
Thiopurine dose [mg/kg]:	1.70 [0.66]	1.63 [0.64]	1.81 [0.68]	0.805

6-TGN, 6-thioguanine nucleotides; BFI, body fat index.

Table 4. Relationship between BMI, categorical 6-TGN levels and 6-MMPN levels.

	BMI < 25	25 ≤ BMI < 30	BMI ≥ 30	p-Value
	$n = 80$	$n = 32$	$n = 20$	-
6-TGN pmol/8 x 10^8 RBCs	284 [198–428]	209 [150–296]	189 [142–266]	0.0148
Sub-therapeutic	28 [35%]	19 [59%]	13 [65%]	0.029
Therapeutic	34 [43%]	10 [31%]	4 [20%]	
Supra-therapeutic	18 [23%]	3 [9%]	3 [15%]	
Thiopurine dose per kg	1.69 [0.63]	1.62 [0.73]	1.83 [0.63]	0.660
MMPN:TGN ratio	2.35 [0.77–7.30]	2.65 [1.02–6.80]	10.64 [3.61–18.04]	0.034
MMPN:TGN ratio < 11	68 [85%]	26 [81.3%]	10 [50%]	0.016
MMPN:TGN ratio ≥ 11	12 [15%]	6 [19%]	10 [50%]	
TPMT [$n = 89$] Mean [SD]	34.61[8.06]	34.21 [6.61]	35.64 [5.58]	0.820

6-TGN, 6-thioguanine nucleotides; MMPN, methyl mercaptopurine nucleotides; RBCs, red blood cells; TPMT, thiopurine methyl transferase.

The increase in 6-TGN nucleotides observed in smokers is intriguing. The effects of smoking on disease pathogenesis and recurrence are well known[38] but its effect on response to drugs is less well studied. Active smoking did not influence response to thiopurines in a retrospective study of 163 IBD patients treated with thiopurines, but active smokers were more likely to develop adverse events with thiopurines.[39] This suggests an as yet unreported interaction between smoking and thiopurine metabolism. Interestingly, smoking has been reported to upregulate xanthine oxidase [XO] activity in gastric mucosa[40] and pulmonary endothelial cells.[41] XO is involved in thiopurine metabolism, though up-regulation of XO would be expected to lead to a reduction in 6-TGN levels. It is possible that smoking may affect other enzymes involved in thiopurine metabolism and this would explain our observation as well as the increased incidence in smokers of adverse events with thiopurines. Further studies are warranted to confirm our observations. Finally, the association of reduced TGN levels with corticosteroid use is likely a reflection of need for steroids in patients with sub-therapetic thioguanine nucleotide levels.

We acknowledge that our study has some limitations. The major limitation is the retrospective nature of our study, which hinders any assessment of causality in the observed interaction between BMI, smoking, and 6-TGN nucleotides. It is well known that thiopurine metabolite levels are subject to fluctuation and, due to our study design, we are unable to confirm if the differences observed could have been accounted for merely by laboratory variations in 6-TGN levels. However, we would have expected this phenomenon to be spread uniformly across the various groups. The repeated sampling on a small proportion of the patients introduced intra-patient correlation, causing dependence between observations which violated the modelling assumptions. As only 20 patients gave multiple observations, it was not feasible to analyse the data using a linear mixed effects model; however, the alternative method that was adopted took this intra-patient correlation into account.

Thiopurine metabolite testing was undertaken at the discretion of the clinician. This could have introduced some bias as clinical testing would have been typically undertaken in the setting of lack of response. Our findings need to be replicated in a prospective cohort with metabolite testing at regular intervals. Finally, it is well known that BMI is an imperfect marker of obesity and does not discriminate between adipose tissue and muscle mass. We have included body fat index to circumvent this limitation, but prospective studies may consider the use of more widely validated indices, such as fat free mass index or skeletal muscle index, by inclusion of biometrical impedance analysis.

In conclusion, we report novel factors that influence 6-TGN nucleotide levels. These may explain some of the variations in results seen among efficacy trials of thiopurines. Design of future clinical trials of thiopurines in IBD should consider the effect of smoking and BMI on thiopurine metabolism.

Author contributions

SP, AK, and MD were involved in data collection and drafting of the manuscript. RA and RJ were involved in data analysis. PC and CP were involved in drafting and final revision of the manuscript. SS was involved in data collection, analysis, drafting, and revision of the manuscript.

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Conflicts of interest

SSP, RA, and RJ have no conflicts of interest. CP is/has been on the speaker bureau and/or an advisory board member for Dr Falk pharmaceuticals, Ferring, MSD, Takeda, Warner Chilcott, Hospira,

and Abbvie. MD has received an educational grant from Abbvie and MSD and speaker honorarium from Abbvie. PC has received speaker fees from Dr Falk pharmaceuticals, MSD, and Ferring, and educational support from Dr Falk, MSD, Ferring, and Given. SS has received speaker fees from MSD, Actavis, Abbvie, Dr Falk pharmaceuticals, and Shire, and educational grants from MSD, Abbvie, and Actavis, and is an advisory board member for Abbvie, Dr Falk pharmaceuticals, and Vifor pharmaceuticals.

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