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***TPMT* but not *ITPA* gene polymorphism influences the risk of azathioprine intolerance in renal transplant recipients**

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Abstract

Purpose Thiopurine drugs have to be withdrawn in 10–30% of cases due to side effects, and it has been presented that genetic factors may be responsible for some of reported toxicity cases. Among polymorphic enzymes of thiopurines' metabolic pathway, thiopurine S-methyltransferase (TPMT) has been studied most extensively, and some recent studies point to inosine triphosphate pyrophosphohydrolase (ITPA) polymorphism as an additional toxicity risk factor.

Methods The aim of the current study was to evaluate an association between *TPMT* and *ITPA* gene polymorphisms and drug intolerance in a cohort of 157 renal transplant recipients treated with azathioprine (AZA). Each subject was genotyped for the presence of variant *TPMT* (*2, *3A, *3B, and *3C) and *ITPA* (94C>A and IVS2+21A>C) alleles. **Results** Mean AZA dose, mean white-blood-cell count, and platelet count in the course of treatment were lower in carriers of variant *TPMT* alleles compared to patients with *TPMT* wild-type genotype. Leukocyte numbers fell below $4.0 \times 10^9/L$ in 41.2% of *TPMT* heterozygous renal transplant recipients, compared to only 18.0% of wild-type patients ($P < 0.01$). In contrast, *ITPA* genotype did not influence AZA dose, hematological parameters, or leucopenia risk.

Conclusions Our results suggest that routine genotyping of renal transplant recipients for *TPMT* variants may be useful

in reducing the risk of AZA-related myelotoxicity, but there is not enough evidence to introduce *ITPA* testing into clinical practice.

Keywords *TPMT* · *ITPA* · Azathioprine · Renal transplantation · Pharmacogenetics

Introduction

Purine analogue drugs such as azathioprine (AZA), 6-mercaptopurine (6-MP), or 6-thioguanine (6-TG) have been in clinical use for decades as immunosuppressive, immunomodulatory, and cytotoxic agents. They are used to treat patients with acute lymphoblastic leukemia, inflammatory bowel disease, rheumatoid arthritis, some dermatological conditions, and in transplantation medicine. Unfortunately, thiopurine drugs have to be withdrawn in 10–30% of cases due to side effects; among the most serious are bone-marrow toxicity and hepatotoxicity [1, 2].

It has been argued that genetic factors may be responsible for some of reported toxicity cases. Among polymorphic enzymes of thiopurines' metabolic pathway, thiopurine S-methyltransferase (TPMT) has been studied most extensively. TPMT catalyzes the S-methylation of anticancer and immunosuppressant thiopurine drugs to inactive metabolites, and TPMT deficiency is associated with severe, sometimes even fatal hematopoietic toxicity after standard doses of thiopurine drugs since TPMT-deficient patients accumulate very high concentrations of toxic thioguanine nucleotides in tissues [3, 4]. Approximately 1 in 300 Caucasian patients is homozygous for variant *TPMT* allele (most commonly *TPMT**3A, *TPMT**2, or *TPMT**3C) and is at high risk of severe bone-marrow suppression [5]. About 10% of the population is heterozy-

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gous for a variant allele and experiences intermediate *TPMT* activity [2, 6]. The clinical impact of heterozygosity in the *TPMT* gene is not so obvious, but some studies have shown an association between heterozygosity and AZA or 6-MP-related myelotoxicity [7].

Inosine triphosphate pyrophosphohydrolase (ITPA) catalyzes the pyrophosphohydrolysis of inosine triphosphate (ITP) to inosine monophosphate (IMP). Deficient ITPA activity leads to intracellular accumulation of ITP, which has not been associated with any pathology but would, under thiopurine therapy, lead to accumulation of unusual thioinosine metabolites (thio-ITP) with the potential for adverse metabolic effects [8]. ITPA activity is also genetically determined: patients homozygous for a 94C>A missense mutation (Pro32Thr) are characterized by no enzyme activity, while homozygotes for the other functional polymorphism in intron 2 (IVS2+21A>C) and compound heterozygous subjects express about 30% of the wild-type median value [9, 10].

The aim of the current study was to evaluate the possible association between *ITPA* gene polymorphism and drug intolerance in a cohort of renal transplant recipients treated with AZA as a part of a standard immunosuppressive regimen. Additionally, we aimed to verify the results of our previous study investigating the impact of *TPMT* variants on AZA toxicity [11] in a larger patient group.

Patients and methods

Peripheral blood samples were collected from 157 renal transplant recipients who met the study criteria. The patients underwent kidney transplantation from cadaveric donors from 1995 to 2005 in the transplantation department of County Hospital, Szczecin, Poland, and received AZA as a part of standard immunosuppressive therapy (AZA, cyclosporine, and prednisone) after organ transplantation. All the subjects were of Polish nationality and Caucasian origin. Patients were excluded from the study if AZA was replaced by another immunosuppressant (mycophenolate mofetil) for reasons other than myelotoxicity, failure to maintain graft function during the first year after transplantation or allopurinol medication (a drug that has been shown to interact with AZA).

All subjects were initially given AZA (Imuran, Glaxo Wellcome) at an oral dose of approximately $2.5 \text{ mg kg}^{-1} 24 \text{ h}^{-1}$, which was reduced to $1.5 \text{ mg kg}^{-1} 24 \text{ h}^{-1}$ during the first week of treatment. The AZA dose was modified in case of myelotoxicity as follows: it was reduced by 50% if a patient's WBC count fell below $4.0 \times 10^9/\text{L}$, and in the case of severe leukopenia (defined as WBC count below $3.0 \times 10^9/\text{L}$), AZA was withdrawn until white-blood-cell count recovery, but

maintenance doses did not exceed $1.0 \text{ mg kg}^{-1} 24 \text{ h}^{-1}$. AZA dose was also modified for other reasons, such as the drug hepatotoxicity, viral infection (HBV, CMV), or if the patient experienced a graft rejection episode, continuous anemia, or thrombocytopenia.

All patients were given cyclosporine A (Sandimmun or Neoral). The initial dose was approximately $7 \text{ mg kg}^{-1} 24 \text{ h}^{-1}$, and it was modified according to drug whole-blood level monitoring during the course of treatment. Additionally, in some cases, a decrease in cyclosporine dose precipitated by its toxicity (nephrotoxicity, neurotoxicity) was compensated for by an increased dose of AZA. The patients included in the study were also administered prednisone (Encorton, Polfa Pabianice) according to the standard immunosuppressive regimen and, for the first period after transplantation, also acetylsalicylic acid (Polopiryna, Polfarma) at dose of 75 mg/day for the first month, cefuroxime sodium (Zinacef, Glaxo Wellcome), and cotrimoxazole (Biseptol, Polfa Pabianice). Some patients were also administered other drugs for concomitant diseases (hypertension, diabetes).

Characteristics of the study patients are given in Table 1. In the current study the following parameters were analyzed: AZA dose, white blood cells (WBC), red blood cells (RBC), and platelet (PLT) counts, cyclosporine dose and whole blood level, as well as creatinine and blood urea nitrogen (BUN) levels. Leukopenia (defined as WBC count below $3.0 \times 10^9/\text{L}$) and hepatotoxicity, defined as serum alanine transaminase levels greater than twice the upper normal limit (45 U/L), were also recorded.

The local ethics committee of the Pomeranian Medical University in Szczecin, Poland, approved the protocol of the study. One hundred and five patients had participated in our previous study on *TPMT* polymorphism [11].

Genomic DNA was extracted from 450 μL of whole-blood samples using a nonorganic and nonenzymatic extraction method. Genotyping of each subject for the presence of *TPMT* gene variants (238G>C for *TPMT**2, 460G>A for *TPMT**3B, and 719A>G for *TPMT**3C, and both 460G>A and 719A>G for *TPMT**3C) was performed

Table 1 Characteristics of patients included in the study ($n=157$)

Characteristic	Value
Age (years)	39.9 ± 12.4 (12–69)
Sex	
Women	68 (43.6%)
Men	88 (56.4%)
Body weight (kg)	68.4 ± 13.9 (35–110)
Acute rejection episodes	26 (16.7%)
Early dialysis after transplantation (<5 days)	34 (21.8%)

Values are mean $\pm$ SD (range) or number (%)

Table 2 *TPMT* and *ITPA* allele frequencies in the studied group

	Allele frequency	
	Number	Percentage
<i>TPMT</i>		
*1 (wt)	295	93.9
*2	2	0.6
*3A	15	4.8
*3C	2	0.6
<i>ITPA</i>		
Wt	257	81.8
94A	20	6.4
IVS2 +21C	37	11.8

using PCR-based assays described by the authors in detail elsewhere [12]. Functional SNPs in *ITPA* gene (94C>A - rs1127354 and IVS2+21A>C - rs7270101) were genotyped with PCR/RFLP assay, using *XmI* endonuclease for allele discrimination [13]. One subject was found to be homozygous for *TPMT* variant allele and excluded from the study, since it is well documented that all *TPMT*-deficient patients develop severe leucopenia after 4–8 weeks of AZA treatment.

Results

Frequencies of *TPMT* and *ITPA* alleles in the studied group are given in Table 2. The genotype frequency distribution did not show a significant deviation from Hardy-Weinberg equilibrium. For further investigation, based on genotyping results, the patients were divided into groups. Clinical parameters were compared between *TPMT* wild-type homozygotes and heterozygotes. In case of *ITPA* genotype, each SNP (94C>A and IVS2+21A>C) was first analyzed separately (as the impact of these on enzyme activity is not equal) and then the effect of compound genotype was studied.

In all groups AZA doses and hematological parameters (WBC, RBC, PLT) were analyzed on the day of transplantation, and 1, 3, 6, and 12 months after surgery. *TPMT* wild-type homozygous and heterozygous patients were administered similar AZA doses at the beginning of immunosuppressive therapy (2.19 ± 0.61 and 2.23 ± 0.69 mg kg⁻¹ 24 h⁻¹, respectively), which was reduced to approximately 1.5 mg kg⁻¹ 24 h⁻¹ according to a standard immunosuppressive regimen and modified in case of leucopenia, AZA hepatotoxicity, viral infection, continuous anemia, thrombocytopenia, or graft rejection episode.

Table 3 AZA dose and hematological parameters in *TPMT* wild-type homozygous (wt/wt) and heterozygous patients (wt/var) during the first year after renal transplantation

	Wt/wt (n=139)	Wt/var (n=17)	P value ^a
Mean AZA dose (mg kg ⁻¹ 24 h ⁻¹) (% of initial dose)			
On the day of transplantation	2.19±0.61 (100)	2.23±0.69 (100)	0.816
1 month after transplantation	1.55±0.51 (70.8)	1.42±0.78 (63.6)	0.347
3 months after transplantation	1.53±0.52 (69.8)	1.22±0.65 (54.7)	0.028
6 months after transplantation	1.51±0.52 (68.9)	1.30±0.69 (58.2)	0.146
12 months after transplantation	1.46±0.51 (66.7)	1.22±0.72 (54.7)	0.079
RBC (10 ¹² /L)			
On the day of transplantation	3.46±0.67	3.45±0.65	0.951
1 month after transplantation	3.70±0.63	3.91±0.71	0.212
3 months after transplantation	4.11±0.71	3.83±1.02	0.165
6 months after transplantation	4.33±0.78	4.35±0.83	0.954
12 months after transplantation	4.22±0.73	4.44±0.76	0.253
WBC (10 ⁹ /L)			
On the day of transplantation	10.10±3.23	9.72±3.91	0.651
1 month after transplantation	7.64±2.81	7.31±2.68	0.646
3 months after transplantation	7.76±2.48	6.51±2.10	0.048
6 months after transplantation	7.26±2.05	6.41±1.14	0.096
12 months after transplantation	6.99±2.23	5.63±1.38	0.016
PLT (10 ⁹ /L)			
On the day of transplantation	188.9±79.4	191.8±69.6	0.883
1 month after transplantation	272.3±91.6	217.6±82.3	0.017
3 months after transplantation	240.4±113.1	236.3±81.5	0.853
6 months after transplantation	243.0±105.4	233.2±70.4	0.615
12 months after transplantation	229.6±89.0	233.1±82.7	0.872

AZA Azathioprine RBC red blood cell count, WBC white blood cell count, PLT platelet count, wt wild type, var variant

^a Student's *t*-test

During subsequent months of treatment, mean AZA dose administered to *TPMT* wild-type homozygous patients was maintained at approximately 66–70% of initial dose, while in heterozygous patients mean dose was decreased in the course of treatment (to about 55% of initial dose after 12 months). This difference reached statistical significance ($P=0.028$) 3 months after transplantation. The percentage of patients who had their AZA dose reduced for reasons other than leucopenia was similar in all patient groups. Some differences in hematological parameters were also observed: mean WBC count after 3 and 12 months, as well as PLT count after 1 month, were significantly lower in patients carrying *TPMT* variant allele (Table 3). In contrast, no association was observed between patients' *ITPA* genotype and either AZA dose nor blood morphology (Table 4).

Leucopenia episodes, occurring independently from viral infections, as well as hepatotoxicity cases, were recorded in the course of treatment. Eight of 17 (41.2%) *TPMT* heterozygous renal transplant recipients experienced a fall in leukocyte numbers to below $4.0 \times 10^9/L$, compared to 24 of 139 (18.0%) wild-type patients ($P<0.01$, Table 5). *ITPA* genotype did not influence leucopenia risk. Although patients carrying two variant alleles (var/var) were more likely to develop leucopenia than wild-type subjects (37.5 vs. 20.4%) that difference was not significant, possibly due to low frequency of *ITPA* variant alleles (Table 5). No association was observed between *TPMT* or *ITPA* genotype and hepatotoxicity risk.

Additionally, a comparison was made between AZA doses in the second week of treatment (after AZA dosage reduction, according to the standard immunosuppressive regimen) and 12 months after kidney transplantation. In 52.9% of *TPMT* heterozygous patients AZA dose was reduced at 12 months, compared to 28.0% of wild-type homozygotes ($P=0.05$). In case of *ITPA*, the differences were not statistically significant (Table 6).

Additionally, all the parameters were analyzed separately in relation to *ITPA* genotype in a subgroup of patients homozygous for wild-type *TPMT* allele ($n=139$) to test *ITPA* polymorphism as an independent risk factor. No significant differences were observed, except for the lack of patients heterozygous for *ITPA* variant allele with the AZA dose increase (0 of 33), which was significantly more frequent in *ITPA* wild-type homozygotes (14 of 121, $P<0.05$). Leucopenia risk was increased in patients carrying two *ITPA* variant alleles (var/var) compared to wild-type subjects (42.8 vs. 17.4%, $OR=3.67$), but that difference was still not significant. Statistical analysis revealed that AZA dose in IVS2 +21A>C variant allele carriers was slightly increased compared to noncarriers after 1 month of the therapy (1.72 vs 1.47 mg kg⁻¹ 24 h⁻¹, $P<0.05$), but this finding may be accidental, as most AZA adverse effects

appear later in the course of treatment along with drug dose adjustment.

In all study groups creatinine, BUN, and cyclosporine levels were similar in the course of treatment (data not shown). Contrary to our previous study, cyclosporine whole-blood concentrations were also found to be similar in patients with different *TPMT* genotype.

Discussion

The results of our study confirm the association of *TPMT* gene polymorphism with AZA toxicity in renal transplant recipients. Increased risk of thiopurine drug toxic effects in patients heterozygous for one of the common *TPMT* variant alleles was controversial in earlier studies, with some investigators showing an association between heterozygosity and AZA or MP-related myelotoxicity, and some finding no association [2]. However, retrospective studies on larger patients groups [14–16], as well as a large prospective study [17], show that patients with intermediate *TPMT* activity are at significantly higher risk of thiopurine drug toxicity and drop-out from therapy. Those findings are also confirmed by the results of our study: *TPMT* heterozygous patients were more likely to undergo AZA dose reduction or develop leucopenia in the course of treatment, and mean WBC count was lower compared with *TPMT* homozygotes. The results strongly support the necessity of *TPMT* genetic testing before initiation of thiopurine drug therapy.

Even though the impact of *ITPA* genetic variants on enzyme activity is unquestionable and well characterized [8–10], the clinical significance of *ITPA* polymorphism does not seem so obvious. We did not observe any association between *ITPA* polymorphism and AZA toxicity in the studied group of renal transplant recipients. Conclusions of some other studies were also negative, as no predictive value of *ITPA* genotyping for the development of AZA side effects was found in inflammatory bowel disease (IBD) patients [18–20]. These observations were contrary to some other studies in IBD patients, including an original study by Marinaki et al. [13], where *ITPA* 94A allele (enzyme deficiency-associated) was significantly associated with adverse drug reactions. Von Ahsen et al. [21] found that patients carrying the *ITPA* 94A allele were at higher risk for drop-out from AZA therapy. Zelinkova et al. [16] observed an increased risk of leucopenia, but not hepatotoxicity in *ITPA* 94A>C heterozygotes. In a recent prospective study, *ITPA* 94A allele only predicted flu-like symptoms but not all AZA toxic effects [17]. Similarly, in a recent study by Stocco et al. in all patients treated with mercaptopurine, *ITPA* 94A allele was found to be a significant determinant of severe febrile neutrope-

Table 4 AZA dose and hematological parameters during the first year after renal transplantation in relation to patients' *ITPA* genotype

	94C>A			IVS2 +21A>C			ITPA compound genotype		
	Wt/wt (n=137)	Variant A carriers (n=19)	P value	Wt/wt (n=124)	Variant C carriers (n=32)	P value	Wt/wt (n=108)	Variant allele carriers (n=48)	P value ^a
Mean AZA dose, mg kg ⁻¹ 24 h ⁻¹ (% of initial dose)									
On the day of transplantation	2.21±0.62 (100)	2.07±0.41 (100)	0.366*	2.16±0.59 (100)	2.30±0.64 (100)	0.256*	2.18±0.61 (100)	2.21±0.58 (100)	0.772*
1 month after transplantation	1.52±0.55 (68.8)	1.61±0.56 (77.8)	0.281*	1.51±0.54 (69.9)	1.63±0.58 (70.9)	0.258*	1.49±0.54 (68.3)	1.63±0.57 (73.8)	0.149*
3 months after transplantation	1.50±0.55 (67.9)	1.49±0.48 (72.0)	1.000*	1.48±0.54 (68.5)	1.55±0.56 (67.4)	0.516*	1.48±0.55 (67.9)	1.53±0.52 (69.2)	0.614*
6 months after transplantation	1.49±0.56 (67.4)	1.45±0.50 (70.0)	0.833*	1.46±0.55 (67.6)	1.57±0.53 (68.3)	0.325*	1.47±0.57 (67.4)	1.53±0.51 (69.2)	0.541*
12 months after transplantation	1.43±0.56 (64.7)	1.42±0.47 (68.6)	0.957*	1.41±0.54 (65.3)	1.52±0.57 (66.1)	0.299*	1.42±0.56 (65.1)	1.49±0.53 (67.4)	0.454*
RBC (10 ¹² /L)									
On the day of transplantation	3.43±0.66	3.59±0.59	0.361	3.45±0.60	3.47±0.82	0.881	3.43±0.60	3.51±0.75	0.479
1 month after transplantation	3.71±0.63	3.82±0.76	0.514	3.70±0.65	3.83±0.64	0.329	3.67±0.62	3.84±0.69	0.145
3 months after transplantation	4.06±0.76	4.16±0.71	0.592	4.05±0.73	4.17±0.83	0.437	4.02±0.73	4.19±0.79	0.203
6 months after transplantation	4.35±0.75	4.24±1.02	0.577	4.32±0.81	4.44±0.69	0.422	4.31±0.76	4.40±0.83	0.533
12 months after transplantation	4.25±0.70	4.19±0.99	0.725	4.23±0.74	4.32±0.76	0.545	4.21±0.68	4.30±0.86	0.507
WBC (10 ⁹ /L)									
on the day of transplantation	10.04±3.28	10.24±3.56	0.82	9.85±3.34	10.78±3.08	0.161	9.78±3.28	10.67±3.30	0.134
1 month after transplantation	7.49±2.65	8.41±	0.19	7.49±2.64	8.03±3.34	0.355	7.40±2.52	8.05±3.28	0.324
3 months after transplantation	7.57±2.38	7.95±	0.53	7.56±2.49	7.83±2.38	0.593	7.56±2.41	7.75±2.58	0.882
6 months after transplantation	7.11±1.86	7.57±	0.35	7.04±1.93	7.64±2.18	0.134	7.00±1.75	7.54±2.41	0.236
12 months after transplantation	6.77±2.08	7.34±	0.29	6.81±2.22	6.94±2.09	0.761	6.79±2.14	6.93±2.30	0.945
PLT (10 ⁹ /L)									
on the day of transplantation	191.2±67.5	193.5±91.3	0.897	192.0±70.0	189.6±73.0	0.870	192.2±65.7	189.8±80.4	0.855
1 month after transplantation	225.9±85.9	208.4±77.4	0.404	217.0±82.9	249.2±88.7	0.072	218.8±83.9	233.7±86.4	0.336
3 months after transplantation	238.2±84.6	226.9±92.9	0.602	233.5±83.3	249.9±93.8	0.356	234.7±81.7	241.5±93.9	0.657
6 months after transplantation	234.8±71.6	231.4±96.2	0.857	232.7±72.2	240.6±85.7	0.609	233.9±67.5	235.3±89.5	0.913
12 months after transplantation	234.4±83.2	220.7±83.9	0.503	228.0±84.0	249.5±79.1	0.197	231.2±84.9	235.7±80.2	0.758

AZA Azathioprine, RBC red blood cell count, WBC white blood cell count, PLT platelet count, wt wild type, var variant

^a Student's *t*-test, except *Mann-Whitney test

Table 5 Comparison of clinical parameters of patients classified by *TPMT* and *ITPA* genotype

	Leucopenia episodes				Hepatotoxicity			
	Number	Percentage	OR (95% CI)	<i>P</i> value	Number	Percentage	OR (95% CI)	<i>P</i> value ^a
<i>TPMT</i>								
Wt/wt (<i>n</i> =139)	24	18.0%	4.26 (1.49–12.15)	0.008	15	10.8%	0.52 (0.06–4.17)	1.000
Var/wt (<i>n</i> =17)	8	41.2%			1	5.9%		
<i>ITPA</i> 94C>A								
Wt/wt (<i>n</i> =137)	29	21.2%	0.70 (0.19–2.56)	0.765	13	9.5%	1.78 (0.45–6.96)	0.417
Var/wt (<i>n</i> =19)	3	15.8%			3	15.8%		
<i>ITPA</i> IVS2 +21A>C								
Wt/wt (<i>n</i> =124)	23	18.5%	1.72 (0.70–4.20)	0.230	12	9.7%	1.33 (0.49–1.44)	0.743
Var/wt + var/var (<i>n</i> =32)	9	28.1%			4	12.5%		
<i>ITPA</i> compound								
Wt/wt (<i>n</i> =108)	22	20.4%			10	9.3%		
Wt/var (<i>n</i> =40)	7	17.5%	0.82 (0.32–2.12)	0.817	5	12.5%	1.4 (0.44–4.38)	0.551
Var/var (<i>n</i> =8)	3	37.5%	2.34 (0.52–10.57)	0.367	1	12.5%	1.4 (0.15–12.55)	0.561
Variant carriers (<i>n</i> =48)	10	20.8%	1.03 (0.44–2.38)	1.000	6	12.5%	1.4 (0.48–4.10)	0.573

OR Odds ratio, CI confidence interval, wt wild type, var variant

^a Fisher's exact test, compared to wt/wt genotype

nia, but the effect of *TPMT* was much more pronounced [22].

It was suggested that *TPMT* polymorphism is the major determinant of toxicity risk if thiopurine dose is not

adjusted for *TPMT* genotype, whereas in *TPMT*-adjusted thiopurine therapy, *ITPA* polymorphism may become important. As AZA dosage was not *TPMT*-adjusted in the current study, our results are consistent with the recent

Table 6 AZA dose change 1 year after transplantation compared to drug dose in the second week of treatment in patients classified by *TPMT* and *ITPA* genotype

Genotype	AZA dose								
	Reduced			Equal			Increased		
	Number	Percentage	<i>P</i> value	Number	Percentage	<i>P</i> value	Number	Percentage	<i>P</i> value ^a
<i>TPMT</i>									
Wt/wt (<i>n</i> =139)	39	28.0%		86	61.9%		14	10.1%	
Var/wt (<i>n</i> =17)	9	52.9%	0.050	7	41.1%	0.120	1	5.9%	1.000
<i>ITPA</i> 94C>A									
Wt/wt (<i>n</i> =137)	42	30.6%		80	58.4%		15	10.9%	
Var/wt (<i>n</i> =19)	6	31.5%	1.000	13	68.4%	0.463	0	0.0%	0.218
<i>ITPA</i> 94C>A IVS2 +21A>C									
Wt/wt (<i>n</i> =124)	37	29.8%		75	60.5%		12	9.7%	
Var/wt + var/var (<i>n</i> =32)	11	34.4%	0.669	18	56.3%	0.689	3	9.4%	1.000
<i>ITPA</i> compound									
Wt/wt (<i>n</i> =108)	31	28.7%		65	60.2%		12	11.1%	
Wt/var (<i>n</i> =40)	16	40.0%	0.233	23	57.5%	0.851	1	2.5%	0.186
Var/var (<i>n</i> =8)	1	12.5%	0.442	5	62.5%	1.000	2	25.0%	0.293
Variant carriers (<i>n</i> =48)	17	35.4%	0.453	28	58.3%	0.861	3	6.3%	0.557

AZA Azathioprine, wt wild type, var variant

^a Fisher's exact test

findings by Stocco et al. Analysis performed in the subset of patients sharing the same *TPMT* genotype (wt/wt) suggested that patients carrying two *ITPA* variant alleles might be at higher risk of leucopenia (OR=3.67). However, the difference was not significant, probably due to time of observation (12 months), while significant differences in the aforementioned study were revealed much later in the course of treatment [22]. Since the frequency of *ITPA* variant genotype (especially two variant allele carriers) is relatively low, the current study does not have enough power to detect some mild effects that cannot be excluded.

The contrasting results of different studies may come from variability of drug dosing regimens, insufficient number of patients included in some studies, and—also of some importance—different frequency of *ITPA* variants in studied populations. Other drug co-medications and drug interactions may also be responsible for some discrepancies between our results and the aforementioned studies, as this is the first study on *ITPA* polymorphism in renal transplant recipients. No conclusion can be drawn for patients deprived of ITPA activity (homozygous for 94A variant), as none were found in the studied population. If any side effects of thiopurines are associated with ITPA activity that group of patients should be at the highest risk. However, results of different studies are contrary: the 94AA homozygotes tolerated AZA well in studies of de Ridder et al. [23] and Zelinkova et al. [16], whereas Marinaki et al. [13] reported that all three 94AA homozygotes suffered from side effects.

Our results suggest that routine genotyping of renal transplant recipients for *TPMT* variants may be useful in reducing the risk of AZA-related myelotoxicity, but there is not enough evidence to introduce *ITPA* testing into clinical practice.

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