Sudan Journal of Medical Sciences
Volume 19, Issue no. 3, DOI 10.18502/sjms.v19i3.14194
Production and Hosting by KnE Publishing

Research Article

Thiopurine S-methyl Transferase (TPMT) Enzyme Level in Healthy Sudanese Population

Rayan Khalid[1]*, Nahla Hashim Hassan Erwa[2], Elshibli Mohamed Elshibli[3], and Imad Fadl-Elmula[4]

[1]Department of Clinical Genetics and Immunology, Assafa college, Khartoum, Sudan
[2]Clinical Immunology Unit, Soba University Hospital and Immunology Unit, Department of Medical Microbiology and Parasitology, Faculty of Medicine, University of Khartoum, Sudan
[3]Department of Biostatics, Al Neelain Stem Cell Research Center, Al Neelain University, Khartoum, Sudan
[4]Department of Clinical Genetics, Al Neelain Stem Cell Research Center, Al Neelain University, Khartoum, Sudan

Abstract

Background: Thiopurine drugs have limited use due to their toxicity, related to the enzyme thiopurine S-methyl transferase (TPMT) activity, which varies between individuals. This is the first study in Sudan, which aimed to assess the TPMT phenotypic status of healthy Sudanese volunteers.

Methods: A total of 177 healthy volunteers from Sudan were included in the study. TPMT enzymatic activities were measured using the ELISA serum protocol. We used SPSS to analyze the data and determined enzyme level categories and normal range with Z scores and quartile tests. The Sudan Medical Specialization Board (SMSB) Ethical Committee approved the study.

Results: There were 117 males and 60 females among the volunteers, with ages ranging from 16 to 70 years and a mean age ± SD of 28.0 ±1 0.2, median = 24. Most candidates were from the Afro-Asiatic linguistic group (64.5%), followed by Nilo-Saharan (18.6%) and Niger-Kordofanian (16.9%). The TPMT enzyme level ranged between 0.17 and 9.5 ng/ml, with a mean of 2.26 ± 0.75 ng/ml. The quartile classification included very low enzyme (<0.76 ng/ml) seen in 4 candidates (2.3%), intermediate low (0.76-1.4 ng/ml) seen in 34 (19.2%), the normal range (1.5 – 3.75 ng/ml) seen in 119 (67.2%), and high enzyme activity (>3.76 ng/ml) seen in 20 (11.3%). No significant correlations between age, sex, and ethnic groups were recorded.

Conclusion: The normal TPMT enzyme activity is between 1.5 and 3.76 ng/ml. A higher prevalence of TPMT deficiency was recorded and compared with international studies. Pretreatment screening using serum ELISA test for TPMT enzyme activity should be used to predict the risk of toxicity.

Keywords: thiopurine S-methyl transferase, TPMT enzyme level, thiopurine drugs, ELISA kits, human TPMT

Corresponding Author: Rayan Khalid; email: rynkhalid@yahoo.com

Received: 21 September 2023
Accepted: 14 December 2023
Published: 30 September 2024

Production and Hosting by KnE Publishing

Editor-in-Chief:
Prof. Nazik Elmalaika Obaid Seid Ahmed Husain, MD, M.Sc, MHPE, PhD.

1. Introduction

An individual's response to a drug can vary, which can be entirely influenced by their genetic profile since genetic variation affects drug metabolism and responses. Determining and understanding the relation between the interpretation of the gene expression (genotype) and drug response (phenotype) is the key to understanding and predicting drug efficacy and/or toxicity, enabling dose optimization, and/or omitting certain drugs with very high toxicity risk and severe morbidity [1, 2].

Thiopurine S-methyl transferase (TPMT) is a cytosolic enzyme expressed globally in the human tissues with the highest levels in the liver, heart, and erythrocytes, and lower lung and brain concentrations [3]. TPMT enzyme is responsible for the inactivation of antimetabolite of thiopurine drugs, such as 6-mercaptopurine, 6-thioguanine, and azathioprine, by catalyzing the S-methylation of sulfhydryl groups in thiopurine drugs using S-Adenylyl-L-methionine (SAM) as a cofactor and methyl donor [4]. All these drugs have been widely used in treating immune-mediated diseases, for example, Crohn's disease and rheumatoid arthritis, acute lymphoblastic leukemia, and organ transplantation. Unfortunately, the TPMT enzyme activity exhibits genetic heterogeneity due to the genetic polymorphisms of the *TPMT* gene, where heterozygous defect allele gives rise to the intermediate phenotype. In contrast, homozygous defect alleles lead to very low or absent TPMT enzyme activity [5, 6]. This was evident in a previous phenotype study, which showed trimodality for red cell TPMT enzyme activity with high enzyme activity in 88.6% of the individuals with intermediate training in 11.1%. In comparison, 0.3% had undetectable activity [7]. The enzyme level varies with the ethnic origin of the population, that is, different *TPMT* variant was seen in 10% (20 of 199) of Caucasians, 2% (2 of 99) of Southwest Asians, 4.7% (9 of 192) of Chinese populations, and 14.8% (32 of 217) of African population [8, 9]. Worldwide, 2 out of 300 individuals are estimated to have deficient TPMT enzyme activity due to genetic mutation in the *TPMT* gene [10]. When treated with conventional doses of azathioprine or mercaptopurine, these individuals would be at risk of developing severe, potentially life-threatening bone marrow suppression. Most developed countries combine genotyping and/or phenotyping techniques to asses TPMT enzyme activity before starting treatment with thiopurine drugs to avoid severe toxicity [11]. Most developed countries use it to evaluate TPMT phenotype and genotype. On the other hand, low- and middle-income countries with limited health facilities, including Sudan, omit pretreatment assessment of TPMT enzyme level altogether.

To the best of our knowledge, no previous study has been conducted on TPMT phenotype and/or genotype profiling among the Sudanese population. Thus, the current study has become crucial in contributing to designing informed national guidelines for using thiopurine drugs in Sudan. Such policies may include phenotyping (enzyme level) to reduce morbidity and mortality in Sudanese patients receiving thiopurine medications.

2. Material and Methods

One hundred and seventy seven Sudanese healthy volunteers were randomly included in the present cross-sectional study from February to September 2022 in Khartoum state. The racial-ethnic composition of the individuals in this study was

according to the tribal, linguistic classification (Afro-Asiatic, Nilo-Saharan, and Niger-Kordofanian) [12]. All individuals suffering from chronic liver disorders, chronic kidney diseases, advanced heart failure, recent blood transfusions, or gross obese volunteers with a body mass index of over 30 were excluded from the study.

Demographic data, including name, age, sex, and father and mother's tribes, were recorded from each participant using a predesigned questionnaire.

The sample size was determined according to the equation: {$n=N\ x/((N-1)\ E2\ +\ x)$, *and* $x=Z(c/100)2r(100-r)$}, where n = sample size; N is the population size = 43,849,260; r is the fraction of responses = 13 %; $Z(c/100)$ is the critical value for the confidence level; and c = 95.

A total of 5 ml of peripheral venous blood was collected from all participants, following the universal precautions for preventing blood-borne pathogens. Of the 5 ml blood, 3 ml were placed in a plain vacutainer tube for 10–20 mins before centrifugation for 20 mins at 2000–3000 RPM. For measuring the serum TPMT enzyme activity, the supernatant layer was then collected without sediment and stored in the refrigerator (2–8°C) 5 days before the measurement of TPMT enzymatic activity, following the manufacturing instruction of used ELISA kits Human TPMT from Bioassay Technology laboratory (BT LAB), Shanghai, China, with catalog number E6266Hu. The kits contain positive, negative, and standard controls.

To create the standard curve, we plotted the average OD for each standard on the Y-axis and the concentration on the X-axis. Then, we used curve-fit software to perform the calculations and determine the best-fit line through the points on the graph. This line was determined through regression analysis.

The results were analyzed using the SPSS program version 23. The continuous variables were described as mean ± standard deviation (mean ± SD). The categorical variables were expressed as numbers and percentages. For the comparison of the continuous variables, *P*-value < 0.05 was considered statistically significant. As the data were not normally distributed, the Kolmogorov-Smirnov test was used to normalize the data to calculate the normal range. For normalization of the results, we used Z score $[z = (x - \mu) / \sigma]$, where z = standard score; x = observed value; μ = mean of the sample; σ = standard deviation of the model. The data were processed in four quartiles equations [{Q1 = {$(n + 1)/4$}; Q2 = {$(n + 1)/2$}; Q3 = {$3(n + 1)/4$}] with the first quartile 25% from smallest to largest numbers. The second quartile ranged between 25.1% and 50% (till median), whereas the third quartile was between 51% and 75% above the median, followed by the fourth quartile equaling 25% of the largest numbers.

The mean serum TPMT level was the dependent variable. The students' *t*-test was used to compare mean serum TPMT levels according to the sex group (male/female). The ANOVA test was used for age categories classified into four categories (16–19, 20–39, 40–59, and ≥60 years). The Sudanese ethnic groups were stratified into Afro-Asiatic, Nilo-Saharan, and Niger-Kordofanian. The enzyme levels of TPMT were classified into four groups (high, normal, intermediate, low, and very low) using Fisher's exact test and Spearman for cross-tabulation correlation analysis.

3. Results

Of the 177 participants, 117 (66.1%) were males and 60 (33.9%) were females, with the participants

aged between 16 and 70 years. According to the participants' age groups, 18 (10.2%) were teenagers aged between 16 and 19 years, 134 (75.7%) were young adults with ages ranging between 20 and 39 years, 20 (11.3%) were middle-aged adults ranging between 40 and 59 years, and only 5 (2.8%) volunteers were senior adults aged ≥60 years. According to the tribal linguistic origin, 114 (64.4%) were Afro-Asiatic tribes, 33 (18.6%) were Nilo-Saharan, and 30 (16.9%) were Niger-Kordofanian tribes (Table 1).

The results of TPMT enzyme activity showed that the serum TPMT enzyme level ranged between 0.17 and 9.5, and the serum TPMT activity mean was 2.26 ± 0.75 ng/ml, calculated using the standard score (Z score) for result normalization.

According to cutoff values of Serum TPMT enzyme level by ELISA sandwich technique, using quartile classification, TPMT enzyme status was classified into four groups: very low activity group with serum levels <0.76 ng/ml, intermediate low activity group with serum levels between 0.76 and 1.4 ng/ml, normal activity group with serum levels between 1.5 and 3.76 ng/ml, and high enzyme activity with serum levels >3.76 ng/ml (Table 2).

Most participants, 119 (67.2%), had normal TPMT activity, while 34 (19.2%) had an intermediate TPMT enzyme activity, 20 (11.3%) showed high TPMT activity, and only four (2.3%) revealed very low enzyme activity (Table 2).

The percentages of serum TPMT level groups among male participants were very low in 2.6% of individuals, intermediate low in 16.2%, normal in 67.5%, and high in 13.7%. In female participants, these TPMT level groups were assigned for 1.7%, 25%, 66.7%, and 6.6%, respectively. TPMT enzyme level categories and sex cross-tabulation show that normal serum TPMT enzyme level is the most frequent category in both sex groups. Accordingly, the Fisher's test showed no statistically significant relation between the two categories (*P*-value = 0.339) (Table 3).

Results also showed that the percentages for serum TPMT enzyme level classes among participants according to their ethnicity groups were assigned as follows: among Afro-Asiatic tribes, very low serum TPMT enzyme levels were presented in 2.6%, intermediate low serum TPMT enzyme levels were seen in 20.2%, normal serum TPMT enzyme level presented in 67.5%, and high serum TPMT enzyme level presented in 9.7%. These TPMT enzyme level groups were assigned for 0.0%, 12%, 72.7%, and 5% in Nilo-Saharan tribes and 3.4%, 23.3%, 60%, and 13.3%, respectively in Niger-Kordofanian tribes. No statistically significant relation between the two variables in TPMT level categories and ethnic groups cross-tabulation and the Fisher's test was observed, *P*-value (0.729) (Table 3).

Statistical analysis of the percentages for serum TPMT level classes in different participants' age groups was consigned as follows: among teenagers, very low TPMT levels were presented in 5.6%, intermediate low TPMT levels were seen in 11.1%, normal TPMT levels presented in 77.8%, and high TPMT levels were seen in 5.6%. These TPMT level groups were assigned for 2.2%,21.6%, 66.4%, and 9.7%, respectively, in young adults; 0.0%, 10%, 65%, and 25% in middle-aged adults; and 0.0%, 20%, 60%, and 20%, respectively, in senior adults. Serum TPMT level categories and age group cross-tabulation showed no statistically significant relation between those two variables using the Spearman test (*P*-value = 0.122; Table 3).

The student's test approved that there was no significant difference in the enzyme mean among

males (2.33 ± 0.75) and females (2.13±.74) with *P*-value = 0.262 (Table 4). The ANOVA test showed no significant difference between the means of TPMT among the Afro-Asiatic tribe group (2.25 ± 0.75), Nilo-Saharan (2.34 ± 0.75), and Niger-Kordofanian (2.22 ± 0.76), with a *P*-value = 0.820. Moreover, the ANOVA test showed no significant differences between the means of TPMT among the teenagers group (2.43 ± 0.71), young adults (2.20 ± 0.75), middle-aged adults (2.45 ± 0.76), and senior adults (2.44 ± 0.93) with a *P*-value = 0.452 (Table 4).

4. Discussion

Pharmacogenetics advances in understanding the relationship between genetics and drug metabolism have made drug treatments increasingly tailored to individual patients. Many medications now include information about the contribution of genetic variation in modulating drug metabolism and/or response in product monographs [13]. Thiopurine drugs are an excellent example, widely used in treating autoimmune disease, inflammatory bowel disease, acute lymphoblastic leukemia, and preventing rejection after solid organ transplant. TPMT is the enzyme that metabolizes thiopurines, so a typical application of personalized medicine is testing for TPMT enzyme status before starting treatment with thiopurine drugs. Mutations in the *TPMT* gene can reduce the activity of the protein, resulting in toxic levels of the drug and leading to bone marrow suppression [14]. Although several worldwide studies investigated the polymorphism of the *TPMT* gene (genotype) and TPMT enzyme activity (phenotype), fewer studies were done in African populations.

Several human TPMT phenotyping techniques were used in the past to determine enzyme activity, including the ones based on radiolabeled 14c-methyl-s-adenosyl-methionine, high-performance liquid chromatography (HPLC), qualitative immunoassays (rapid immunomigration), and quantitative (enzyme-linked immunosorbent). Unfortunately, most of these methods are unpopular since some use radioactive isotopes known for their health hazard effects, whereas some are labor-intense and expensive. In the present study, we used an ELISA method, which utilizes an antibody developed by BIOLOGIX Research Corporation. The technique utilizes an antibody specific to the riboside of 6-methyl mercaptopurine. Although several available kits are on the market, this method has not yet become routine. We opted for this approach due to its cost-effectiveness and capacity to gauge TPMT expression activity. Nevertheless, there exists a chance of misidentification of enzyme activity when employing phenotypic analysis for patients undergoing therapy that disrupts in vitro reaction or triggers enzyme activity. Additionally, the outcomes may not be entirely precise in patients who have recently undergone blood transfusions [15, 16]. However, most studies using ELISA methods chose whole blood or RBC protocol, in contrast to our methodology, which utilized a serum-based protocol to avoid false results that are usually produced by the variation in the hemoglobin level, percentage of young RBCs, and the effect of white cell activity when using whole blood protocol. The result of the present study, however, agreed with what has been reported in previous studies which used different methods. This may support the screening for TPMT using serum ELISA before starting thiopurine therapy [17].

The results showed sex TPMT enzyme level variation, as 13.7% of males expressed a high TPMT enzyme activity compared with only 6.6%

Table 1: Frequency distribution of demographic (sex, ethnic, and age) groups (n = 177).

		Number of individuals	Percentage (%)
Sex	Male	117	66.1%
	Female	60	33.9%
Ethnic groups	Afro–Asiatic	114	64.4%
	Nilo–Saharan	33	18.6%
	Niger–Kordofanian	30	16.9%
Age groups (yrs)	Teenagers (16–19)	18	10.2%
	Young adults (20–39)	134	75.7%
	Middle-aged adults (40–59)	20	11.3%
	Senior adults (≥60)	**5**	2.8%

Table 2: The frequency distribution of categorical serum TPMT enzyme levels (n = 177).

Categories	Cutoff values (ng/ml)	Frequency	Percentage (%)
Very low	<0.76	4	2.3
Intermediate low	0.76–1.4	34	19.2
Normal	1.5–3.76	119	67.2
High	>3.76	20	11.3

Table 3: Cross-tabulation of categorical serum TPMT enzyme levels and demographic (sex, ethnicity, age) groups.

Demographic groups		Serum TPMT enzyme levels categories				Total	P-value
		Very low	Intermediate low	Normal	High		
Sex groups	Male	1.7%	10.7%	44.6%	9.0%	66.1%	0.34
	Female	0.6%	8.5%	22.6%	2.3%	33.9%	
	Total	2.3%	19.2%	67.2%	11.3%	100%	
Ethnic groups	Afro-Asiatic	1.7%	13.0%	43.5%	6.2%	64.4%	0.73
	Nilo-Saharan	0.0%	2.3%	13.6%	2.7%	18.6%	
	Niger-Kordofanian	0.6%	4.0%	10.2%	2.3%	16.9%	
	Total	2.3%	19.2%	67.2%	11.3%	100%	
Age groups	Teenagers	0.6%	1.1%	7.9%	0.6%	10.2%	0.12
	Young adults	1.7%	16.4%	50.3%	7.3%	75.7%	
	Middle-aged adults	0.0%	1.1%	7.3%	2.8%	11.3%	
	Senior adults	0.0%	0.6%	1.7%	0.6%	2.8%	
	Total	2.3%	19.2%	67.2%	11.3%	100%	

of females, revealing a high TPMT enzyme activity. This may indicate habit differences between the two sexes, for example, smoking is more common in males than females, eventually leading to physiological polycythemia, which may result in false readings. Generally, no significant difference was observed in the mean TPMT activity between the three ethnic groups. However, a slightly higher percentage of enzyme levels were seen in the young adult and middle-aged adult groups of the Sudanese population in this study. This can be explained by the popularity of smoking among these predominantly male age groups [18].

Table 4: Mean, SD, and median of serum TPMT enzyme level among sex, tribes, and age groups.

		TPMT level			
		Mean	**Standard deviation**	**Median**	**P-value**
Sex	Male	2.33	0.75	2.57	0.262
	Female	2.13	0.74	1.84	
Tribe group	Afro-Asiatic	2.25	0.75	2.50	0.820
	Nilo-Saharan	2.34	0.75	2.63	
	Niger- Kordofanian	2.22	0.76	1.93	
Age groups (yrs)	Teenagers (16–19)	2.43	0.71	2.65	0.452
	Young adults (20–39)	2.20	0.75	2.43	
	Middle-aged adults (40–59)	2.45	0.76	2.64	
	Senior adults (60 or more)	2.44	0.93	2.82	

The other exciting result was the absence of low TPMT enzyme levels in Nilo-Saharan linguistic tribes, young adults, and middle-aged groups of three tribes. Genotyping would help to elucidate the whole picture, especially in a heterogeneous population like Sudan. Moreover, most previous studies have combined phenotyping/genotyping tests to determine TPMT enzyme activity to show the status of *TPMT* gene alleles and detect those with high enzyme activity resulting from functional duplication or overexpression of pseudogenes [1].

Of note, genetic differences exist in the prevalence of polymorphisms at the *TPMT* gene between sub-Saharan Africans and Caucasians, as previously reported [19]. The results of the present study agree with that notion, as there is a lower TPMT activity in 2.3% of our population compared to <1% of Caucasians. The same conclusion was reached for the percentage of individuals with intermediate low TPMT enzyme levels, as our study showed 19.2% of our population compared with 10–14% of the Caucasian population [19, 20]. Research in the US found no differences between Black and White individuals. Genetic admixture may explain this. Despite a large sample size, similar results were observed in comparisons between Caucasians and South Asians. Pretreatment assessment of TPMT activity allows some patients to avoid thiopurine drugs that might cause significant harm. In contrast, in other instances, patients might safely receive treatment at a more appropriate dose [21, 22]. Based on both previous and current research findings, it is morally questionable to administer thiopurine drugs to a patient without first checking their TPMT status. Such treatment poses a 1% risk of death from bone marrow suppression and a fourfold increase in suppression for heterozygotes. Multiple economic studies have shown the cost-effectiveness of thiopurine treatment compared to nontreatment [23]. TPMT screening before treatment is cost-effective when using phenotyping instead of genotyping. Furthermore, phenotyping and metabolite monitoring allow for personalized dosing, leading to better outcomes, cost savings, and improved quality of life [24]. This approach to patient care is beneficial and cost-effective, reducing the need for costly inpatient treatment. The assessment of TPMT activity by phenotype and/or genotype is an excellent example of the role of pharmacogenetics in modern clinical practice. The prevalence of TPMT deficiency in the

Sudanese, done for the first time, in a multiethnic population is higher than reported in many studies [25]. TPMT enzyme activity is significantly lower in Sub-Saharan Africans than in Caucasian and South Asian groups. This information might facilitate safer and more effective prescribing of azathioprine for patients. A further study including genotyping is crucial to complete the picture and may open the door for better and safer clinical practice [20].

This is the first-ever study in Sudan that attempted to address the TPMT enzyme activity among normal Sudanese individuals. Moreover, it is also the first in English literature that used serum TPMT enzyme activity ELISA protocol. Hence, the results of the present study are needed to introduce a wide use of thiopurine drugs in Sudan.

Although the present study showed interesting findings on the TPMT enzyme activity in the Sudanese population, it was limited to the phenotypic characteristics. A complementary genotype characterization would provide more information about the gene allele of each individual, especially those with very high enzyme activity.

5. Conclusion

The study suggested a normal TPMT enzyme activity in the Sudanese population ranges between 1.5 and 3.76 ng/ml. It also confirmed that the Sudanese (sub-Saharan) population has an increased percentage of low intermediate and very low TPMT enzyme activity compared with Caucasians and South Asians. The increased frequency of low TPMT enzyme activity in the study population justifies prethiopurine treatment screening of TPMT enzyme activity level. The serum TPMT enzyme ELISA protocol has proven quick, reliable, affordable, and inexpensive.

Declarations

Acknowledgments

The authors want to express their sincere gratitude to Dr. Shereen Faisal Abdelrahman for her technical assistance. The staff at Assafa College volunteered in the present study; without their help, this work could not be completed.

Ethical Considerations

The ethical approval was obtained from the scientific committee at the Sudan Medical Specialization Board (SMSB) and the Educational Development Center (EDC) ethical committee. Each participant had to sign written informed consent before enrolling in the study. The samples were coded and kept anonymous.

Competing Interests

Authors declare no personal or financial competing interests with any individual or organization.

Availability of Data and Material

All data of this study is available upon request.

Funding

This project is self-funded.

Abbreviations and Symbols

TPMT: Thiopurine S-methyl transferase

SMSB: Sudan Medical Specialization Board

SAM: S-Adenylyl-L-methionine

BT LAB: Bioassay technology laboratory

EDC: Educational Development Center

ELISA: Enzyme-linked Immunoassay

HPLC: High-performance liquid chromatography

References

[1] Abaji, R., & Krajinovic, M. (2017). Thiopurine S-methyltransferase polymorphisms in acute lymphoblastic leukemia, inflammatory bowel disease and autoimmune disorders: Influence on treatment response. *Pharmacogenomics Pers Med, 10,* 143–156.

[2] Ames, M. M., Selassie, C. D., Woodson, L. C., Loon, J. A. V., Hansch, C., & Weinshilboum, R. M. (2002). *ACS publications*. American Chemical Society.

[3] Ameyaw, M. M., Collie-Duguid, E. S., Powrie, R. H., Ofori-Adjei, D., & McLeod, H. L. (1999). Thiopurine methyltransferase alleles in British and Ghanaian populations. *Human Molecular Genetics, 8*(2), 367–370. https://doi.org/10.1093/hmg/8.2.367

[4] Ansari, A. (2003). *Treatment of zero and intermediate TPMT patients with a tailored dose of azathioprine - Research Portal*. King's College.

[5] Azad, M., Kaviani, S., Soleymani, M., Nourouzinia, M., & Hajfathali A. (2009). Common polymorphism's analysis of thiopurine S-methyltransferase (TPMT) in Iranian population. *Yakhteh Medical Journal, 11*(3), 311–316.

[6] Coelho, T., Andreoletti, G., Ashton, J. J., Batra, A., Afzal, N. A., Gao, Y., Williams, A. P., Beattie, R. M., & Ennis, S. (2016). Genes implicated in thiopurine-induced toxicity: Comparing TPMT enzyme activity with clinical phenotype and exome data in a paediatric IBD cohort. *Scientific Reports*, *6*(1), 34658. https://doi.org/10.1038/srep34658

[7] Collie-Duguid, E. S., Pritchard, S. C., Powrie, R. H., Sludden, J., Collier, D. A., Li, T., & McLeod, H. L. (1999). The frequency and distribution of thiopurine methyltransferase alleles in Caucasian and Asian populations. *Pharmacogenetics, 9*(1), 37–42. https://doi.org/10.1097/00008571-199902000-00006

[8] Cooper, S. C., Ford, L. T., Berg, J. D., & Lewis, M. J. (2008). Ethnic variation of thiopurine S-methyltransferase activity: A large, prospective population study. *Pharmacogenomics, 9*(3), 303–309. https://doi.org/10.2217/14622416.9.3.303

[9] Dobón Berenguer, B., Hassan, H. Y., Laayouni, H., Luisi, P., Rica no Ponce, I., Zhernakova, A., Wijmenga C., Tahir, H., Comas, D., Netea, M. G., & Bertranpetit, J. (2015). The genetics of East African populations: A Nilo-Saharan component in the African genetic landscape. *Scientific Reports, 5,* 9996. 2015.

[10] Hon, Y. Y., Fessing, M. Y., Pui, C. H., Relling, M. V., Krynetski, E. Y., & Evans, W. E. (1999). Polymorphism of the thiopurine S-methyltransferase gene in African-Americans. *Human Molecular Genetics, 8*(2), 371–376. https://doi.org/10.1093/hmg/8.2.371

[11] Lennard, L., Cartwright, C. S., Wade, R., & Vora, A. (2015). Thiopurine dose intensity and treatment outcome in childhood lymphoblastic leukaemia: The influence of thiopurine methyltransferase pharmacogenetics. *British Journal of Haematology, 169*(2), 228–240. https://doi.org/10.1111/bjh.13240

[12] Maitland, M. L., Vasisht, K., & Ratain, M. J. (2006). TPMT, UGT1A1 and DPYD: Genotyping to ensure safer cancer therapy? *Trends in Pharmacological Sciences, 27*(8), 432–437. https://doi.org/10.1016/j.tips.2006.06.007

[13] McLeod, H. L., Pritchard, S. C., Githang'a, J., Indalo, A., Ameyaw, M. M., Powrie, R. H., Booth, L., & Collie-Duguid, E. S. (1999). Ethnic differences in thiopurine methyltransferase pharmacogenetics: Evidence for allele specificity in Caucasian and Kenyan individuals. *Pharmacogenetics, 9*(6), 773–776. https://doi.org/10.1097/00008571-199912000-00012

[14] Oliveira, E., Quental, S., Alves, S., Amorim, A., & Prata, M. J. (2007). Do the distribution patterns of polymorphisms at the thiopurine S-methyltransferase locus in sub-Saharan populations need revision? Hints from Cabinda and Mozambique. *European Journal of Clinical Pharmacology, 63,* 703–706. https://doi.org/10.1007/s00228-007-0310-8

[15] Otterness, D., Szumlanski, C., Lennard, L., Klemetsdal, B., Aarbakke, J., Park-Hah, J. O., Iven, H., Schmiegelow, K., Branum, E., O'Brien, J., & Weinshilboum, R. (1997). Human thiopurine methyltransferase pharmacogenetics: Gene sequence polymorphisms. *Clinical Pharmacology and Therapeutics, 62*(1), 60–73. https://doi.org/10.1016/S0009-9236(97)90152-1

[16] Payne, K., Newman, W., Fargher, E., Tricker, K., Bruce, I. N., & Ollier, W. E. R. (2007). TPMT testing in rheumatology: Any better than routine monitoring? *Rheumatology (Oxford, England)*, *46*(5), 727–729. https://doi.org/10.1093/rheumatology/kel427

[17] Relling, M. V., Hancock, M. L., Rivera, G. K., Sandlund, J. T., Ribeiro, R. C., Krynetski, E. Y., Pui, C. H., & Evans, W. E. (1999). Mercaptopurine therapy intolerance and heterozygosity at the thiopurine S-methyltransferase gene locus. *Journal of the National Cancer Institute, 91*(23), 2001–2008. https://doi.org/10.1093/jnci/91.23.2001

[18] Priest, V. L., Begg, E. J., Gardiner, S. J., Frampton, C. M. A., Gearry, R. B., Barclay, M. L., Clark, D. W. J., Hansen, P. (2006). Pharmacoeconomic analyses of azathioprine, methotrexate and prospective pharmacogenetic testing for the management of inflammatory bowel disease. *Pharmacoeconomics, 24*(8):767–781.

[19] Richard, V. S., Al-Ismail, D., & Salamat, A. (2007). Should we test TPMT enzyme levels before starting azathioprine? *Hematology (Amsterdam, Netherlands), 12*(4), 359–360. https://doi.org/10.1080/10245330701283959

[20] Schaeffeler, E., Fischer, C., Brockmeier, D., Wernet, D., Moerike, K., Eichelbaum, M., Zanger, U. M., & Schwab, M. (2004). Comprehensive analysis of thiopurine S-methyltransferase phenotype-genotype correlation in a large population of German-Caucasians and identification of novel TPMT variants. *Pharmacogenetics, 14*(7), 407–417. https://doi.org/10.1097/01.fpc.0000114745.08559.db

[21] Shin, J., Kayser, S. R., & Langaee, T. Y. (2009). Pharmacogenetics: From discovery to patient care. *American Journal of Health-System Pharmacy, 66*(7), 625–637. https://doi.org/10.2146/ajhp080170

[22] Szumlanski, C., Otterness, D., Her, C., Lee, D., Brandriff, B., Kelsell, D., Spurr, N., Lennard, L., Wieben, E., & Weinshilboum, R. (1996). Thiopurine methyltransferase pharmacogenetics: Human gene cloning and characterization of a common polymorphism. *DNA and Cell Biology, 15*(1), 17–30. https://doi.org/10.1089/dna.1996.15.17

[23] Weinshilboum, R. M., & Sladek, S. L. (1980). Mercaptopurine pharmacogenetics: Monogenic inheritance of erythrocyte thiopurine methyltransferase activity. *American Journal of Human Genetics, 32*(5), 651–662.

[24] Woodson, L. C., Ames, M. M., Selassie, C. D., Hansch, C., & Weinshilboum, R. M. (1983, November). Thiopurine methyltransferase. Aromatic thiol substrates and inhibition by benzoic acid derivatives. *Molecular Pharmacology, 24*(3), 471–478.

[25] Zeglam, H. B., Benhamer, A., Aboud, A., Rtemi, H., Mattardi, M., Saleh, S. S., Bashein, A., & Enattah, N. (2015). Polymorphisms of the thiopurine S-methyltransferase gene among the Libyan population. *The Libyan Journal of Medicine, 10*(1), 27053. https://doi.org/10.3402/ljm.v10.27053