Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.elsevier.com/locate/poamed

Original research article

Allele frequencies of thiopurine S-methyltransferase (TPMT) variants in the Nigerian population

Ayorinde Adehin [a,*], *Oluseye O. Bolaji* [a], *Martin A. Kennedy* [b], *Babatunde A. Adeagbo* [a]

[a] *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria*

[b] *Department of Pathology and Carney Centre for Pharmacogenomics, University of Otago, Christchurch, New Zealand*

ARTICLE INFO

Article history:
Received 30 March 2016
Accepted 22 June 2016
Available online 24 August 2016

Keywords:
TPMT
Nigerians
Thiopurine
Genotype

ABSTRACT

Introduction: Thiopurine S-methyltransferase (TPMT) methylates clinically relevant thiopurine drugs most of which are noted for adverse reactions in certain users, largely due to polymorphisms in the *TPMT* gene.

Aim: This study investigated the prevalence of functionally relevant *TPMT* alleles in the Nigerian population.

Material and methods: One hundred eighty unrelated subjects consisting of 123 males and 57 females from the main Nigerian ethnicities (44 Igbo, 101 Yoruba, 23 Hausa and 12 from other minor ethnic groups) were genotyped for *TPMT**2, *3B, *3C and *4 alleles using the iPLEX genotyping assay technique. The genotype calls were validated with Sanger sequencing in a random set of samples and the acquired data were assessed for Hardy–Weinberg equilibrium using the Fisher's exact test.

Results and discussion: Defective *TMPT* alleles were found in individuals representing 10% of the study population. *TPMT**3C constituted 9.4% (95% CI, 5.6–14.7) of all alleles detected, with one homozygote and 17 heterozygotes recorded. The prevalence of the *TPMT**3C allele in the population conformed with Hardy–Weinberg equilibrium. *TPMT**2, 3B and *4 were, however, not detected in the population.

Conclusions: *TPMT**3C was the only defective allele identified in Nigerians and may hence be the major underlying genetic contributor to adverse reactions due to thiopurine drugs in the population.

* *Correspondence to*: Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Obafemi Awolowo University, Ile Ife, Osun State, Nigeria. Tel.: +234 8022013470.
E-mail addresses: ayoadehin@yahoo.com, aadehin@oauife.edu.ng (A. Adehin).

http://dx.doi.org/10.1016/j.poamed.2016.06.007

1. Introduction

Thiopurine S-methyl transferase (TPMT) plays a crucial role in the metabolism of clinically relevant thiopurine drugs such as azathiopurine, 6-mercaptopurine and 6-thioguanine. These drugs, used in the management of hematological malignancies, dermatological conditions and as immunosuppressants, have been noted for adverse reactions such as leucopoenia, hepatotoxicity and pancreatitis when administered in standard doses in certain users subsequently identified as carriers of defective *TPMT* gene copies.[1–3] Codominant expression of inherited *TPMT* genes has previously been reported[4] and about 39 defective alleles are currently known[5] with varied prevalence across populations and ethnicities. *TPMT*2* (238G>C), **3A*, **3B* (460A>G) and **3C* (719A>G) are the most significant of these alleles owing to their widespread prevalence in reported studies, although another less studied intronic variant (rs12201199) with a notably high prevalence of over 50% in Africans has been correlated with cisplatin ototoxicity.[6] Nevertheless, *TPMT*2*, **3A*, **3B* and **3C* account for about 95% of the defective variants in Caucasians[5,6] whereas only **3A* and **3C* have been reported in a few African populations studied to date and none of the rare variants like *TPMT*4*, for instance, have been reported in Africans.[7–9] A high genotype–phenotype correlation reported to be over 90%,[5] albeit with noted discrepancies owing to the impact of a number of non-genetic factors,[10] makes *TPMT* genotype status a reliable indicator of clinical phenotype and thus a guide for the safe administration of thiopurine drugs in most populations.

2. Aim

Studies of prevalent *TPMT* variants have often characterized most populations where genotype-guided dosing of TMPT-associated drugs have become a standard. Nigeria, with a population of over 140 million people, consists of three main ethnicities (*Hausa*, *Igbo*, *Yoruba*) which may be further distinctly subdivided into about 374 others.[11] Such huge diversity, often characteristic of African populations in general and previously reported to impact wide genetic diversity,[12] warrants the need for this study which attempts to address the paucity of data on the prevalence of common clinically-relevant *TMPT* variants in the population.

3. Material and methods

3.1. Study population

The study population ($n = 180$) consisted of 123 male and 57 female healthy volunteers who were required to provide written informed consent after the study had previously been approved by the ethics committee of the Obafemi Awolowo University Teaching Hospital, Nigeria. These subjects, all unrelated, were drawn from different ethnicities in Nigeria which included *Igbo* ($n = 44$), *Yoruba* ($n = 101$), *Hausa* ($n = 23$) and other minor ethnic groups ($n = 12$). Each participant provided 5 mL of whole blood which was collected in EDTA tubes and stored at −20°C until analysis.

3.2. DNA extraction and iPLEX™ genotype analysis

Genomic DNA was extracted from 400 μL of whole blood using the phenol–chloroform method and further diluted to 10 ng/μL with ultrapure water for the iPLEX genotyping assay. The multiplex polymerase chain reaction (PCR), iPLEX assay and extension primers for *TPMT*2*, **3B*, **3C* and **4A* were designed using Assay Design Suite v 2.0 (Agena BioScience, CA), and these primers, other reagents and equipment used were from Agena Bioscience (San Dieago, CA, USA).

Multiplex PCR amplification of targets at a final volume of 5 μL was carried out using 0.8 μL ultrapure water, 0.5 μL of 10× PCR reaction buffer, 0.4 μL of 2-mM $MgCl_2$, 0.1 μL of 500-μM dNTP mix, 1 μL of primer mix (0.5 μM each), 0.2 μL of polymerase (5 U/μL) and 2 μL of genomic DNA. Thermocycling condition included a denaturation at 94°C for 15 min, followed by 45 cycles at 94°C for 15 s, 56°C for 30 s and 72°C for 1 min. A final extension was done 72°C for 3 min and thereafter the reaction placed on 4°C hold.

Dephosphorylation of the PCR product was carried out by adding 2 μL of a shrimp alkaline phosphatase (SAP) reaction mix consisting of 1.53 μL of ultrapure water, 0.17 μL SAP buffer, 0.3 μL of SAP enzyme (1.7 U/μL) to 5 μL of the multiplex PCR product. This was then incubated at 37°C for 40 min in a thermocycler followed by the denaturing of SAP at 85°C for 5 min.

The iPLEX primer extension assay was then carried out in mix consisting of 0.755 μL of ultrapure water, 0.2 μL of iPLEX buffer, 0.2 μL of iPLEX termination mix, 0.804 μL of primer mix and 0.04 μL of iPLEX enzyme. This was cycled using a two-step 200 short cycles program which started with denaturation step at 94°C for 30 s; an annealing at 52°C for 5 s and extension at 80°C for 5 s which was repeated another four times. This described process was repeated for another 39 times, followed by a final extension at 72°C for 3 min and the product subsequently held at 4°C.

The final extension product was desalted with a resin mix and 20 μL of the product dispensed onto a Spectro II array chip for analysis using the Bruker mass spectrometer. Visual quality checks were made of the generated peaks in addition to the non-template control prior to report generation using Typer 4 analysis software. The iPLEX analysis was carried out by Uniservices, University of Auckland (Auckland, New Zealand). Validation of the nucleotide calls was further carried out by Sanger sequencing of products generated after amplifying target sequences from a set of randomly selected genomic DNA samples.

3.3. Statistical analysis

Using the Fisher's exact test, the genotype frequencies of *TPMT* alleles were assessed for Hardy–Weinberg equilibrium and also compared with those of other populations. A $P < 0.05$ implied statistical significance.

4. Results

Carriers of defective *TMPT* alleles in the population were at a frequency of 0.10 (18/180; 95% CI, 0.060–0.153) with *TPMT*3C* accounting for all variant alleles detected. Heterozygous carriers of *TPMT*3C* were at a frequency of 0.094 (17/180; 95% CI, 0.056–0.147) while homozygous *TMPT*3C* carriers were at a frequency of 0.006 (1/180; 95% CI, 0.000–0.031). *TPMT*2*, **3B* and **4* were not detected in the study population. The prevalence of alleles in the ethnicities studied is presented in Table 1. Inheritance of the *TPMT*3C* allele conformed with Hardy–Weinberg equilibrium as assessed using Fisher's exact test and a random genotype validation with Sanger sequencing confirmed consistency of called genotypes.

5. Discussion

Polymorphism in the *TPMT* gene and its implication for thiopurine drugs' toxicity has been one of the most successful applications of pharmacogenetics in clinical practice as the current trend requires that *TPMT* genotype be known before drug administration. The prevalence of these common *TPMT* alleles which are well-correlated with adverse responses in patients is also known to vary across populations and ethnicities.[5,9] A summary table showing the identified allele in the present study, previously known to result in decreased TPMT activity,[5,12] alongside data from some other populations is presented in Table 2. The *TPMT*3C* (rs1142345) allele, identified in the present study and in all the few other African populations[7–9,13,15] studied to date, appears to be a common defective allele in Africans and African-Americans except in Moroccans.[14,16] However, the prevalence of the *TPMT*3C* allele in Nigerians is only comparable to reported frequencies in Ghanaians and Kenyans, but significantly higher than values reported for Egyptians, Libyans, Tunisians, African-Americans, Caucasians and Asians. The *TPMT*3A* allele which carries two mutations, one in exon 7 (G460A) and the other in exon 10 (A719G), has been previously reported some African population[7,9] and is known to inflict the largest decrease in TPMT activity.[21] This allele was not detected in the present study and neither were the *TPMT*3B* (460G>A) and **4* alleles. This study also reports on the absence of the *TPMT*2* allele in this population. This allele, reported to result from a single nucleotide transversion in exon 5 (G238C) of the *TPMT* gene,[22]

Table 1 – Summary data of studied TPMT alleles in the Nigerian population.

Ethnic groups	N	Allele frequency (95% CI)			
		TPMT*2	TPMT*3B	TPMT*3C	TPMT*4
Igbo	44	0.000	0.000	0.057 (0.019, 0.128)	0.000
Yoruba	101	0.000	0.000	0.054 (0.026, 0.095)	0.000
Hausa	23	0.000	0.000	0.043 (0.005, 0.148)	0.000
Others*	12	0.000	0.000	0.042 (0.001, 0.211)	0.000
Total	180	0.000	0.000	0.053 (0.032, 0.081)	0.000

* This group consisted of: Isoko – 1 person, Bini – 4, Urhobo – 2, Ebira – 3, Ibibio – 2.

Table 2 – Prevalence of observed TPMT alleles in Nigerians and some other populations.

Populations	Number of subjects	Allele frequencies TPMT*3C	References
Nigerians	180	0.053	Present study
Other African populations			
Egyptians	200	0.013*	7
Ghanaians	217	0.076	8
Libyans	246	0.010*	9
Kenyans	101	0.054	13
Moroccans	103	0.000*	14
Tunisians	208	0.014*	15
African-Americans	248	0.024*	16
Asians			
Japanese	192	0.008*	17
Chinese (Uygur)	160	0.016*	18
Koreans	400	0.009*	19
Vietnamese	159	0.028	19
Caucasians			
Italians	103	0.010*	20
Germans	1222	0.004*	21

* Significantly different frequencies compared with the present study population at 95% CI using the Fisher's exact test.

is known to exert greater reduction in enzyme activity than the *TPMT*3C* allele[21,23] and is generally less frequent in populations of the world. Heterozygous carriers of low-activity-impacting *TPMT* alleles detected in the present study will be expected to show intermediate intolerance to standard thiopurine therapy consequently necessitating appropriate dose adjustment, whereas administration of thiopurine drugs may not be advisable for the homozygous carriers in the population.

6. Conclusions

The present study identified *TPMT*3C* as the main defective allele in the Nigerian population accounting for 100% of the variant alleles detected. The prevalence of this allele was also observed to be similar across the main ethnicities in the Nigerian population and may thus be the only *TPMT* variant critical for the dosing of thiopurine drugs in the population.

Conflict of interest

None declared.

REFERENCES

1. Fazel-Najafabadi E, Vahdat Ahar E, Fattahpour S, Sedghi M. Structural and functional impact of missense mutations in TPMT: an integrated computational approach. *Comput Biol Chem*. (59 Pt A):2015;(59 Pt A):48–55.
2. Stocco G, Martelossi S, Barabino A, et al. TPMT genotype and the use of thiopurines in paediatric inflammatory bowel disease. *Dig Liver Dis*. 2005;37(12):940–945.
3. Dhawan A, Ruwali M, Pant MC, Rahman Q, Parmar D. Association of genetic variability in enzymes metabolizing chemotherapeutic agents with treatment response in head and neck cancer cases. *Asia Pac J Clin Oncol*. 2016. http://dx.doi.org/10.1111/ajco.12446 [Epub ahead of print].
4. Farfan MJ, Salas C, Canales C, et al. Prevalence of TPMT and ITPA gene polymorphisms and effect on mercaptopurine dosage in Chilean children with acute lymphoblastic leukemia. *BMC Cancer*. 2014;28:299.
5. Chouchana L, Narjoz C, Roche D, et al. Interindividual variability in TPMT enzyme activity: 10 years of experience with thiopurine pharmacogenetics and therapeutic drug monitoring. *Pharmacogenomics*. 2014;15(6):745–757.
6. Ross CJ, Katzov-Eckert H, Dubé MP, et al. Genetic variants in TPMT and COMT are associated with hearing loss in children receiving cisplatin chemotherapy. *Nat Genet*. 2009;41(12):1345–1349.
7. Hamdy SI, Hiratsuka M, Narahara K, et al. Genotype and allele frequencies of TPMT, NAT2, GST, SULT1A1 and MDR-1 in the Egyptian population. *Br J Clin Pharmacol*. 2003;55 (6):560–569.
8. Ameyaw MM, Collie-Duguid ES, Powrie RH, Ofori-Adjei D, McLeod HL. Thiopurine methyltransferase alleles in British and Ghanaian populations. *Hum Mol Genet*. 1999;8(2):367–370.
9. Zeglam HB, Benhamer A, Aboud A, et al. Polymorphisms of the thiopurine S-methyltransferase gene among the Libyan population. *Libyan J Med*. 2015;10:27053.
10. Clayton TA, Lindon JC, Cloarec O, et al. Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature*. 2006;440(7087):1073–1077.
11. National Population Commission, Abuja, Nigeria. 2008 Nigeria Demographic and Health Survey (NDHS Report). http://www.population.gov.ng/index.php/surveys. [accessed 19.03.16].
12. Tishkoff SA, Reed FA, Friedlaender FR, et al. The genetic structure and history of Africans and African Americans. *Science*. 2009;324(5930):1035–1044.
13. McLeod HL, Pritchard SC, Githang'a J, et al. Ethnic differences in thiopurine methyltransferase pharmacogenetics: evidence for allele specificity in Caucasian and Kenyan individuals. *Pharmacogenetics*. 1999;9 (6):773–776.
14. Janati Idrissi M, Samri I, Khabbal Y, El Hassouni M, Ouldim K. TPMT alleles in the Moroccans. *Clin Res Hepatol Gastroenterol*. 2015;39(4):e55–e56.
15. Melaouhia S, Fékih M, Garat A, et al. Allele frequency of inosine triphosphate pyrophosphatase (ITPA) and thiopurine-S-methyl transferase (TPMT) genes in the Tunisian population. *Clin Res Hepatol Gastroenterol*. 2012;36 (2):178–184.
16. Hon YY, Fessing MY, Pui CH, Relling MV, Krynetski EY, Evans WE. Polymorphism of the thiopurine S-methyltransferase gene in African-Americans. *Hum Mol Genet*. 1999;8(2):371–376.
17. Hiratsuka M, Inoue T, Omori F, Agatsuma Y, Mizugaki M. Genetic analysis of thiopurine methyltransferase polymorphism in a Japanese population. *Mutat Res*. 2000;448 (1):91–95.
18. Zhang JP, Guan YY, Xu AL, et al. Gene mutation of thiopurine S-methyltransferase in Uygur Chinese. *Eur J Clin Pharmacol*. 2004;60(1):1–3.
19. Lee SS, Kim WY, Jang YJ, Shin JG. Duplex pyrosequencing of the TPMT*3C and TPMT*6 alleles in Korean and Vietnamese populations. *Clin Chim Acta*. 2008;398(1–2):82–85.
20. Rossi AM, Bianchi M, Guarnieri C, Barale R, Pacifici GM. Genotype–phenotype correlation for thiopurine S-methyltransferase in healthy Italian subjects. *Eur J Clin Pharmacol*. 2001;57(1):51–54.
21. Tai HL, Krynetski EY, Yates CR, et al. Thiopurine S-methyltransferase deficiency: two nucleotide transitions define the most prevalent mutant allele associated with loss of catalytic activity in Caucasians. *Am J Hum Genet*. 1996;58 (4):694–702.
22. Evans WE, Hon YY, Bomgaars L, et al. Preponderance of thiopurine S-methyltransferase deficiency and heterozygosity among patients intolerant to mercaptopurine or azathioprine. *J Clin Oncol*. 2001;19 (8):2293–2301.
23. Krynetski EY, Schuetz JD, Galpin AJ, Pui CH, Relling MV, Evans WE. A single point mutation leading to loss of catalytic activity in human thiopurine S-methyltransferase. *Proc Natl Acad Sci U S A*. 1995;92(4):949–953.