Mahindroo et al., J Pharmacogenomics Pharmacoproteomics 2017, 8:1
DOI: 10.4172/2153-0645.1000167

Journal of Pharmacogenomics & Pharmacoproteomics

Case Report Open Access

Thiopurine Methyltransferase Genotype Testing in Paediatric Patients in South Australia: A Retrospective Audit into Prescribing Practices

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Abstract

Background: Thiopurines are used to treat a number of medical conditions including inflammatory bowel disease, acute lymphoblastic leukemia and severe eczema in children. Pre prescription identification of variant alleles helps reduce thiopurine related adverse events. The aim of the study was to explore the clinical utility of TPMT genotyping in a paediatric population in South Australia, specifically the uptake of testing and whether the results are guiding appropriate dosing of thiopurines in keeping with current established international guidelines.

Methods: A retrospective audit was conducted reviewing all patients below the age of 18 years who underwent TPMT genotyping in South Australia during the 10-year period between January 2004 and January 2014. Data regarding demographics and prescribing practices was collected from the medical records of 260 paediatric patients.

Results: Paediatric gastroenterologists requested 67% of the TPMT genotypes performed. Loss of function alleles were confirmed in almost 9% of cases. There were positive correlations between adverse events and whether the test was used correctly ($p<0.011$) and with subspecialty unit ($p<0.001$). Oncology recorded the largest percentage of adverse events 63.5% whilst only comprising 16.5% of the total dataset.

Conclusion: The safest prescribing practice in all groups of patients is to ensure the TPMT gentyope is performed prior to administration and dosing is guided by the results and established guidelines.

Keywords: Thiopurine methyltransferase; Pediatric; Genotype; Audit

Introduction

The immunosuppressive agent Thiopurines (e.g. azathioprine, 6-mercaptopurine and Thioguanine) are used to treat a variety of medical conditions including Inflammatory Bowel Disease (IBD), Acute Lymphoblastic Leukemia (ALL) and severe eczema [1,2]. Adverse effects of Thiopurines include myelosuppression, hepatotoxicity and pancreatitis [3]. The metabolism of Thiopurines is predominantly directed through the enzyme Thiopurine Methyltransferase (TPMT) that converts the drugs to their inactive metabolites 6 thioguanine nucleotides (6-TGN) and 6-methylmercaptopurine (6-MMP). Blood concentrations of 6-TGN and 6-MMP are also important predictors for hepatotoxicity in patients with inflammatory bowel disease [4].

As of January 2016 there are 53 identified alleles (single nucleotide polymorphisms) of the TPMT gene [5]. Variance in genotype closely correlates to the level of TMPT activity in the blood (phenotype) [6]. Genotyping and Phenotyping both have high specificity; however, genotyping has greater sensitivity [7]. The most common genotypes are: Wild type (TPMT *1) which encodes normal enzyme activity, variant alleles (TPMT *2, *3A, *3B, *3C or *4) that determine intermediate or low activity. Individuals with homozygous variant alleles are at highest risk for thiopurine related adverse effects [8]. In paediatric patients diagnosed with ALL, heterozygotes require more frequent mercaptopurine dose adjustment due to myelosuppression than do wild type genotypes [9,10]. A metanalysis of eleven studies using Azathioprine in the treatment of autoimmune disease established the association between TPMT polymorphisms and myelosuppression and gastric intolerance [11].

As with all pharmacogenomics, there is significant variability in genotype amongst ethnic groups. The frequency of two inactive alleles is 1% in the sub-Saharan African population, 0.3% for Caucasians and perhaps even lower for the Asians [12-14]. In a study of Korean paediatric patients with IBD, 5.5% were heterozygotes and 0.9% was homozygous [15]. In contrast, 7.8% of paediatric patients with IBD examined in a study from Slovakia were heterozygotes [16] In Chilean children with ALL the frequency of heterozygotes was 8% [17]. These variations become increasing relevant as migratory patterns of ethnic groups reshape the genetic fingerprint of ethnically diverse nations such as Australia.

Pre prescription TPMT genotyping is an example of pharmacogenomics in clinical practice. The benefits to both heterozygous and homozygous null individuals with regards to reduction of significant adverse effects through appropriate dosing have been well established [18]. As Thiopurines are an important aspect of chemotherapy for ALL, assessment of TPMT genotype is essential to balance the risks of adverse effects with the risk of incomplete treatment or secondary malignancy. There is no evidence of increased risk of relapse in children suffering from ALL who have been prescribed lower doses of thiopurines following TPMT testing; however, these children may have a reduction in secondary malignancy [19].

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Received January 11, 2017; **Accepted** February 09, 2017; **Published** February 17, 2017

Citation: Mahindroo S, Fletcher J, Hissaria P, Hakendorf P, Nicholson S (2017) Thiopurine Methyltransferase Genotype Testing in Paediatric Patients in South Australia: A Retrospective Audit into Prescribing Practices. J Pharmacogenomics Pharmacoproteomics 8: 167. doi: 10.4172/2153-0645.1000167

The Clinical Pharmacogenetics Implementation Consortium (CPIC) provides peer reviewed and evidence based guidelines for Thiopurine dosing. Recommendations include; normal starting dose for homozygous wild type or normal enzyme activity, 30-70% of target dose for heterozygotes or intermediate enzyme activity. Finally, consider alternative therapy or a significant reduction in thiopurine starting dose and reduction in frequency of administration for those with two non-functional alleles [20,21]. There is however, a wide variation in clinical practice guidelines across different specialty units [22].

TPMT genotyping prior to thiopurine treatment has been shown to be a cost effective test and an astute use of healthcare resources [23]. The cost of thiopurine-mediated leucopenia and related hospitalization outweighs the cost of a genetic test [24].

Methods

A retrospective audit was conducted reviewing all patients below the age of 18 years who underwent TPMT genotyping through the immunology department at SA Pathology in South Australia during the 10-year period between January 2004 and January 2014. The aim of the study was to explore the clinical utility of TPMT genotyping in the paediatric population in South Australia, specifically the uptake of testing and whether the results are guiding appropriate dosing of thiopurines in keeping with current established international guidelines.

Genomic DNA was analysed for the TPMT gene variants, *1S,*2, *3A, *3C, *3D, *8, *9, *20, by PCR amplification of exons 5, 7 and 10 followed by direct DNA sequencing using the primers 5'-CTACAgTgAATCTgCgTgC-3', 5'-GTACCAGCATGCACCATG-3', 5'-CAggCTTTAgCATAATTTTC-3' respectively [25]. TPMT variant alleles were classified as defined by the TPMT nomenclature committee [26].

Of the 334 tests performed, 10 were duplicated tests, 27 were ordered from a hospital other than Women's and Children's Hospital and 30 patients were followed up privately. These cases were therefore excluded. The medical records for 7 patients were unable to be accessed. Data was collected from the remaining 260 patients. Women's and Children's Hospital pharmacy data collected between October 2009 and January 2014 revealed that 219 different patients were dispensed Azathiopurine and 99 patients were dispensed Mercaptopurine.

Primary outcome measures were patient demographics (age, gender, primary diagnosis, ethnicity and genotype) and prescribing practices (medical specialty, use of test result, starting dosage, dose modifications and adverse event data). Adverse event data was only reviewed for 1 year following TPMT genotyping. In order to assess specific adverse events (hepatotoxicity and leucopenia) baseline complete blood examination and liver function test data were also recorded.

Data was analysed using Stata software [27]. Categorical and binary variables are presented as numbers and percentages. Fisher's exact test was used to assess for statistical significance where appropriate. A p value of <0.05 was used as indicator of statistical significance.

Results

260 patient records dating from between January 2004 to January 2014 were reviewed. 145 were male and 115 were female. Table 1 demonstrates the population demographics by ethnicity, genotype, diagnoses and subspecialty unit. Gastroenterologists ordered the largest number of TPMT genotype tests, comprising just over 67% of the total. Figure 1 displays the number of tests ordered by subspecialty unit over the examined period, note the earlier years (2004-2007) was uninformative.

96% of patients had a baseline complete blood examination prior to genotyping with 7% of these being abnormal. Similarly, 93% of patients had baseline liver function tests performed, with 36% being abnormal. In only 32% of patients, were the results of the test i.e., the starting dose of thiopurine was modified according to their TPMT genotype.

Incomplete data were available for 51/260 cases. In 47 cases thiopurine was not prescribed in spite of the test being administered. 2 cases were non-compliant with the prescribed drug, 1 moved interstate and 1 was transitioned to adult care after prescription.

In 75% of cases, co administered medications were prescribed and 90% of these were immunosuppressive. These were likely to be contributory to the adverse events recorded.

There were 44 adverse events (Table 2). Of the adverse events identified, 28/44 was as a result of myelosuppression, 3 from leucopenia, 3 from neutropenia and 4 from hepatotoxicity, there was 1 case of pancreatitis and 1 of a drug eruption. The remaining were non-specific complaints e.g. abdominal pain, tantrums, headaches and dizziness. There was no correlation found between any specific TPMT alleles and adverse events ($p=0.974$).

Following prescription, a dose adjustment was made in 165 cases. However, somewhat surprisingly, in only 66% of these adjustments did the prescriber actually use the TPMT genotype to guide adjustment?

Following an adverse event, in the majority of cases the medication was either ceased or held for a period of time following adverse reaction. No patient was directly hospitalized due to the adverse event and none died as a direct result.

In 42 cases the initial dose of thiopurine prescribed was too high; in 128 it was too low, predominantly in gastroenterology patients.

Discussion

The rate of heterozygous genotype in this data set was 8.8%. This is similar to rates seen in other parts of the world [12-16]. There were no homozygous variant genotypes found in this cohort of patients. A statistically significant correlation has been found between genotype and ethnicity; however, given the small numbers in the ethnicity dataset, further analysis than this will not be accurate (Table 3) and is beyond the scope of this study. In addition to this, a correlation between

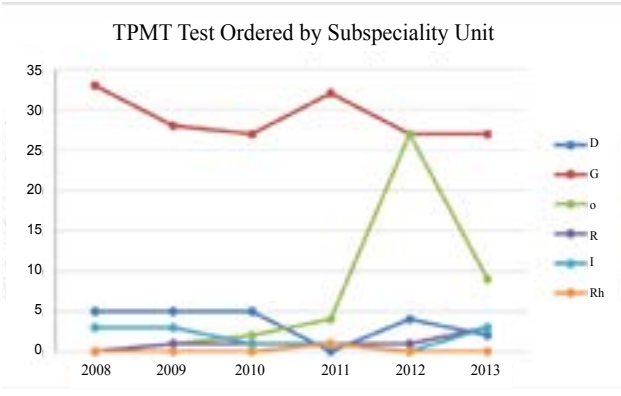

Figure 1: TPMT test ordered by subspecialty unit.

Ethnicity		
Not documented	147	56.5%
African	5	1.9%
Asian	15	5.8%
Caucasian	88	33.9%
Indigenous	5	1.9%
Subspecialty Unit		
Dermatology	22	8.5%
Gastroenterology	175	67.3%
Immunology	11	4.2%
Oncology	43	16.5%
Rheumatology	1	0.4%
Renal	8	3.1%
Diagnosis		
Acute Lymphoblastic Leukemia	38	15%
Autoimmune Hepatitis	16	6%
Crohn's Disease	99	38%
Severe Eczema	33	13%
Ulcerative Colitis	52	20%
Other	22	8%
Genotype		
*1/*1	149	57.3%
*1/*3A	13	5%
*1/1s	74	28.5%
*1/*2	1	0.4%
*1/*3C	2	0.8%
*1S/*1S	13	5.4%
Other	7	2.7%
*1s/3A	3	1.2%
*1S/3C	2	0.8%
*1S/349G>A	1	0.4%
*1/ *8	1	0.4%

Table 1: Demographics N (260) percentage.

subspecialty unit and ethnicity implies certain ethnic groups may be more prone to certain diseases; this is consistent with previous findings [28-30].

There was no direct correlation between adverse events and genotype, ethnicity (Table 2) and whether patients who had adverse events had their thiopurine doses adjusted following the genotype becoming available (p=0.759).

There is a statistically significant variation in practice between subspecialty units regarding whether the TPMT genotype was used prior to prescription of a thiopurine (p<0.001). Oncology patients were treated using predetermined protocols to guide dosing rather than the TPMT genotype, in many cases the genotype was ordered after the patient had been on the thiopurine for months. Gastroenterology prescribers awaited the test result prior to commencing treatment (with Azathioprine) and in the cases where treatment was initiated prior to the result it was always at a low dose (0.5 mg/kg/day) with a view to be increased in due course. Similarly, dermatology and immunology prescribers awaited the test results prior to prescription and if appropriate commenced at high dose immediately (2 mg/kg/day). This is in keeping with established international guidelines [2]. Gastroenterologists were the only subspecialty unit to consistently order and monitor 6-thioguanine nucleotide (6-TGN) levels, in keeping with research that confers ideal of drug therapy [31].

Adverse events were less likely to occur in patients whose dose was determined according to TMPT genotyping (11.7% *vs.* 26.5% p=0.011).

	No Adverse Events (*n*=165)	**Adverse Events (*n*=44)**	***P***
Genotype result used for dose prescription	-	-	**0.011**
Yes	68 (41%)	9 (20%)	
No	97 (59%)	35 (80%)	
Subspecialty Unit	-	-	**0.000**
Dermatology	10 (6.1%)	2 (4.6%)	-
Immunosuppressive Medication			**0.126**
Yes	134 (81%)	40 (91%)	-
No	31 (19%)	4 (9%)	-
Genotype	-	-	**-**
			0.974
*1/*1	92 (55.7%)	24(54.6%)	-
*1/*3A	9 (5.5%)	2 (4.6%)	-
*1/1s	49(29.7%)	14(31.8%)	-
*1/*2	1 (0.6%)	0	-
*1/*3C	1 (0.6%)	0	-
*1S/*1S	9 (5.5%)	2 (4.6%)	-
Other	4 (2.4%)	2 (4.6%)	-
	-	-	**0.879**
Wild Type	141 (85%)	38 (86%)	-
Heterozygous Variant	24 (15%)	6 (14%)	-
Ethnicity	***N*=70**	***N*=15**	**0.505**
African	3 (4.3%)	0	**-**
Asian	7 (10%)	3 (10%)	-
Caucasian	57 (81.4)	12 (80%)	-
Indigenous	3 (4.3%)	0	-

Table 2: No adverse events (n=165) adverse events (n =44).

Variant prescribing practices were associated with an increased risk of adverse drug reactions (P<0.001), with oncology recording the largest numbers of adverse events, over 63.5% whilst only comprising less than 20% of the total patient dataset. There are however some key confounders here including initial diagnosis, additional immunosuppressive and chemotherapy medications.

A proportion of patients received additional medications (alongside thiopurine); these were more likely to be immunosuppressive. The addition of an immunosuppressive did not increase the rate of adverse events, such an association may be found with an increased sample size however (Table 2). As expected, a correlation between subspecialty unit and additional immunosuppressive use was found, (p=0.004) with most Oncology and Gastroenterology patients on more than one immunosuppressive at any point in time.

It was unable to be determined from the record whether treatment success or failure was the result of inadequate thiopurine levels.

Conclusion

The CPIC guidelines suggest reducing the doses for patients with one nonfunctional TPMT allele and significantly reduced doses for those with malignancy and two non-functional alleles. This is to be down alongside ongoing monitoring for adverse effects [20,21]. This study has identified some key areas of deviation from established guidelines and offers the opportunity to reflect and improve on our prescribing practices.

	African	Asian	Caucasian	Indigenous	*P* value
Subspecialty Unit	-	**-**	-		**0.001**
Dermatology	0	4	7	0	-
Astroenterology	5	7	67	2	-
Immunology	0	3	6	0	-
Oncology	0	0	8	2	-
Renal	0	1	0	1	**-**
Genotype	-	**-**	-	-	**0.021**
*1/*1	1	8	52	4	-
*1/*3A	0	0	6	0	-
*1/1s	3	6	23	0	**-**
*1/*2	0	0	1	0	-
*1/*3C	1	1	0	0	-
*1S/*1S	0	0	5	0	-
Other	0	0	1	1	-

Table 3: Ethnic variations.

Acknowledgements

Research was commenced following approval from Women's and Children's Hospital Human Research Ethics Committee (WCH HREC). The author wishes to acknowledge Women's and Children's Hospital medical records department staff. Sean Turner, Director of Pharmacy, Women's and Children's Hospital, Adelaide. Dr. David Moore and Dr. Richard Couper: Paediatric Gastroenterology Department. Dr. Patrick Quin and Dr. Damian Chen: Paediatric Allergy/Immunology Department.

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