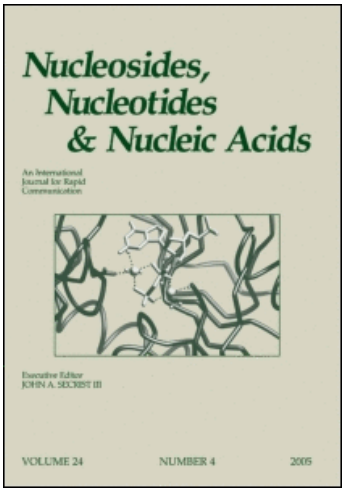

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Mutation in the *ITPA* Gene Predicts Intolerance to Azathioprine

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ABSTRACT

Inosine triphosphate pyrophosphatase (ITPase) deficiency occurs with polymorphic frequencies in Caucasians and results in the benign accumulation of the inosine nucleotide ITP. In 62 patients treated with azathioprine for inflammatory bowel disease, the *ITPA* 94C>A deficiency-associated allele was significantly associated with adverse drug reactions (OR 4.2, 95% CI 1.6–11.5, p = 0.0034). Significant associations were found for flu-like symptoms (OR 4.7, 95% CI 1.2–18.1, p = 0.0308), rash (OR 10.3, 95% CI 4.7–62.9, p = 0.0213) and pancreatitis (OR 6.2,CI 1.1–32.6, p = 0.0485). Polymorphism in the *ITPA* gene thus predicts AZA intolerance. Alternative immunosuppressive drugs, particularly 6-thioguanine, should be considered for AZA-intolerant patients with ITPase deficiency.

Key Words: Azathioprine; 6-Mercaptopurine; ITPA; ITPase; Inosine triphosphate pyrophosphatase; Thiopurine; Methyltransferase; Side effects.

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INTRODUCTION

Azathioprine (AZA) is widely used in the treatment of chronic inflammatory diseases, haematological malignancies and in transplantation. Polymorphisms in the thiopurine methyltransferase (*TPMT*) gene predict adverse drug reactions (ADR) in 5 to 10% of patients treated with thiopurine drugs,[1] thus the majority of side-effects are unexplained.[1–3] Inosine triphosphate pyrophosphatase (ITPase) deficiency is a benign condition occurring with polymorphic frequency in Caucasians and is characterized by the accumulation of inosine triphosphate (ITP) in erythrocytes.[4] Patients homozygous for a 94C>A missense mutation (Pro32 to Thr) have zero erythrocyte ITPase activity while heterozygotes averaged 22.5% of the control mean. Homozygotes for a second polymorphism in intron 2 (IVS2 + 21A>C) averaged 60% of the control mean.

ITP is formed from IMP, a central intermediate in purine metabolism and in normal cells, ITPase recycles ITP back to IMP. The thiopurine 6-mercaptopurine (6-MP), of which AZA is the pro-drug, is activated through a thio-IMP intermediate. We hypothesised that thio-ITP might accumulate in ITPase deficient patients treated with thiopurine drugs, resulting in toxicity.

METHODS

Ethical permission for the study was granted by the Ethics Committee of Guy's and St Thomas' NHS Trust. A consecutive series of 62 Caucasian IBD patients were identified retrospectively from those referred to the Purine Research Laboratory for TPMT phenotyping because of ADR experienced on AZA therapy. Controls were a consecutive series of 68 Caucasian patients attending the IBD clinic at Guy's and St Thomas' Hospitals treated with AZA for a minimum of 3 months without suffering any ADR. Hepatotoxicity was defined by alanine transaminase greater than twice the upper normal limit (50 IU/l); pancreatitis by severe abdominal pain and serum amylase >800 IU/l; neutropenia by a neutrophil count of $< 2.0 \times 10^9$ cells. Sixteen patients with flu-like symptoms included 5 patients with myalgia as a prominent symptom. Patients experiencing headaches or severe abdominal pain with normal amylase were grouped as 'other.' All patients were genotyped for ITPase 94C>A and IVS2 + 21A > C mutations,[4] TPMT*3A, TPMT*3C and TPMT*2 mutations and phenotyped for erythrocyte TPMT activity.[3] Association between ADR and mutations in each gene was tested using a two-sided Fisher's exact test; odds ratios (OR) and 95% confidence intervals (CI) were calculated from contingency tables.

RESULTS

The association between ADR to AZA therapy and the *ITPA* 94C>A allele was significant (OR 4.2, 95% CI 1.6–11.5, p = 0.0034, Table 1). Furthermore, there was a significant association between the 94C>A mutation and flu-like symptoms (OR 4.7, 95% CI 1.2–18.1, p = 0.0308), rash (OR 10.3, 95% CI 4.7–62.9, p = 0.0213) and

Table 1. Association of adverse drug reaction with a *TPMT* or *ITPA* deficiency-associated genotype in patients treated with azathioprine.

		TPMT *3A + C	*ITPA* 94C>A	*ITPA* IVS2 + 21A>C
All side effects (n = 62)	Odds ratio	1.7	4.2	0.7
	p-value	p = 0.4360	p = 0.0034	p = 0.6403
	N° of heterozygotes	10	15	8
	N° of homozygotes	0	3	1
Flu-like symptoms (n = 16)	Odds ratio	0.3	4.7	1.0
	p-value	p = 0.3372	p = 0.0308	p = 1.0000
	N° of heterozygotes	0	4	3
	N° of homozygotes	0	1	0
Rash (n = 6)	Odds ratio	4.4	10.3	0.9
	p-value	p = 0.1528	p = 0.0213	p = 1.0000
	N° of heterozygotes	2	3	0
	N° of homozygotes	0	0	1
Pancreatitis (n = 8)	Odds ratio	1.3	6.2	0.6
	p-value	p = 1.0000	p = 0.0485	p = 1.0000
	N° of heterozygotes	1	3	1
	N° of homozygotes	0	0	0
Nausea and vomiting (n = 13)	Odds ratio	5.5	3.1	0.4
	p-value	p = 0.0206	p = 0.1529	0.4480
	N° of heterozygotes	5	1	1
	N° of homozygotes	0	2	0
Hepatotoxicity (n = 4)	Odds ratio	2.9	10.3	0.5
	p-value	p = 0.3824	p = 0.0584	p = 1.0000
	N° of heterozygotes	1	2	0
	N° of homozygotes	0	0	0
Other (n = 4)	Odds ratio	0.9	3.4	4.2
	p-value	p = 1.0000	p = 0.3419	p = 0.1894
	N° of heterozygotes	0	1	2
	N° of homozygotes	0	0	0
Neutropenia (n = 11)	Odds ratio	0.9	1.0	0.4
	p-value	p = 1.0000	p = 1.0000	p = 0.6781
	N° of heterozygotes	1	1	1
	N° of homozygotes	0	0	0
Controls (n = 68)	N° of heterozygotes	7	6	10
	N° of homozygotes	0	0	3

p-value for testing against controls, using Fisher's exact test.
$p < 0.05$ considered significant.

pancreatitis (OR 6.2, CI 1.1–32.6, p = 0.0485). By contrast, the IVS2 + 21A>C mutation did not predict ADR.

Mean TPMT activity did not differ significantly between controls (10.7 ± 2.9 U) and the ADR group (10.7 ± 2.7 U, p = 0.1812, two sided t-test). A heterozygous *TPMT* genotype was associated with nausea and vomiting only (Table 1, OR 5.5, 95% CI

1.4–21.3, p = 0.0206). Combining *TPMT* and *ITPA* 94C>A genotypes did not substantially alter the risk of experiencing an ADR (OR 3.5, 95% CI 1.6–7.6, p = 0.0023). Excluding *TPMT* heterozygous genotypes from the ADR cohort strengthened the association between ADR and the *ITPA* 94C>A polymorphism (OR 5.3, 95% CI 1.9–14.6, p = 0.0010).

AZA therapy was continued at full dose in only 5 of 62 ADR patients. Twenty one were re-challenged with a reduced AZA dose. The frequency of heterozygous or homozygous *ITPA* 94C>A genotypes did not differ significantly between those patients tolerating AZA at a reduced dose and those remaining intolerant.

DISCUSSION

Although 3 of 6 patients with *ITPA* 94C>A alleles tolerated a reduced AZA dose, further studies are needed to determine whether therapeutic efficacy is decreased. An alternative approach, to AZA dose reduction is to treat 94C>A heterozygotes with 6-thioguanine. 6-Thioguanine is activated via a thio-GMP intermediate rather than thio-IMP, thus thio-ITP would be unlikely to accumulate in ITPase deficient patients. Indeed, thioguanine has been used successfully in patients with 6-MP/AZA related toxicity.[5]

We recognise that the ADR group may be biased by the retrospective nature of the study towards patients with normal TPMT activity. Patients with an intermediate TPMT phenotype measured prior to AZA therapy may not start therapy because of fears of toxicity, or may not experience an ADR if AZA therapy is initiated at a reduced dose. *TPMT* or *ITPA* variant genotypes were found in 45% of patients with ADR. Unknown mutations in the *TPMT* or *ITPA* genes or other loci, and drug–drug interactions[1] may explain toxicity in some wildtype patients.

In conclusion, this retrospective cohort controlled study reports an association between the *ITPA* 94C>A polymorphism and ADR to AZA therapy. Prospective studies are needed to confirm these results in IBD and other clinical entities where AZA and 6-MP are of therapeutic benefit.

REFERENCES

1. Lennard, L. TPMT in the treatment of Crohn's disease with azathioprine. Gut **2002**, *51*, 143–146.
2. Sandborn, W.; Sutherland, L.; Pearson, D.; May, G.; Modigliani, R.; Prantera, C. Azathioprine or 6-mercaptopurine for inducing remission of Crohn's disease. Cochrane Database Syst. Rev. **2000**, *2*, CD000545.
3. Ansari, A.; Hassan, C.; Duley, J.; Marinaki, A.; Shobowale-Bakre, E.M.; Seed, P.; Meenan, J.; Yim, A.; Sanderson, J. Thiopurine methyltransferase activity and the use of azathioprine in inflammatory bowel disease. Aliment. Pharmacol. Ther. **2002**, *16*, 1743–1750.
4. Sumi, S.; Marinaki, A.M.; Arenas, M.; Fairbanks, L.; Shobowale-Bakre, M.; Rees,

D.C.; Thein, S.L.; Ansari, A.; Sanderson, J.; De Abreu, R.A.; Simmonds, H.A.; Duley, J.A. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. Hum. Genet. **2002**, *111*, 360–367.

5. Dubinsky, M.C.; Hassard, P.V.; Seidman, E.G.; Kam, L.Y.; Abreu, M.T.; Targan, S.R.; Vasiliauskas, E.A. An open-label pilot study using thioguanine as a therapeutic alternative in Crohn's disease patients resistant to 6-mercaptopurine therapy. Inflamm. Bowel Dis. **2001**, *7*, 181–189.