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The impact of thiopurine S-methyltransferase polymorphisms on azathioprine dose 1 year after renal transplantation

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Abstract Azathioprine metabolism is influenced by activity of the enzyme thiopurine S-methyltransferase (TPMT), which varies markedly between individuals. In this study we examined the influence of TPMT gene polymorphisms on azathioprine dose 1 year after renal transplantation. TPMT coding and promoter genotypes were determined using PCR-based assays. Azathioprine dose, white cell count, and intercurrent events throughout the first year after renal transplantation were ascertained from contemporaneous clinical notes. All patients analysed ($n=172$) received an initial azathioprine dose of 1.5 mg/kg per day. Twelve individuals with one variant TPMT coding allele were detected (*3A $n=11$, *3C $n=1$). Of these, 58% required azathioprine dose reduction because of leucopenia, compared to only 30% of homozygous wild-type patients ($P=0.04$). A significant correlation between the presence of ≥11 variable number tandem repeats (VNTRs) in the TPMT promoter and reduction in azathioprine dose was also identified ($P=0.001$). We concluded that when azathioprine is administered at an initial dose of 1.5 mg/kg per day, both coding and promoter TPMT polymorphisms influence the dose tolerated.

Keywords Thiopurine methyltransferase · Genetic polymorphism · Azathioprine · Renal transplantation · Pharmacogenetics

Introduction

The clinical response to many prescribed drugs is highly variable. Some of this variation can be attributed to genetic polymorphisms in enzymes involved in their metabolism [1]. Understanding the molecular basis of this variability has the potential to improve clinical prescribing, as determination of an individual's specific genotype might facilitate awareness of prevention of drug toxicity and optimisation of drug dosing [2]. Azathioprine remains a cornerstone of immunosuppressive therapy, despite the development of newer agents. It is widely used in transplantation and for the treatment of autoimmune diseases and inflammatory bowel disease, and its active metabolite, 6-mercaptopurine (6MP), remains an important anti-leukaemic agent. Azathioprine metabolism is influenced by genetic variation in the enzyme thiopurine S-methyltransferase (TPMT). This enzyme is present in most tissues and catalyses the S-methylation of aromatic and heterocyclic sulfhydryl compounds [3].

Although its endogenous substrate is unknown and its biological role uncertain, TPMT plays a pivotal role in the metabolism of the thiopurine group of drugs, including azathioprine and 6MP (reviewed in [4]). These drugs mediate their anti-proliferative effects through conversion into 6-thioguanine nucleotides, a process catalysed by hypoxanthine-guanine phosphoribosyl-

transferase. When incorporated into DNA, 6-thioguanine nucleotides are directly cytotoxic and also suppress de novo purine synthesis [5]. Thiopurine drugs are also metabolised by two other competing pathways: they may be oxidised into 6-thiouric acid by xanthine oxidase or, alternatively, may be converted into inactive 6-methyl compounds by TPMT. Thus, the metabolism of thiopurines by TPMT shunts the drug down the methylation pathway and away from the active pathway. Excessive availability of azathioprine for conversion into active 6-thioguanine nucleotides, either through drug overdose [6, 7] or because of deficiency in TPMT [8, 9], results in significant myelotoxicity. As thioguanine nucleotide concentration is reciprocally related to leukocyte count, azathioprine toxicity is monitored clinically by regular measurement of circulating white cells.

TPMT activity has a trimodal distribution, with 89% of individuals exhibiting high activity, 11% intermediate activity, and 1 in 300 functional deficiency [3]. Much of this variation can be attributed to polymorphisms in the TPMT gene [10]. Several coding-region polymorphisms have been identified, and the resulting variant proteins generally have diminished activity when compared with the wild-type protein, at least in part because of more rapid degradation [11]. These polymorphisms influence clinical response to thiopurine drugs: individuals that are homozygous for coding polymorphisms are at very high risk of azathioprine toxicity [8, 9]; conversely, individuals with very high levels of TPMT activity might be inadequately treated at conventional doses [12].

The clinical impact of TPMT coding-region heterozygosity remains controversial: while some studies have shown an association between TPMT heterozygosity and leucopenia induced by azathioprine or 6MP [13, 14, 15], others have found no association [16, 17, 18]. Furthermore, significant variability of TPMT activity has been noted amongst individuals without coding-region variants. One explanation might be the existence of a variable number of tandem repeats (VNTRs) polymorphism in the transcription factor-binding site of the TPMT promoter. This polymorphism consists of repeating sequences of a 17–18 basepair unit and is thought to affect binding of the transcription factor Sp1 [19]. The relationship between promoter polymorphisms and TPMT activity is controversial.

An inverse relationship between in vitro TPMT activity and total number of repeats has been documented [19] although, in other studies, the association has been found to be weak [20, 21] or absent [22]. Additional studies have suggested that the number of specific motifs within the VNTR structure is more important than the number of repeats in influencing enzyme activity [21]. The impact of promoter polymorphisms on drug-induced adverse events in vivo is unknown.

The aim of this study was to determine the effect of coding and promoter polymorphisms of the TPMT gene on changes in azathioprine dose after renal transplantation.

Methods

Patients and controls

This study was approved by the Central Oxford Research Ethics Committee. Individuals who received a kidney transplant at the Oxford Transplant Centre between January 1995 and December 1997 were included in this study if they had been treated with azathioprine. Clinical charts and case notes, including contemporaneously recorded drug doses and haematological parameters, were reviewed retrospectively by a single individual who was blind to the genotyping results. For each patient, records for the first year after transplantation were examined and pertained to a minimum of 45 medical and haematological assessments. Of the patients included in this study, 95% were of Caucasoid ethnic origin.

After transplantation, all patients received cyclosporin, azathioprine, and prednisolone according to a standard immunosuppressive regimen. Only patients who were highly sensitised (IgG panel reactive antibodies >85%) received anti-thymocyte globulin (ATG) at induction. Cyclosporin was administered, either as Sandimmune or Neoral, to maintain a trough plasma level of 150–300 ng/ml for the first 6 months and 75–150 ng/ml thereafter (Neoral) or 100–200 ng/ml thereafter (Sandimmune). Azathioprine was available as 10, 25, 50, and 100 mg tablets. The initial azathioprine dosage was calculated by weight at the time of transplant and was approximated as closely to 1.5 mg/kg per day as possible. All patients were treated according to a clinical protocol that dictated that this dose be maintained throughout the first year unless leucopenia or other adverse event developed: if the white cell count fell below 4.0×10^9/l, azathioprine dose was reduced by 50%, and if it fell below 3.0×10^9/l, azathioprine was omitted. After recovery of the white cell count, the protocol specified reintroduction of azathioprine to a maximum of 1.5 mg/kg, although occasionally this did not occur. Azathioprine dose was not adjusted for changes in body weight after transplantation.

All patients received aspirin 75 mg daily starting immediately before and continuing for 1 month post-transplantation to prevent renal vein thrombosis. All patients also received co-trimoxazole prophylaxis for 3 months after transplantation. During the epoch under investigation (1995–1997), no patient received prophylactic ganciclovir to prevent CMV infection. Patients at high risk of infection (donor CMV positive and recipient

CMV negative, or both donor and recipient CMV negative) were screened weekly by direct detection of CMV antigen in peripheral blood leukocytes [23]. Patients taking allopurinol were excluded from the study because of its interaction with azathioprine.

A control population comprising 218 UK Caucasoid cadaveric renal allograft donors were also genotyped for TPMT polymorphisms.

Genotyping methodology—TPMT coding polymorphisms

A unified polymerase chain reaction with sequence-specific primers (PCR-SSP) system was established, which allowed simultaneous detection of TPMT alleles *1, 2, *3A, *3B, *3C, *3D, *4, *5, *6 and *7. Primer sequences and mix composition are listed in Table 1. The total volume of the PCR reaction mixture was 8 μl and consisted of 68 mmol/l Tris base pH 8.8; 16.9 mmol/l ammonium sulphate, 1.9 mmol/l magnesium chloride; 0.1% v/v Tween 20; 93 μmol/l of each of dATP, dTTP, dGTP and dCTP; between 0.1 and 0.01 μg DNA; and 0.1177 units of *Taq* polymerase (Advanced Biotechnology, London, UK). Primer concentrations were optimised for each reaction. DNA samples were amplified in MJ Research PTC-200 thermal cyclers. Cycling parameters were as follows: 1 min at 96°C followed by five cycles of 96°C for 25 s, 70°C for 45 s and 72°C for 45 s, followed by 21 cycles of 96°C for 25 s, 65°C for 50 s and 72°C for 45 s, followed by four cycles of 96°C for 25 s, 55°C for 60 s and 72°C for 120 s.

Following PCR, 5 μl of loading buffer, consisting of 0.25% Orange G, 30% v/v glycerol, and 0.5x TBE buffer (89 mmol/l Tris base, 89 mmol/l boric acid, 2 mmol/l EDTA, pH 8.0), was added to each reaction mix. PCR products were subjected to electrophoresis in 1.0% agarose gels containing 0.5 μg/ml ethidium bromide for 30–35 min at 15 V/cm in 0.5x TBE buffer, visualised with UV illumination, and photographed with a Polaroid Land camera. To verify successful DNA amplification, all primer mixes included a control primer set, which amplifies a non-polymorphic 796 bp fragment of HLA-DRB1 [24]. With the exception of the rare *7 allele, for which we were unable to obtain control DNA, all assays were validated with DNA of known TPMT genotype (kindly provided by Professor R. Weinshilboum, Mayo Foundation, Minnesota, USA and Professor William E. Evans, St Jude Children's Research Hospital, Tennessee, USA).

Genotyping methodology—promoter polymorphisms

The number of VNTRs was determined using primers listed in Table 1 [25]. The buffer and PCR conditions were used as above, and PCR products underwent electrophoresis through 4% agarose gel containing 0.5 μg/ml ethidium bromide for 180 min at 15 V/cm in 0.5xTBE buffer. The number of VNTRs was determined by comparison with control samples known to be homozygous for each VNTR (confirmed by sequencing), which were run in every gel.

Study design and statistical analysis

Phenotype, genotype and allele frequencies were determined by allele counting for all TPMT polymorphisms. Associations between categorical variables were assessed by chi-square (with Yates' continuity correction, where appropriate) and Fisher's exact tests, using KnowledgeSEEKER (Angoss, UK) or EpiInfo 2000. The unpaired *t*-test was used to compare the means of continuous variables between two groups.

Results

Two hundred and twenty-eight individuals received a kidney transplant at the Oxford Transplant Centre between January 1995 and December 1997. One hundred and eighty-one were initially included in this study, while 47 individuals were excluded from analysis because of lack of 1-year follow up (death $n=14$, graft failure $n=12$, and transfer to another centre $n=3$); an alternative initial immunosuppressive regimen (mycophenolate/cyclosporin/prednisolone as part of a multicentre trial $n=10$; identical twin transplant, no immunosuppression $n=1$) or because no DNA was available ($n=7$). No major adverse events attributable to azathioprine use, including bone marrow aplasia or hepatotoxicity, were recorded. In addition, no patient's azathioprine had been decreased or discontinued because of gastrointestinal intolerance.

During the course of the first year, 71 individuals experienced a white cell count $<4.0\times10^9$/l on at least one occasion (39%). By the first annual review, 16 patients had had their azathioprine dose reinstated to baseline, while 55 were on a lesser maintenance dose. An additional nine individuals had had their azathioprine dose changed for other reasons (substitution of cyclosporin by high-dose azathioprine because of cyclosporin toxicity $n=3$; substitution of azathioprine by mycophenolate mofetil because of recurrent rejection $n=2$; potential drug interaction with allopurinol $n=3$; and gastrointestinal bleeding $n=1$). Because the rationale for changing azathioprine dose in these nine patients was unrelated to any adverse events potentially associated with TPMT genotype, they were excluded from further analysis. Thus, data pertaining to 172 individuals was analysed. Two aspects of azathioprine dosage were

Table 1 TPMT primer mixes

Reaction no.	Allele	Amino acid	Nucleotide	Sense primers sequence 5′-3′ Sequence 5′-3′	Concentration (μmol/l)	Antisense primer Sequence 5′-3′	Concentration (μmol/l)	Size (bp)	Reference
1	TPMT *2	Pro 80	C 238	CTGCATGTTCTTTGAAACCCT	1.2	ACTGTGTCCCCGGTCTGG	1.2	162	[39]
2	TPMT *1/*3A/*3B/ *3C/*3D/*4/*5/*6/*7	Ala 80	G 238	CTGCATGTTCTTTGAAACCCT	1.2	ACTGTGTCCCCGGTCTGC	1.2	162	
3	TPMT *3B/*3A/*3D	Thr 154	A 460	GACATGATTTGGGATAGAGGAA	2.4	CCTTATAGCCTTACACCCAGG	2.4	259	[9, 36]
4	TPMT *1/*2/*3C/*4/ *5/*6/*7	Ala 154	G 460	ACATGATTTGGGATAGAGGAG	2.4	CCTTATAGCCTTACACCCAGG	2.4	258	
5	TPMT *3C/*3A/*3D	Cys 240	G 719	GAATTGACTGTCTTTTTGAAAA-GTTATG	3.6	GAAATATTTTTTTAATTGTAC-AGGTAACACATG	3.6	363	[9, 36]
6	TPMT *1/*2/*3B/*4/ *5/*6/*7	Tyr 240	A 719	GAATTGACTGTCTTTTTGAAAAG-TTATA	3.6	GAAATATTTTTTTAATTGTAC-AGGTAACACATG	3.6	363	
7	TPMT *3D	Stop 98	T 292	CTGCATGTTCTTTGAAACCCT	4.8	GAAAGATTCTGCTCTGT-AAAAAATTA	4.8	224	[40]
8	TPMT *1/*2/*3A/ *3B/*3C/*4/*5/ *6/*7	Glu 98	G 292	CTGCATGTTCTTTGAAACCCT	4.8	AGATTCTGCTCTGT-AAAAAATTC	4.8	221	
9	TPMT *4	Exon 10 Splice Site	AA	AACATGTTACTCTTTCTTGTTT-CAA	4.8	TAATTGTACAGGTAA-CACATGC	4.8	442	[40]
10	TPMT *1/*2/*3A/ *3B/*3C/*3D/*5/ *6/*7	Exon 10 Splice Site	AG	AACATGTTACTCTTTCTTGTTT-CAG	4.8	TAATTGTACAGGTAA-CACATGC	4.8	442	
11	TPMT *5	Ser 49	C 146	GGGTGTGTGTCTGAGCAAG	2.4	AAGGAAAGTATCTAAATGC-TTCTTTG	2.4	199	[40]
12	TPMT *1/*2/*3A/ *3B/*3C/*3D/*4/ *6/*7	Leu 49	T 146	GGGTGTGTGTCTGAGCAAG	3.6	AAGGAAAGTATCTAAATGC-TTCTTTA	3.6	199	
11	TPMT *6	Phe 180	T 539	CCTCAAGCACACAGGCATC	2.4	TAAGAAAGAACACACAGG-AGAA	2.4	409	[40]
12	TPMT *1/*2/*3A/ *3B/*3C/*3D/*4/ *5/*7	Tyr 180	A 539	CCTCAAGCACACAGGCATC	2.4	TAAGAAAGAACACACAGG-AGAT	2.4	409	
13	TPMT *7	Glu 227	G 681	GATGCTTTTGAAGAACGACAG	1.2	TAATTGTACAGGTAACAC-ATGC	1.2	382	[40]
14	TPMT *1/*2/*3A/ *3B/*3C/*3D/ *4/*5/*6	His 227	T 681	TGATGCTTTTGAAGAACGACAT	1.2	TAATTGTACAGGTAACA-CATGC	1.2	383	
	Promoter VNTR			GCTCCGCCCTGCCCATTT	2.0	GCCTCCGCCACCAATGAC	2.0		[41]

Table 2 TPMT coding region genotypes in renal transplant recipients and controls

Genotype	Renal transplant recipients		Controls	
	($n=172$)	(%)	($n=218$)	(%)
TPMT*1/*1	160	93.0	204	94.0
TPMT*1/*2	0	0.0	1	0.5
TPMT*1/*3A	11	6.4	12	5.0
TPMT*1/*3C	1	0.6	1	0.5

examined: firstly, the incidence of azathioprine dose reduction 1 year after transplantation, and secondly, the quantitative change in azathioprine dose compared with the initial dose.

TPMT-coding polymorphisms

The frequency of TPMT coding-region polymorphisms amongst renal transplant recipients was 3.5%: 11 of 172 individuals carried the TPMT*3A allele, while one individual of Afro-Caribbean ancestry carried the TPMT*3C allele (Table 2). No TPMT variant homozygotes, compound heterozygotes, or other genotypes, were detected. A similar allele and genotype frequency was found in the control group of 218 cadaveric transplant donors. An example of gel electrophoresis of PCR-SSP products from a wild-type/TPMT*3A heterozygote control sample is shown in Fig. 1.

Initial azathioprine dose was equivalent amongst TPMT-coding heterozygotes and TPMT wild-type individuals and approximated 1.5 mg/kg per day (Table 3). One year after transplantation, individuals who carried a TPMT-coding polymorphism were more likely than TPMT wild-type homozygotes to have had their maintenance azathioprine dose reduced ($P=0.04$). However, the mean change in azathioprine dose at 1 year (expressed as percentage of initial dose) was not significantly different in TPMT heterozygotes when compared with TPMT wild-type homozygotes. Mean leukocyte count at 1 year was equivalent in both groups, as was the incidence and severity of acute rejection, suggesting broadly equivalent functional immunosuppression.

A number of factors influence peripheral blood leukocyte count after transplantation, including ATG administration and CMV infection. Nine individuals

▶

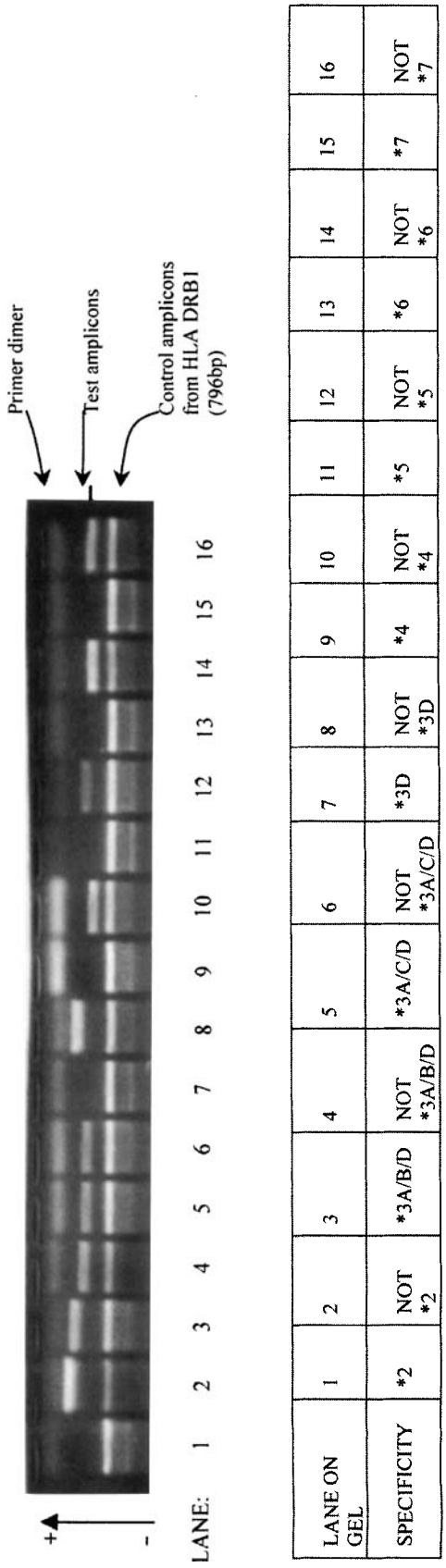

Fig. 1 PCR-SSP electrophoresis gel from wild-type/TPMT*3A heterozygote DNA. In ascending order of migration distance, control bands and primer dimers are present in each lane. Between these two bands are TPMT allele-specific amplicons generated by PCR-SSP. The lack of such an amplicon indicates the absence of the allele

Table 3 Effect of TPMT polymorphisms on azathioprine dose and clinical parameters 1 year after transplantation (*AZA* azathioprine, *NS* not significant)

Parameter	Coding TPMT genotype			Promoter TPMT genotype		
	TPMT wt./variant ($n=12$)	TPMT wt./wt. ($n=160$)	*P*	≤ 10 Repeats ($n=135$)	≥11 Repeats ($n=22$)	*P*
Mean AZA dosage at baseline (mg/kg per day)	1.58 (SD 0.12)	1.53 (SD 0.14)	NS	1.54 (SD 0.15)	1.53 (SD 0.11)	NS
Number of individuals whose AZA dose had been decreased 1 year after transplantation	7 (58%)	48 (30%)	0.04	34 (25%)	13 (59%)	0.001
AZA dose 1 year after transplantation (expressed as percentage of initial dose)	67.9% (SD 34.1)	82.6% (SD 30.7)	NS	84.6% (30.4)	68.9% (31.2)	0.03
Mean leucocyte count at 1 year ($\times 10^9$cells/l)	6.10 (SD 1.48)	6.76 (SD 2.04)	NS	6.89 (SD 2.10)	6.02 (SD 1.62)	NS
Number of individuals experiencing one or more acute rejection episodes	6 (50%)	68 (43%)	NS	56/135	11/22	NS
Mean serum creatinine at 1 year (μmol/l)	146 (SD 64)	173 (SD 83)	NS	168 (SD 64)	200 (SD 158)	NS

experienced leucopenia associated with ATG administration (as defined by first occurrence within 7 days of ATG administration), and 31 individuals experienced leucopenia associated with CMV infection (as defined by first occurrence within 30 days of two positive CMV antigenaemia tests). TPMT-coding heterozygotes were not more likely than TPMT wild-type homozygotes to experience leucopenia associated with ATG or CMV (data not shown). However, in the subgroup of individuals who experienced a fall in peripheral leukocyte count that was unrelated to CMV or ATG, 5/31 were TPMT variant heterozygotes, compared with 5/101 individuals who did not experience a fall in their peripheral blood leukocyte count ($P=0.04$).

TPMT promoter genotype

The number of potential VNTR genotypes complicates analysis of the effect of polymorphisms in the TPMT promoter [20]. However, an inverse relationship between TPMT activity and number of VNTRs on both chromosomes has been documented [19, 20]. We therefore analysed our data on the basis of total number of VNTRs, corresponding to the sum of repeats on both chromosomes, rather than specific genotype. An arbitrary threshold of ≥11 promoter VNTRs vs ≤ 10 VNTRs was chosen. In order to avoid the confounding effect of coding polymorphisms we included, in this analysis, only individuals who did not carry any coding-region polymorphisms (wild-type homozygotes, *1/*1).

The TPMT VNTR allele frequencies and total VNTR counts of renal transplant recipients and controls are shown in Tables 4 and 5. No significant differences were identified. One year after transplantation, renal transplant recipients with ≥11 promoter VNTRs were more likely to have had their azathioprine dose decreased than were those with ten or fewer promoter VNTRs ($P=0.001$, Table 3). This was reflected in the

Table 4 TPMT promoter polymorphisms—TPMT promoter allele frequencies. Promoter polymorphism frequencies are shown for TPMT coding region homozygote wild types only. Insufficient DNA was available for promoter polymorphism analysis in three patients and 30 controls. Total VNTR counts were calculated as the sum of VNTR on both chromosomes

Parameter	Renal transplant recipients		Controls	
	($n=314$)	(%)	($n=348$)	(%)
VNTR*3	1	0.3	2	0.6
VNTR*4	181	57.6	192	55.2
VNTR*5	131	41.7	124	35.6
VNTR*6	30	9.6	5	1.4
VNTR*7	9	2.9	19	5.5
VNTR*8	10	3.2	3	0.6
VNTR*9	0	0	0	0

Table 5 TPMT promoter polymorphisms—total VNTR counts

No. of repeats	Renal transplant recipients		Controls	
	($n=157$)	(%)	($n=174$)	(%)
7	1	0.6	2	1.1
8	46	29.3	58	33.3
9	51	32.5	67	38.5
10	37	23.5	24	13.8
11	9	5.7	8	4.6
12	11	7.0	12	6.9
13	1	0.6	1	0.6
14	0	0.0	1	0.6
15	0	0.0	0	0.0
16	1	0.6	1	0.6

quantitative change in azathioprine dose at 1 year ($P=0.03$) and remained true when individuals who experienced leucopenia associated with CMV or ATG were excluded from analysis (8/26 individuals who experienced leucopenia unrelated to ATG or CMV carried ≥11 promoter VNTRs compared with 7/94 individuals who did not experience leucopenia, $P=0.001$). Mean white cell count at annual review and acute rejection rates were similar in both groups.

Discussion

Azathioprine remains an important and widely used immunosuppressant. However, azathioprine dosing is limited by the idiosyncratic incidence of adverse events such as leucopenia and bone marrow aplasia. In an attempt to avoid these complications patients may receive inadequate doses of azathioprine, with important clinical consequences [26]. A number of tests have been developed to optimise azathioprine dosing: functional assays of TPMT enzyme activity are available [27, 28], but results may be influenced by a number of factors, including blood transfusions, intercurrent medication, and uraemia [29], which limits their usefulness prior to renal transplantation.

Therapeutic monitoring of the active metabolites of azathioprine, particularly red blood cell 6-thioguanine nucleotides, has also been advocated as a means of assessing drug efficacy and toxicity [30]. This might be particularly useful in monitoring therapeutic non-compliance [31]. TPMT-coding polymorphisms are correlated with in vitro enzyme activity [19, 32, 33], and molecular genotyping is a useful surrogate for functional testing. Genotyping is quicker and cheaper than functional studies and is not influenced by environmental factors. Concern has been expressed that screening for all TPMT-coding polymorphisms would necessitate multiple PCR analysis or extensive sequencing [33]. However, the genotyping method described in this paper allows the majority of coding polymorphisms to be determined in a single assay format.

In addition, the primers have been deliberately designed to work under conditions widely used for HLA typing [34], and, thus, TPMT genotype can be easily determined at the same time as HLA typing in patients that are awaiting transplantation. A minor disadvantage of this assay, as for others [19, 32], is that it is unable to distinguish a *3A/wt heterozygote individual from a *3B/*3C compound heterozygote, a distinction that is of functional relevance. However, this combination is very rare [35], and it is unlikely to be a significant problem in clinical practice. The allele frequency of TPMT-coding polymorphisms in the cohort of British renal transplant recipients studied here was 3.5%, which is similar to that reported in other studies [19, 33]. The commonest TPMT variant allele was TPMT*3A, while TPMT*3C was detected in an individual of Afro-Caribbean origin [36, 37]. A single TPMT*2/wt heterozygote was detected amongst the control cohort, and the rare alleles TPMT*3B, *4, *5, *6, and *7 were not detected.

While it is well established that individuals homozygous for TPMT-coding polymorphisms are likely to develop severe or even fatal haematological toxicity on standard doses of thiopurine drugs [8, 9], there is less consensus as to the clinical importance of TPMT-coding heterozygosity. Coding heterozygotes are generally unable to tolerate azathioprine dosages of 2–3mg/kg per day, developing leucopenia within 4 weeks of the initiation of therapy [13, 15]. However, at lesser dosages the effect of TPMT heterozygosity is variable: Dubinsky et al. showed that heterozygosity failed to influence leucopenic episodes in a study of paediatric patients with inflammatory bowel disease that were receiving 6MP at a dosage of 1.25 mg/kg per day [17].

Similar results were observed in a study of patients with systemic lupus erythematosus [16], where three heterozygote individuals tolerated azathioprine uneventfully at a dosage of 0.4–1.9 mg/kg. Furthermore, in a study of children with acute lymphoblastic leukaemia, who were treated with 6MP at 75 mg/m^2 per day, TPMT heterozygotes and wild types exhibited no difference in percentage of weeks when therapy was curtailed because of leucopenia [18]. In contrast, in a similar study that used the same dosages of 6MP, coding heterozygotes required significantly more treatment breaks, although, ultimately, the average dose tolerated was only 15% lower than the dose tolerated by wild-type individuals [14].

In the study described here, all individuals received an identical immunosuppressive regimen governed by a standard protocol, which included azathioprine at 1.5 mg/kg per day, and were intensively monitored throughout the first year after transplantation. Adverse events were limited to moderate leucopenia, and no episodes of hepatotoxicity or significant gastrointestinal

disturbance were documented. However, the incidence of leucopenia was high, with 39% of individuals suffering a drop in their white cell count to fewer than 4.0×10^9/l on at least one occasion. This is much higher than usually attributed to azathioprine use in other clinical contexts [38] and probably reflects the fact that azathioprine is only one of many determinants of white cell count in patients receiving a moderate dose of azathioprine after kidney transplantation.

To determine the effect of TPMT genotype, we examined the number of individuals that had had their azathioprine dose changed, 1 year after transplantation: this time point was chosen because the majority of individuals are clinically stable at this point and receiving an established immunosuppressive regimen. TPMT-coding heterozygotes were more likely than TPMT wild-type individuals to have had their maintenance azathioprine dose reduced at this time. This remained true if the subgroup of individuals who experienced leucopenia related to ATG administration or CMV infection were excluded. However, this effect was small and did not translate into a significant difference in quantitative change in azathioprine dose between the two groups. While TPMT promoter polymorphisms have not previously been studied in a clinical context, their contribution to TPMT activity has been thought to be relatively small in comparison with that of coding polymorphisms [20]. This study revealed a significant correlation between carriage of ≥11 VNTRs and alteration of azathioprine dose after 1 year, both in terms of incidence and in extent of dose reduction.

In summary, this study demonstrates that, when azathioprine is initially administered at a dosage of 1.5 mg/kg per day, both coding and promoter TPMT polymorphisms contribute to the dose tolerated. The effect of TPMT coding heterozygosity was small, and 50% of heterozygote individuals were able to sustain their initial azathioprine dose without adjustment during the first year after transplantation. An important finding was that TPMT promoter genotype also contributed to tolerance of azathioprine. This might explain the considerable variation in TPMT activity in coding homozygotes and warrants further investigation.

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