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The frequency and significance of thiopurine *S*-methyltransferase gene polymorphisms in azathioprine-treated renal transplant recipients

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SIR, Interindividual variation in the metabolism of azathioprine, commonly used post-transplant, can be due to the presence of genetic polymorphisms affecting the thiopurine *S*-methyltransferase gene (*TPMT*).[1] Standard dosing of azathioprine can expose patients with *TPMT* variants to a larger burden of immunosuppression over the duration of transplant.[2] The aim of this retrospective study was to define the frequency and significance of *TPMT* nonfunctional mutations in a renal transplant population.

This study was primarily carried out in Beaumont Hospital, the national renal transplant centre in Ireland. Additional patients were recruited from the Adelaide and Meath Hospital, Tallaght, Dublin. Ethical approval for this study was received. Patients older than 16 years of age were interviewed and examined by one observer (F.J.M.). Data were collected using a standardized questionnaire as previously described.[3]

Renal transplant recipients (RTR) with and without skin cancer were genotyped for *TPMT*2* (G238C), *TPMT*3A* (G460A, A719G), *TPMT*3B* (G460A) and *TPMT*3C* (A719G). Genomic DNA was isolated from a peripheral venous blood sample and genotyping was performed using the Amplifluor™ method by K Biosciences (http://www.kbioscience.co.uk). Categorical variables were tested using χ^2 and Mantel–Haenszel statistics while continuous variables were tested using Student's *t*-test and Wilcoxon two-sample test. In the analyses, older age, longer duration since transplantation, fairer skin type and higher total sun exposure score ($P < 0{\cdot}0001$) were shown to be predictors of increased skin cancer risk. The association of *TPMT* variants with skin cancer risk was therefore initially tested using a Cochrane–Armitage trend test and adjusted odds ratios (ORs) were then calculated based on a logistic regression, factoring in the above covariates.

The study population of 407 patients (249 men, 158 women) had a mean age of 48·3 years and a mean duration of 10·4 years since transplant. All patients in the study had received a cadaveric renal transplant. Most RTR were receiving triple drug immunosuppression. A total of 332 patients (82%) had received azathioprine post-transplantation, including 244 who had received it for >5 years. All patients were standard dosed with azathioprine, commencing on a dose of 2·5 mg kg^{-1} daily from the date of transplant. Most patients were skin types I and II (71%). The remainder were skin types III and IV, and four patients were skin type V. A total of 147 patients, 36% of RTR in the study, had a prior history of nonmelanoma skin cancer (NMSC).

Thirty-two of the 407 patients (7·9%) possessed a single *TPMT* nonfunctional allele: *TPMT*3A*, n = 28; *TPMT*3B*, n = 1; *TPMT*3C*, n = 3. Haematological toxicity necessitated cessation of azathioprine in 20% of RTR with a *TPMT* variant allele, thereby limiting the power of the study. There was a nonsignificantly increased risk of NMSC post-transplant in those possessing a variant *TPMT* allele (OR 1·07, 95% confidence interval, CI 0·51–2·26), which was greater when only patients who had received azathioprine for >5 years were considered (OR 1·22, 95% CI 0·52–2·84) (Table 1). A stronger association with skin cancer risk was seen when adjusted for age, gender, time since transplant, skin type and sun exposure score (OR 2·61, 95% CI 0·60–11·44); however, it remained statistically nonsignificant.

Table 1 Test of association for skin cancer risk with thiopurine *S*-methyltransferase gene (*TPMT*) genotype in renal transplant recipients on azathioprine for >5 years (n = 244)

TPMT variant	No history of skin cancer (n = 142)		History of skin cancer (n = 102)		P-value (adjusted)	Odds ratio (adjusted)	95% CI (adjusted)
TPMT wild-type (n = 217)[a]	128	59%	89	41%	0·6493 (0·2044)	1·22 (2·61)	0·52–2·84 (0·60–11·44)
*TPMT*3A*/*TPMT*3B*/*TPMT*3C* (n = 24)	13	54%	11	46%			

Test of association using Cochrane–Armitage trend test and logistic regression. Adjusted P-value, odds ratio and 95% confidence interval (CI) are based on a logistic regression with covariates age, gender, time since transplant, skin type and sun exposure score. Counts and percentages are based on the available data in a particular category. [a]Genotypic information not available on three individuals.

The 7·9% frequency of *TPMT* variants in Irish caucasian RTR is a lesser frequency than the 10% frequency of *TPMT* variants reported for British caucasian populations[4,5] but is greater than the frequencies described in more recent studies in French, German and Polish caucasian populations.[6–8] While there were no *TPMT* homozygotes within the study population, the one in five heterozygotes for *TPMT* who dropped their blood counts would potentially have also been the most susceptible to skin cancer risk if maintained on a long-term dose of azathioprine.

While it is generally accepted that long-term azathioprine in conjunction with ultraviolet radiation are synergistic in the production of skin cancers,[9] the supporting evidence in the literature is somewhat conflicting.[10] The balance of evidence suggests that longer durations of exposure to azathioprine, as occurs following transplantation, is a contributing factor to skin cancer risk. This risk may not manifest in patients treated with azathioprine for shorter durations or in the absence of other immunosuppressant drugs. This study suggests that possessing a variant *TPMT* gene may contribute to skin cancer risk in azathioprine-treated transplant patients but that such risk is overshadowed by other environmental and genetic factors known to predispose to skin cancer.

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Conflicts of interest: none declared.

Invasive aspergillosis due to subungual onychomycosis during treatment for non-Hodgkin lymphoma

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Sir, Onychomycosis is a common dermatological condition that accounts for approximately 50% of all nail problems. The fungi that cause onychomycosis are dermatophytes (over 90%), moulds and yeasts.[1] Nondermatophyte filamentous fungi (NDF) cause between 2% and 5% of nail infections and yeasts are responsible for 3–5% of cases. Infections are more common in the toenails, although sometimes the fingernails may be involved. *Aspergillus* species can cause superficial onychomycosis, distal-lateral subungual onychomycosis and proximal subungual onychomycosis (PSO) with paronychia.[1]

A 69-year-old woman with high-grade non-Hodgkin lymphoma was treated with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone). During the remission of the lymphoma the patient was admitted to the haematology ward for a peripheral blood stem cell autologous bone marrow transplant. On the third day of chemotherapy the patient was neutropenic and received prophylaxis with oral fluconazole 200 mg daily. On day 13, the patient had fever and wide-spectrum antibiotics were started. A blood culture grew *Staphylococcus aureus*. On day 18, serum *Aspergillus* galactomannan antigen (Platelia *Aspergillus*; Bio-Rad, Marnes la Coquette, France) had an index of 0·927. The nail fold of the little finger was swollen, violaceous and detached from the dorsal surface of the nail plate, which appeared ridged and