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SHORT COMMUNICATION

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Distribution of ITPA P32T alleles in multiple world populations

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Abstract Dose-limiting toxicity from azathioprine treatment affects up to 37% of patients. Screening for thiopurine methyltransferase (TPMT) polymorphisms will prospectively identify approximately 10% of patients. Recently, a polymorphism in the inosine triphosphate pyrophosphatase gene (ITPA) has been associated with severe azathioprine toxicity. We demonstrate here that this proline to threonine substitution at codon 32 in the ITPA gene is found at low frequency in Central/South American populations (1–2%), at a constant frequency across Caucasian and African populations (6–7%), and is highest in Asian populations (14–19%). This data is consistent with previously described allele frequencies in other Caucasian (7%), African (5%), and Asian (11–15%) populations. This data provides a foundation on which prospective screening studies can be planned to identify patients at risk for severe toxicity from azathioprine therapy.

Keywords Azathioprine · Inosine triphosphate pyrophosphatase · Thiopurine methyltransferase · Polymorphism · Population

Introduction

Azathioprine (AZA), the pro-drug of mercaptopurine, is commonly used in organ transplant patients and for the treatment of chronic inflammatory disease, dermatologic disorders, and rheumatic diseases. Severe toxicity to AZA therapy occurs in up to 37% of patients (Black et al. 1998). Polymorphisms in the thiopurine methyltransferase (TPMT) gene have been associated with dose-limiting AZA toxicity. These polymorphisms have significant ethnic variation in frequency (1–11%) (Ameyaw et al. 1999; Collie-Duguid et al. 1999; Hon et al. 1999; McLeod et al. 1999) and account for approximately 10% of observed AZA toxicity (Black et al. 1998; McLeod and Siva 2002).

Cao and Hegele (2002) identified an inosine triphosphatase deficiency phenotype. This phenotype is characterized by an abnormal accumulation of inosine triphosphate in erythrocytes leading to a lack of inosine monophosphate (IMP), an intermediate in purine metabolism (Cao and Hegele 2002). Recently, an association with the inosine triphosphate pyrophosphatase gene (ITPA; OMIM# 147520) and AZA intolerance has been defined (Marinaki et al. 2004). The ITPA gene is located on chromosome 20p13 and contains eight exons encoding a 21.5 kDa protein (Lin et al. 2001). The inosine triphosphatase deficiency phenotype is normally benign. However, in individuals treated with AZA, the lack of IMP leads to an accumulation of the metabolite 6-thio-ITP, which is thought to contribute to severe AZA toxicity (Marinaki et al. 2004). Several polymorphisms in the ITPA gene have been described (Cao and Hegele 2002; Sumi et al. 2002). A nonsynonymous SNP, 94C > A, causing a proline to threonine substitution at codon 32, has been identified as the basis of ITPA deficiency and is significantly correlated with AZA toxicity (Cao and Hegele 2002; Marinaki et al. 2004; Sumi et al. 2002). This polymorphism is in a conserved region in the human and mouse genome, supporting its importance in ITPA activity (Lin et al. 2001). Preliminary studies found ITPA P32T in British Caucasians (7%), Africans (5%), and Asians (11–15%) (Cao and Hegele 2002). Using pyrosequencing, we have determined the frequency of this clinically relevant polymorphism in multiple world populations.

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Table 1 ITPA 94C > A (P32T) genotype and allele frequencies in multiple world populations

Population	*n*	C/C	C/A	A/A	*p*	*q*
Peruvian	79	77	2	0	0.99	0.01
Mexican	94	91	3	0	0.98	0.02
American Caucasian	95	83	12	0	0.94	0.06
British Caucasian[a]	125	n/a	n/a	n/a	0.93	0.07
African American	55	48	7	0	0.94	0.06
African[a]	60	n/a	n/a	n/a	0.95	0.05
Ghanaian	81	72	9	0	0.94	0.06
East Indian[a]	60	n/a	n/a	n/a	0.89	0.11
Chinese[a]	60	n/a	n/a	n/a	0.85	0.15
Han Chinese	94	63	27	4	0.81	0.19
Filipino	55	40	15	0	0.86	0.14

[a]Data from Cao and Hegele (2002)

Subjects and methods

ITPA P32T was analyzed in 95 American Caucasian, 55 African American, 81 Ghanaian, 91 Han Chinese, 55 Filipino, 94 Mexican, and 79 Peruvian healthy volunteers under a protocol approved by the Washington University Human Studies Committee. Pyrosequencing was carried out as previously described (Rose et al. 2003) with PCR primers forward: 5′-TCGATGAGAAAGGCGGATGA-3′ and reverse: 5′-Biotin-ACGGTCAATTTTCTGTGCCAC-3′ with an annealing temperature of 62°C and pyrosequencing primer: 5′-TTCAGATTCTAGGAGATAAG-3′. χ^2 analysis was performed with the genotype frequencies for all population combinations.

Results and discussion

Frequency of the ITPA threonine allele is constant across Caucasian, African American, and African populations (5–7%). The threonine allele is highest in Asian populations (11–19%) and lowest in Central/South Americans (1–2%) (Table 1; Fig. 1). No significant difference between genotype frequencies was observed between Central/South American populations ($p > 0.05$), between Caucasian, African American, and African populations ($p > 0.05$ in all cases), or between Filipino and Han Chinese populations ($p > 0.05$). Statistically significant differences in genotype frequencies were observed between Central/South American and the other world populations ($p < 0.05$ for all cases) and Asian populations and the other world populations ($p \leq 0.01$ for all cases except Filipino versus African American; $p = 0.05$ and Filipino versus Ghanaian; $p = 0.03$).

Data on TPMT was available in the Ghanaian population (Ameyaw et al. 1999) and demonstrated similar allele frequencies of both TPMT*3C and ITPA P32T variant alleles (0.076 and 0.06, respectively). However, 17% (14/81) Ghanaian individuals had at least one variant allele in either TPMT*3C or ITPA P32T. Consequently, genotyping for both variants would identify a greater number of patients at risk for severe AZA toxicity than genotyping TPMT or ITPA alone. TPMT has a low (1.5–2.3%) variant frequency in Asian populations (McLeod and Siva 2002), whereas the ITPA P32T frequency in Asians is significantly higher (11–19%). ITPA P32T may be a more appropriate marker for strategies to avoid AZA toxicity in Asian populations.

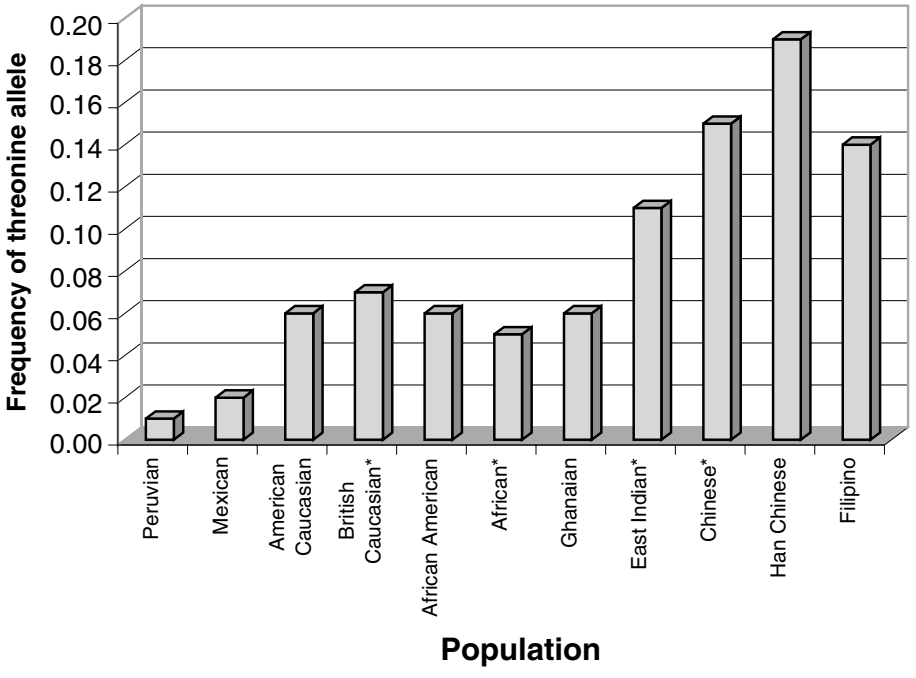

Fig. 1 Distribution of the ITPA P32T threonine allele in multiple world populations. *Data from Cao and Hegele (2002)

Prior to AZA therapy, prescreening patients for TPMT variants is likely to reduce the likelihood of inducing life-threatening toxicity. However, TPMT variants do not identify all patients at risk. Including a screen for ITPA P32T would further improve the chances of identifying at-risk patients and place us further toward the goal of individualizing treatment using genotype-guided therapy.

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