Frequencies of thiopurine S-methyltransferase mutant alleles (*TPMT**2, *3A, *3B and *3C*) in 151 healthy Japanese subjects and the inheritance of *TPMT**3C in the family of a propositus

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Aims To determine the frequencies of four thiopurine *S*-methyltransferase (*TPMT*) mutant alleles, *TPMT*2*, **3A*, **3B* and **3C* in a normal Japanese population.
Methods Genotypes were determined in 151 Japanese subjects and in six family members of a propositus using polymerase chain reaction (PCR)-restriction fragment length polymorphism and allele-specific PCR assays.
Results Only one *TPMT*3C* heterozygote was identified (gene frequency 0.3%). *TPMT*2, *3A* and **3B* were not detected. In addition, *TPMT*3C* was found to have been inherited from the mother and passed on to the son of the propositus.
Conclusions *TPMT*3C* appears to be most prevalent among the known mutant allele of *TPMT* in a Japanese population which may have some relevance for the treatment of Japanese patients with thiopurine drugs.

Keywords: genotype, Japanese, *TPMT*3C*

Introduction

Thiopurine *S*-methyltransferase (TPMT) is a cytoplasmic enzyme that preferentially catalyses *S*-methylation of thiopurine drugs such as the anticancer agents 6-mercaptopurine and 6-thioguanine and the immunosuppressant azathioprine [1]. There is a large interindividual variability in rate of the *S*-methylation of these thiopurine drugs. Caucasian populations show a trimodal distribution, with 89–94% possessing high enzyme activity, 6–11% intermediate activity and 0.3% low activity [2]. Decreased activity of TPMT is associated with severe haematopoietic toxicity after standard doses of these drugs [1]. Moreover, this toxicity can be fatal, as exemplified by a heart transplant patient with low activity of TPMT who was being treated with a standard dosage of azathioprine and died of sepsis as a consequence of repeated leucopenia [3].

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Received 17 August 2000, accepted 24 January 2000.

Variation in TPMT activity has been mainly accounted for by pharmacogenetic factors [1]. There are several mutations in the *TPMT* gene which give rise to low phenotypic enzyme activities. The wild-type allele is designated as *TPMT*1* and the mutant alleles are *TPMT*2* (G238C; Ala80→Pro), *TPMT*3 A* (G460A and A719G; Ala154→Thr and Tyr240→Cys), *TPMT*3B* (G460A; Ala154→Thr) and *TPMT*3C* (A719G; Tyr240→Cys) [4]. These mutant alleles of *TPMT* are present in more than 80% of Caucasian individuals with low TPMT activity [5]. The TPMT*3 A variant enzyme shows negligible activity, while TPMT*3C has moderate activity compared to the wild type, when expressed in yeast or COS-1 cells [4, 6]. The gene products of the *TPMT*2* and **3B* alleles have catalytic activities of one twentieth and one ninth of the wild type allele, respectively [6].

There is a marked interethnic difference in the frequencies of *TPMT* mutant alleles [7–10]. The most prevalent one in the Caucasian population is *TPMT*3A* [7–9], while *TPMT*3C* predominates in Chinese and Ghanaian populations [9, 10]. Other rare mutant alleles have recently been identified in a single family of Northern European ancestry (*TPMT*4*) [11], individuals of unknown ethnic origin (*TPMT*3D* and **5*) [5], a single

Korean individual (*TPMT***6*) [11], a single European Caucasian individual (*TPMT***7*) [12], and a single African-American individual (*TPMT***8*) [8].

In this study, we examined the frequency of four mutant alleles of *TPMT*, namely *TPMT***2*, **3A*, **3B* and **3C*, in 151 Japanese subjects, and also studied the inheritance of *TPMT***3C* in the family of a propositus over three generations.

Methods

One hundred and fifty-one unrelated healthy Japanese volunteers (aged 19–61 years) were recruited from 2000 employees of SRL Inc. (Hachiohji, Japan). Family members ($n=6$; aged 7–69 years) of the one subject possessing a *TPMT* mutant allele were also recruited. They were considered to be healthy as assessed by their medical histories. All subjects were informed both verbally and in writing of the experimental procedure and the purpose of the study. Each subject gave written consent before the study, the protocol of which was approved by the local Institutional Review Board.

Venous blood (3 ml) was obtained from each of the 151 subjects and six family members, and DNA was isolated from peripheral leucocytes using an extraction kit (DNA Extractor WB Kit, WAKO Pure Chemical Industries, Ltd, Osaka, Japan). Genotyping for identification of the mutations was performed by polymerase chain reaction (PCR)-restriction fragment length polymorphism or allele-specific PCR assay using specific primers for the G238C (*TPMT***2*), G460A and A719G (*TPMT***3A,* **3B,***3C*) loci as described by Yates *et al.* [13]. PCR products were directly sequenced or digested with appropriate restriction enzymes to confirm that PCR amplifications were performed correctly. Alleles without any of these mutations were assumed to be the *TPMT* wild-type gene (*TPMT***1*).

Results

Among the 151 Japanese subjects studied, only one subject carrying a mutant *TPMT* allele was identified. He was heterozygous for *TPMT***3C* (**1*/**3C*). *TPMT***2,* **3A* and **3B* were not detected. Therefore, the frequency of mutant alleles of *TPMT* examined in the current study of Japanese subjects was 0.3% (Table 1).

Analysis of the family members of this one subject possessing the *TPMT***3C* variant revealed that his mother and son were heterozygous (**1*/**3C*) and other family members were homozygous wild type (**1*/**1*), indicating that the *TPMT***3C* allele had been inherited from his mother and passed on to his son. All of the members of at least two former generations of the propositus were of Japanese origin.

Discussion

Although the frequencies of *TPMT* mutant alleles have been studied in Caucasian, African and Chinese populations [7–10], limited information is available on the frequencies in the Japanese population. The results of the present study showed that *TPMT***3C* but not *TPMT***2,* **3A* and **3B* was detected in Japanese subjects. The finding is essentially similar to the recent report of Hiratuka *et al.* [14] indicating that *TPMT***3C* was found in 0.8% of 192 Japanese subjects but *TPMT***2,* **3A* and **3B* were not detected. The frequency of *TPMT***3C* found in the present study is compatible with those in British Caucasians (0.3%) [9], American Caucasians (0.2%) [8] and European Caucasians (0.8%) [7], but lower than those in Chinese (2.3%) [9], African Americans (2.4%) [8] and Ghanaian subjects (7.6%) [10] (Table 1). It is noteworthy that the frequency of *TPMT***3C* in Japanese was much lower than that of Chinese although both populations are Oriental races.

Although TPMT*3C shows moderate activity *in vitro* [4, 6], *in vivo* studies [5, 13] indicate that homozygotes of

Table 1 Thiopurine *S*-methyltransferase *(TPMT)* allele frequencies in Japanese compared with other ethnic populations.

Population (n)	*Allele frequencies (%)* *2	*3A	*3C	*Reference*
Japanese (151)	0	0	0.3	Present study
Chinese (192)	0	0	2.3	Collie-Duguid *et al.* [9]
British South-west Asian (99)	0	1.0	0	Collie-Duguid *et al.* [9]
American Caucasian (282)	0.2	3.2	0.2	Hon *et al.* [8]
British Caucasian (199)	0.5	4.5	0.3	Collie-Duguid *et al.* [9]
European (191)	0.5	5.7	0.8	De la Moureyre *et al.* [7]
African Americans (248)	0.4	0.8	2.4	Hon *et al.* [8]
Ghanaian (217)	0	0	7.6	Ameyaw *et al.* [10]

*TPMT*3C* have impaired metabolism of thiopurine drugs, possibly due to intrinsic instability of the enzyme compared with that of TPMT*1 [4, 6]. Therefore, the one heterozygote of *TPMT*3C* found in the present study may have decreased capacity to metabolise thiopurine drugs. In fact, Ishioka *et al.* [15] reported that 3 among 36 patients with rheumatic disease were heterozygous for *TPMT*3C* and had to discontinue azathiopurine treatment due to leucopenia. Since *TPMT*4, *5, *6, *7* and **8* have not been detected in Japanese subjects [16], *TPMT*3C* appears to be most prevalent among the known mutant allele of *TPMT* in a Japanese population.

In conclusion, *TPMT*3C* was present at the frequency of 0.3% but *TPMT*2, *3A* and **3B* were not detected in 151 Japanese subjects. This finding may be of some relevance for the treatment of Japanese patients with thiopurine drugs.

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