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Thiopurine methyltransferase activity in the Jewish population of Israel

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Abstract Objective: 6-Mercaptopurine is used therapeutically for its immunosuppressant and cytotoxic properties. It is deactivated by thiopurine methyltransferase (TPMT), which shows genetic polymorphism in many populations. In North American populations, TMPT activity exhibits a trimodal activity pattern. In Oriental populations, TPMT shows almost a unimodal pattern of activity. The purpose of the present study was to assess the activity of TPMT in a Jewish male population sample in Israel.

Methods: The study was approved by the Israeli Ministry of Health. Blood samples of 2.5 ml were collected in heparinized tubes from 134 males. The red blood cell (RBC) fraction of each individual was washed and hemolyzed. TPMT activity in the RBC hemolysate was determined using a radioactive assay with tritiated S-adenosyl methionine as a methyl donor.

Results: The activity of TPMT ranged from 3.2 nmol/h/ml to 42.9 nmol/h/ml packed RBCs with mean and median activities of 18.6 nmol/h/ml and 17.9 nmol/h/ml packed RBCs, respectively. The distribution frequency of TPMT was very close to the unimodal by analysis of normal distribution.

Conclusion: The pattern of distribution of TPMT in the Jewish population of Israel is closer to that of East Asian populations than European and North American populations. This observation may have relevance for the usage of 6-mercaptopurine and azathioprine as therapeutic agents in the Jewish population.

Key words Thiopurine methyltransferase · TPMT · Pharmacogenetic polymorphism

Introduction

6-Mercaptopurine and its pro-drug azathioprine are used, respectively, for treatment of acute lymphoblastic leukemia (ALL) in children and as an immunosuppressant following organ transplantation. 6-Mercaptopurine (an inactive pro-drug) is activated in vivo to 6-thioguanine nucleotides. The 6-thioguanine nucleotides exert their cytotoxic activity by being incorporated into DNA.

6-Mercaptopurine is detoxified mainly by thiopurine methyltransferase (TPMT) to 6-methylmercaptopurine (6MPP). TPMT is a cytosolic enzyme that mediates the methylation of aromatic and heterocyclic thiols [1]. It is found in the liver, kidney, intestine, and blood cells [1, 2, 3]. A direct correlation between the activity of the enzyme in the liver and in the red blood cells (RBCs) has been shown [4, 5]. Due to the easy accessibility of erythrocytes, the activity of TPMT is usually assessed in these cells. It has been shown that TPMT exhibits pharmacogenetic polymorphism in many populations. In a North American Caucasian population sample, 88.6% of the individuals showed high activity of the enzyme in erythrocytes and homozygosity of the TPMTH allele, 11.1% were heterozygous and had intermediate activity, and 0.3% of the population had very low or undetectable activity [6]. Individuals with very low activity are homozygous for the TPMTL allele. Bimodal or trimodal activity patterns have also been shown in several European and American populations, although variations in the absolute activity of the enzyme between the various populations was observed [4, 7, 8, 9, 10].

In 1993, Chocair reported that the activity of erythrocyte TPMT in a group of 32 Japanese (living in Brazil) was homogeneously high, and all subjects were fast mercaptopurine methylators [10]. Qualitatively, the same pattern was observed also in 119 Chinese living in Singapore [11]. Recently, two studies [12, 13] showed
that the Korean population also seems to exhibit a unimodal pattern of high TPMT activity. It is thus evident that the polymorphic expression of TPMT in the East-Asian populations is different from American Caucasian and European populations.

In the present study we investigated the activity of TPMT in the Jewish population in Israel. Due to reports of possible gender variation in the activity of this enzyme [14, 15], the study was performed only in male volunteers as in the previous studies cited above.

Materials and methods

Blood samples (2.5 ml) were collected in heparinized test tubes from 134 adult Jewish volunteers. The study was approved by the local internal review board and endorsed by the Israeli Ministry of Health.

The samples were centrifuged and the erythrocytes were washed and hemolysed as described by Weinshilboum et al. [16]. The hemolysates were kept at −80°C for about 6 weeks, pending analysis. The thawed hemolysates were incubated with Chelex-100 prior to TPMT assays to chelate magnesium ions. TPMT assays were carried out in triplicate according to the radiometric method of Weinshilboum [16] with tritium labeled S-adenosylmethionine as a methyl donor [8]. In short, the reaction mixture (containing RBC hemolysate, 6-mercaptopurine, [3H]-S-adenosylmethionine dithiobisylitol, and allopurinol) was incubated for 1 h at 37°C. The reaction was stopped by addition of borate buffer (pH 10). Following extraction with toluene-isoamyl alcohol, the radioactivity in the organic phase was assessed. TPMT activity was expressed as nanomoles 6MMP formed per hour per milliliter of packed RBCs. The between-run and within-run coefficients of variance of the assay were 7.45% and 6.74%, respectively. The normal test variable (NTV) [17], probit test, and Kolmogorov-Smirnov test assessed the deviation of TMPT activity from normal distribution in the Jewish population sample.

Results

The activity of TPMT in the Jewish population sample studied ranged from 3.2 nmol 6MMP formed/h/ml to 42.9 nmol 6MMP formed/h/ml RBC (Fig. 1). The mean and median activities of TPMT in the erythrocyte hemolysates were 18.6 nmol/h/ml RBC and 17.9 nmol/h/ml RBC, respectively. The probit plot (Fig. 2A) and the Kolmogorov-Smirnov test (D0.005,140 = 0.081) support the assumption of normal distribution of TPMT activity. The five individuals with slightly negative values in the normal test variable (Fig. 2B), which constitute 3.7% of the population sample, may indicate a minor deviation from normal distribution of TPMT in the Jewish population study.

Discussion

The median activity of TPMT in the Jewish population was almost 18 nmol/h/ml RBC. This median activity is

![Fig. 1](image1.png)  
**Fig. 1** Frequency distribution of thiopurine methyltransferase (TPMT) activity in red blood cells of Jewish population

![Fig. 2](image2.png)  
**Fig. 2** A Probit analysis of thiopurine methyltransferase (TPMT) activity in red blood cells. B Normal test variable analysis of TPMT activity in red blood cells