Thiopurine S-methyltransferase gene polymorphism in pemphigus vulgaris

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Abstract

Introduction: Thiopurine S-methyltransferase (TPMT) genotypes or phenotypes are important as a predictive factor for thiopurine-induced toxicity. Individuals who are TPMT deficient or have intermediate TPMT activity are at risk of developing myelosuppression and life-threatening leucopenia when treated with standard doses of thiopurines; thus, pretreatment identification of these individuals is very important.
Aim: The aim of this study was to analyze the TPMT gene polymorphisms in pemphigus vulgaris patients who were treated with azathioprine.
Material and methods: Fifty-four patients with pemphigus vulgaris were analyzed and treated with azathioprine. The TPMT genotyping of these patients was carried out by an allele-specific PCR, PCR-restriction fragment length polymorphism (RFLP) assay and DNA sequencing.
Results: The distribution of TPMT genotypes was 96.7% as wild-type TPMT*1/TPMT*1, 1.85% as heterozygous TPMT*1/TPMT*3A, and 1.85% as heterozygous TPMT*1/TPMT*2. Fifty-two samples without carrier mutant alleles, described as TPMT*1, had no toxicity when treated with a standard dosage of azathioprine. However, the patient who was characterized as heterozygous TPMT*3A developed severe myelosuppression after the standard doses of azathioprine. The second patient characterized as heterozygous TPMT*2 was then treated with methylprednisolone without any adverse effects.
Conclusions: These data indicate that TPMT gene polymorphism detection might be important in pemphigus vulgaris before the patients are treated with azathioprine.

Key words: thiopurine S-methyltransferase, azathioprine, pemphigus vulgaris, pharmacogenetic.

Introduction

Thiopurine S-methyltransferase (TPMT; EC 2.1.1.67) is a cytoplasmic enzyme that catalyses the S-methylation of aromatic and heterocyclic sulphydryl compounds, such as 6-mercaptopurine (6MP), thioguanine and azathioprine [1, 2].

In the Caucasian population, nearly 90% of individuals have high TPMT activity and 10% are heterozygous carriers of mutant allele, resulting in a decreased TPMT activity. Around 1 in 300 individuals are homozygous for the variant allele, leading to extremely low enzyme activity or complete TPMT deficiency, and consequently, an increased risk of severe or fatal myelosuppression when treated with conventional doses of TPMT-metabolized drugs [3-5]. Thus, it is important to be able to identify those patients who are at risk of these complications. The molecular basis for altering this enzyme activity is well characterized with four variants accounting for up to about 95% of both heterozygous and homozygous patients [6]. There is a significantly ethnic difference in the frequency of these mutant alleles [7-9]. The detection of these polymorphisms is important because they are responsible for large individual variations in thiopurine toxicity [10, 11].

Pemphigus is a rare, chronic and life-threatening autoimmune blistering disease of the skin and mucous membranes [12-14]. Systemic corticosteroids are the mainstay of treatment for pemphigus patients [14, 15]. Steroid treatment has side effects with serious morbidity and mortality especially for old people and for those who have risk factors: hypertension, hyperglycemia, osteoporosis, Cushing syndrome, psychosis, cataract, peptic ulcer and

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infection [13, 15]. For this reason, immune-suppressives which suppress the production of pathogen antibodies are used to decrease the steroid dosage rapidly and/or shorten the curing period. Among these, azathioprine is usually preferred in pemphigus treatment due to relatively higher effects and less side effects as compared with other immune-suppressives [13, 16]. The efficacy and toxicity of azathioprine is dependent on TPMT enzyme activity or genotypes in patients [14, 17].

Aim

Moreover, the prevalence of TPMT genotypes has not been reported in patients with pemphigus vulgaris. In the present study, we aimed to determine the genotypes of TPMT with pemphigus vulgaris before these patients were treated with azathioprine and the frequency of mutant alleles in these patients.

Material and methods

Peripheral blood samples were collected from 54 patients with pemphigus vulgaris who were treated with standard doses of azathioprine (2.5 mg/kg/day) and were under the care of the Dermatology Department, Medicine Faculty Hospital at Cukurova University. Fifty-four patients who were diagnosed with pemphigus vulgaris both histopathologically and immunofluorescently were included in the study. Patients were excluded if reliable data for clinical response or adverse effects could not be obtained. The mean age (range) and body weight (± SD: standard deviation) (range) of the patients were 46.2 ±15.4 (25-91) years and 78.2 ±10.3 (55-105) kg, respectively. Regular blood count and liver function tests of these patients who have the treatment of azathioprine were conducted. Azathioprine treatment of the patients whose blood count decreased and enzymes of liver function increased was terminated.

Genomic DNA samples from 54 patients, including 32 females and 22 males, were prepared from 3 ml of whole blood after written informed consent was obtained from each patient. The study was approved by the Ethics Committee of the Medicine Faculty of Cukurova University. The genomic DNA was extracted from all of the blood samples using an Agencourt Genfind v2 DNA isolation kit (Beckman Coulter, Beverly, USA), according to the manufacturer's protocol. Two known deficient mutations, G460A and A719G, were determined using previously described multiplexed allele-specific PCR-based assays with minor modifications [18]. To determine the G238C mutation, an allele-specific PCR method was used, and additional P719F and P719R primers were used as internal control [2].

Restriction enzymes *MwoI* (HpyF10VI) and *AccI* (FD1734 and FD1484, Fermentas) were used to confirm G460A and A719G mutations, respectively [2]. Also, we directly sequenced the undigested PCR products on an RFLP assay. DNA sequencing was performed with Beckman Coulter CEQ8000, USA.

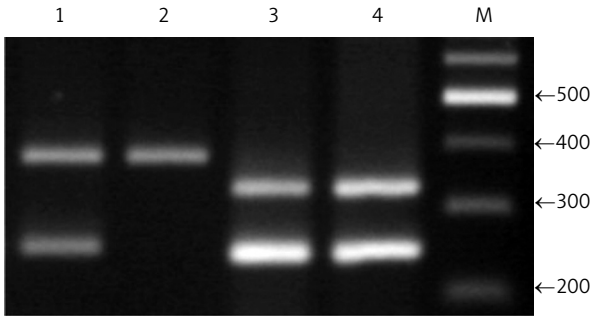

Figure 1. Electrophoresis patterns for TPMT*3A patient analysed by allele specific PCR and multiplexed PCR-ARMS assay. Lane 1, wild-type specific. Lane 2, mutation specific PCR analysis of G238C. Lane 3-4, multiplexed ARMS assay for G460A and A719G. M, marker (DNA ladder, 100-1000 bp)

Results

Among the 54 pemphigus cases, 52 samples showed no mutations at codons 238, 460, and 719 of the TPMT gene, described as TPMT wild-type allele (TPMT*1). These patients who did not have a genetic variation of the TPMT gene had no myelosuppression during treatment with a standard dosage of azathioprine. All patients had regular blood cell counts and liver function tests. A 41-year-old woman diagnosed with pemphigus disease was shown to have bullous oral mucosa, erosions and crusts of the arm, leg and shoulder. This patient has the weight of 60 kg and she started with 2.5 mg azathioprine/kg body weight. After 2 weeks of the azathioprine treatment, WBC value fell to 3400 and since liver enzymes increased up to 120, azathioprine treatment was terminated. Subsequently, her disease was controlled with various doses of oral steroids. Heterozygous TPMT*3A haplotype was detected in the patient (Figures 1-2). The second patient (91-year-old) who showed haplotype TPMT*2 was shown to have widespread lesions in oral mucosa and vermilion of the lips. The patient was treated with methylprednisolone without myelosuppression.

The frequency of both TPMT*2 and TPMT*3A (2/54 patients) in these samples was calculated as 1.85%. The TPMT*3C and TPMT*3B alleles were not detected in any of the Turkish individuals in the Cukurova region, southern Turkey. Furthermore, no homozygous or compound heterozygous for mutant TMPT alleles were found in the study. The distribution of TPMT genotypes was thus 96.3% (52/54) TPMT*1/TPMT*1, 1.85% (1/54) TPMT*1/TPMT*2 and 1.85% (1/54) TPMT*1/TPMT*3A.

The sequences of exon 7 in TPMT*3A patient was revealed as substitution G→A (G460A; GCA→ACA) at codon 154, changing the alanine to threonine. However, the sequences of exon 10 were detected as substitution

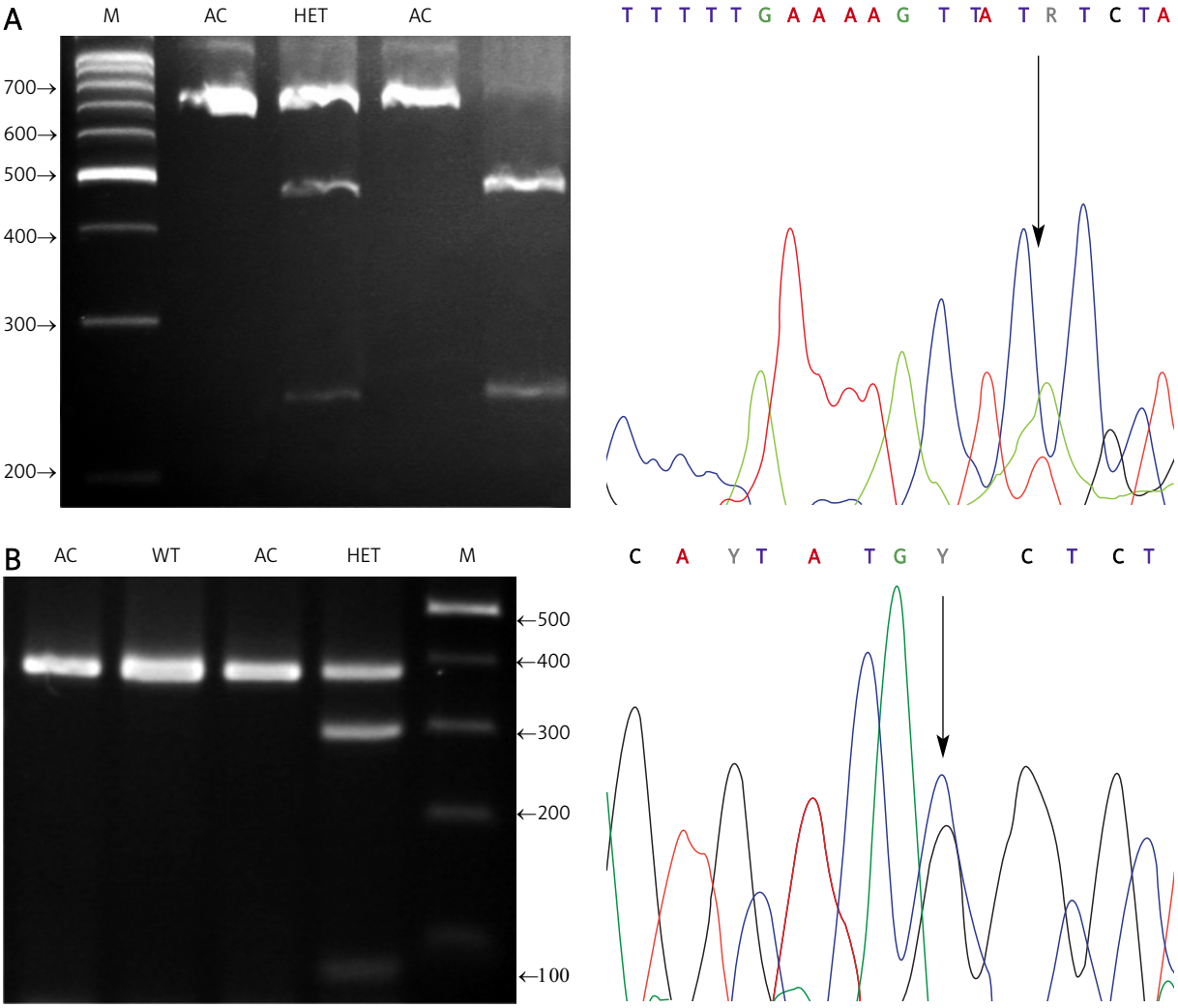

Figure 2. Results of TPMT*3A patients by PCR-RFLP and DNA sequencing. **A.** PCR-RFLP analyses and DNA sequencing of G460A. **B.** PCR-RFLP analyses and DNA sequencing of A719G. M: Marker (DNA ladder, 100-1000 bp), AC: Amplification control, WT: Homozygous wild-type, HET: Heterozygous mutant

A→G (A719G; TAT→TGT) at codon 240, changing the tyrosine to cysteine in the same patient (Figure 2).

Discussion

Early diagnosis of TPMT enzyme activity is essential to avoid potentially life-threatening toxicity when exposed to standard doses of thiopurines [3, 4]. More than 20 alleles of the TPMT gene have been identified and the major mutations causing TPMT deficiency have been defined [19-21]. In patients with 2 nonfunctional alleles, carrier individuals are at increased risk of potentially life threatening toxicity when exposed to standard doses of thiopurine drugs. The heterozygous carriers have to start the drugs with 50-60% of the standard dose [6, 22]. A large number of studies have demonstrated that there is a link between TPMT deficiency and the occurrence of thiopurine-induced toxicity in treatment including systemic lupus erythematosus, Crohn's disease, autoimmune hepatitis, acute leukemia, and inflammatory bowel disease with thiopurine medications [5, 17, 23-25]. Due to individual variations in TPMT activity it plays an important role in azathioprine toxicity and/or therapeutic activity [4]. So, it is important to detect TPMT enzyme phenotypes or genotypes before beginning treatment with azathioprine [6, 26].

The polymorphisms of the TPMT gene have been studied intensively in several populations with several patient groups [27-29]. Aydin-Sayitoğlu *et al.* determined TPMT genotypes in 148 healthy individuals in a Turkish popula-

tion, and found the frequency of TPMT*2, *3A and *3C allele to be 2.0%, 1.0% and 1.4%, respectively [30]. Tumer *et al.* investigated the mutations in 106 Turkish children with ALL and found a frequency of 0.9% TPMT*3A and TPMT*3C for both [31]. However, little is known about pemphigus vulgaris patient groups.

The estimated annual prevalence of pemphigus in the Mediterranean region of Turkey was 0.24 per 100,000 and frequent in the southern provinces of Turkey [13]. In this study, we present the first data on allelic frequencies of the TPMT gene with pemphigus vulgaris patients; a candidate patient group who may undergo azathioprine therapy. A large number of pemphigus vulgaris patients could not be collected at our hospital. With this limitation on the study, the distribution of TPMT*1 wild-type was 96.7% (52 of 54 patients). TPMT*3B and TPMT*3C genotypes and homozygous patients were not detected. TPMT*2 and TPMT*3A variant genotypes were found in 2 of 54 (3.7%) patients with pemphigus vulgaris. The heterozygous TPMT*3A patient had developed agranulocytosis after treatment with standard doses of azathioprine and therapy of the disease was continued with oral steroids. Methylprednisolone was began in the second patient who was characterized as heterozygous TPMT*2. So the patient had no developed myelosuppression. However, 52 patients who were described as TPMT*1/TPMT*1 variant had no myelosuppression during treatment with standard doses of azathioprine.

Conclusions

Pharmacogenetic screening for known TPMT genotypes before azathioprine therapy may be important to prevent fatal myelosuppression in patients with TPMT deficiency. However, we found that the incidence of TPMT polymorphisms in this population was significantly lower than in other Caucasians. Furthermore, this issue should be investigated with a larger population.

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