



Distribution of HLA-B27 and CYP2D6*4 mutations in the middle Black Sea area (Tokat) of Turkey

S. Sahin, L. Aydogan, I. Benli and H. Ozyurt

Department of Biochemistry, Faculty of Medicine,
Gaziosmanpasa University, Tokat, Turkey

Corresponding author: S. Sahin
E-mail: semsettinsahin@hotmail.com

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ABSTRACT. We analyzed distribution of HLA-B27 and CYP2D6*4 mutations in 249 patients from Tokat province in Turkey with symptoms of arthritis, sacroiliac, joint and back pain, using a LightCycler 480 II Real-Time PCR thermal cycler. The Genes-4U was applied for studying HLA-B27 mutation, and the Tib-Molbiol commercial kit was used to examine the CYP2D6*4 mutation. Among the 249 patients, 18.5% had the HLA-B27 mutation. The CYP2D6*4 mutation was found in 22.0% (six homozygotes). Ten patients had both mutations. These frequencies are similar to what has been reported from other populations.

Key words: HLA-B27; CYP2D6*4; Mutation; Real-time PCR

INTRODUCTION

The HLA (human leukocyte antigen) region is the most polymorphic region as defined in IMGT/HLA. This region located in the short arm of chromosome 6 within MHC (major histocompatibility complex) has been extensively studied because of its critical role in organ and stem cell transplantation (Buhler and Sanchez-Mazas, 2011).

The HLA-B27 family contains a great number and subtypes of allele variations whose heterogeneity was previously determined and whose ethnic distribution differs (Khan, 2010). According to a study in which there were HLA-B27 positive children, JIA (juvenile idiopathic arthritis) was related to 8 HLA-B27 allele types (*2702, *2703, *2704, *2705, *2710, *2715, *2717, *2728). Of these, the most common alleles are HLA B*2705, *2710 and *2717 (Stanevicha et al., 2010).

Unlike many complex genetic diseases, a single gene (HLA-B) plays an important role in ankylosing spondylitis (AS). B27 allele contributes up to 40% of all genetic load and is the major factor for many other spondyloarthropathies (SpA) (Reveille, 2006; Brown, 2006). It has been clearly stated that the powerful relation between MHC and AS is HLA-B27 (Thomas and Brown, 2010). In SpA patients, just like in 95% of AS patients carrying B27, HLA-B27 is observed at a high frequency. For this reason, SpA appears as one of the best examples of the diseases related to the HLA marker. Human and transgenic animal model link and relation studies have proven the role of B27 in AS (Taurog, 2009; Thomas and Brown, 2010).

Genetic polymorphisms of drug-metabolizing enzymes show the source of individual variants in treatment response. Among these enzymes, CYP2D6 and CYP2C19 play an important role (Zanger et al., 2008). CYP2D6 is a component of one the major metabolic pathways for central nerve system drugs, many of which are depressants, such as antidepressants, anti-psychootics, opioids and antihistamines (Ingelman-Sundberg et al., 2007).

Up to now, more than 50 human CYP isoenzymes have been determined (Ma et al., 2002). Of these, more than 20, including CYP2A6, CYP2C9, CYP2C19 and CYP2D6, are functionally polymorphic. Therefore, about 40% of CYP linked to drug metabolism involve polymorphic enzymes (Ingelman-Sundberg, 2005). At least, 15 allele variations of CYP2D6 can cause weak-metabolizing phenotypes, but 75% of weak-metabolizing phenotypes are homozygous CYP2D6*4 (Brown et al., 2000). CYP2D6*4 (allele frequency of 20%) was found to be the most common variant in Caucasians (Bradford, 2002).

MATERIAL AND METHODS

In this study, 249 patients from the Middle Black Sea area of Turkey were included, who were seen at Gaziosmanpasa University Health Search and Application Center. The complaints of the patients were arthritis, sacroiliac and joint pains and backaches. HLA-B27 and CYP2D6*4 mutations were studied in these patients. The purpose was explained to them and their written permission was obtained using a "Patient Enlightened Approval Form". A venous blood sample (2 mL) was drawn for routine analysis and placed in tubes containing EDTA. DNA isolation was done from whole blood using the Roche High Pure PCR Template Preparation Kit. HLA-B27 and CYP2D6*4 mutation screening was performed based with the real-time PCR method using a LightCycler 480 II Real-Time PCR thermal cycler.

Using the real-time polymerase chain reaction (RT-PCR) method, the increase in the

fluorescence of the product obtained by the amplification of DNA was followed in real time. Gene polymorphism was determined by analyzing the detailed melting curve of the PCR product obtained. The Genes-4U kit was used for the HLA-B27 mutations and the Tib-Molbiol kit for the CYP2D6*4 mutations.

RESULTS

HLA-B27 and CYP2D6*4 mutations were studied in 249 patients. Based on the results, HLA-B27 mutation was positive in 46 (18.47%) patients. CYP2D6*4 mutation was heterozygous in 49 (19.67%) patients and homozygous in 6 samples (2.4%). Ten patients (4.01%) were found carrying both HLA-B27 and CYP2D6*4 mutations. There were 158 (63.45%) patients with no mutations. Results are given in detail in Table 1.

Table 1. Patient number surveyed for HLA-B27 and CYP2D6*4 mutations and ratios.

Mutation name	Mutation number/ratio (N = 249)		
HLA-B27	Wild type	Positive	
	203 (81.52%)	46 (18.47%)	
CYP2D6*4	Wild type	Heterozygote	Homozygote
	194 (77.91%)	49 (19.67%)	6 (2.40%)

DISCUSSION

Each of the HLA-B27 positive patients could have had a different illness (enthesitis, polyarthritis, oligoarthritis, arthritis, ankylosing spondylitis) in addition to a different level of illness activity (Stanevicha et al., 2010). HLA-B27 is related to SpA which are a group of inflammatory diseases affecting many areas such as skin, ankle and tarsal joints (Brebant et al., 2004).

As a multi-systemic disease characterized by ankylosis of intervertebral, costovertebral and sacroiliac joints, AS shows genetic familial transition, and 95% of AS patients are HLA-B27 positive. Twenty percent of people with HLA-B27 have AS disease (Olivieri et al., 1998; Edwards et al., 2000).

In normal individuals, the HLA-B27 rate was found to be 8-14% (Arnett, 1997; Taurrog and Lipsky, 1998). In people with HLA-B27 and HLA Bw60 combination, risk of ankylosing spondylitis triples. Incidents were reported as 7.3 in 100,000 people (Van der Linden et al., 2005).

The frequency of HLA-B27 has been shown to vary in different ethnic groups. HLA-B27 prevalence was found to be 18-50% in American Indians, 10-16% in Scandinavians, 6-9% in Western Europe, 2-6% in Southern Europe, 6-8% in Pakistanis, 2-6% in Indians, 1% in Japanese, and 1% in Africans (Van der Uden, 1997). This rate varies between 4 and 13% in Caucasian Americans. The rate is 2-3% in African Americans (Khan, 1995; Tay-Kearney et al., 1996). While 95% of AS patient carry HLA-B27, in the general population this rate is 10% in Europeans, 1% in Japanese and 10-50% in American Indians, which is the highest number (Brewerton et al., 1973; Ball and Khan, 2001). In a study conducted in Turkey, HLA-B27 phenotype frequency in a healthy Turkish population was reported to be 6.8% (Gul et al.,

2002). In a study carried out by Gunal et al. (2008) in 112 Turkish AS patients, HLA-B27 was positive in 79 (70%) patients. In our study, the number of patients HLA-B27 positive was 46 (18.47%) out of 249. This number was higher compared to the results reported by Gul et al. (2002) and was lower than the results reported by Gunal et al. (2008). This comparison suggests that patients with complaints of various aches including AS may have a high chance of being positive for HLA-B27.

HLA-B27 and CYP2D6*4 are susceptibility alleles for AS. CYP2D6*4 homozygous patients have a higher risk for AS. This type of high risk was not observed in heterozygotes (Beyeler et al., 1996; Brown et al., 2000). In addition, susceptibility for AS is an important factor for the cure of AS patients (Wagner et al., 1998; Brown et al., 2000).

According to Brown et al. (2002), the fact that there was no relation between observed heterozygote CYP2D6*4 and AS suggests either a direct relationship with polymorphism playing a recessive role or there was a strong connection inequality with the allele playing a recessive role.

While CYP2D6*4 is a predominant defective allele in the Caucasian population, it is rare in the Asian population (1-3%) (Gjerde et al., 2008). In a study conducted in Turkey, CYP2D6*4 homozygote mutation rate was 4% and allele frequency was 0.21 (Koseler et al., 2007). Aydın et al. (2005) found that CYP2D6*4 allele frequency in Turkey was 15.4%. The results of allele frequency and homozygote prevalence of CYP2D6*4 mutation in Turkey are given in Table 2. Allele frequencies in various populations are the following: 21% in Germans, 18% in Americans, 1% in Japanese and 8% in African Americans (Aydın et al., 2005). In our study, the number of patients with CYP2D6*4 mutation was 55 (22.07%): 49 (19.67%) were heterozygous and 6 (2.4%) were homozygous. In all patients, CYP2D6*4 allele frequency was 0.122. When we compared the results of our study with those of Aynacioglu et al. (1999) in which there were 404 patients, and of Aydın et al. (2005) in which there were 140 patients, we observed that allele frequencies and homozygous mutations were close.

Table 2. CYP2D6*4 allele frequency and homozygote prevalence in various studies.

	CYP2D6*4 allele frequency	CYP2D6*4 homozygote prevalence (%)
Aynacioglu et al., 1999 (N = 404)	0.11	1.5
Bozkurt et al. (N = 326)	-	3.4
Aydın et al., 2005 (N = 140)	0.154	2.4
Koseler et al., 2007 (N = 100)	0.21	4.0
Our study (N = 249)	0.12	2.4

We found that although there are various cultural structures in the Middle Black Sea area of Turkey, the distribution of HLA-B27 and CYP2D6*4 mutations was similar to that in other parts of the country.

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