Progress in Nutrition 2021; Vol. 23, N. 3: e2021272 DOI 10.23751/pn.v23i3.11827 © Mattioli 1885

Original article

HLA-B27 and CYP2D6*4 Polymorphism Prevalence Analysis in Turkey

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Abstract. *Background:* HLA-B27 positivity is held responsible for the etiopathogenesis of spondyloarthropathies, some autoinflammatory and neurological diseases, and different types of cancer. CYP2D6 from the cytochrome P450 subfamily group is involved in the metabolism of 25% of the drugs used in the treatment. Knowing that CYP2D6*4 polymorphic alleles may cause different effects on therapeutic drugs, these variant alleles are noteworthy in the lack of response to treatment, observation of adverse drug effects, as well as in the investigation of the etiopathogenesis of many diseases. In this study, we investigated the prevalence of HLA-B27 and CYP2D6*4 in the central Black Sea Region of Turkey for the analysis of ethnicity-related genetic differences in polymorphic cytochrome P450 enzymes and CYP2D6*4 alleles. *Material and Methods:* A total of 1063 healthy individuals were included in the study and HLA-B27 and CYP2D6*4 polymorphisms were analyzed. Genes-4U kit and Tib-Molbiol kit were used. For analysis LightCycler 480 II Real Time PCR thermal cycler was used. *Results:* A total of 1063 samples were studied in the HLA- B27 and CYP2D6*4 polymorphism analysis. HLA-B27 polymorphism-positivity was detected in 86 (8.9%) samples. In CYP2D6*4 polymorphism heterozygous alleles were detected in 203 (19.09%) and homozygous alleles in 26 (2.44%) samples. While 29 (2.71%) samples carried both HLA-B27 and CYP2D6*4 polymorphisms. *Conclusion:* In our study, we determined the frequency of polymorphism and allele frequencies at a rate similar to the data of other healthy populations.

Key words: HLA-B27, cytochrome P450, CYP2D6*4, polymorphism

Introduction

Human leukocyte antigen (HLA-B27) is encoded by the B locus. B locus where many polymorphisms are seen is located in the MHC class1 region of the major histocompatibility complex (1). Although not true for the entire HLA-B27 family, some subtypes have been linked with various diseases (2). Diseases such as ankylosing spondylitis (AS), reactive arthritis (ReA) and psoriatic arthritis (PsA), whose etiopathogenesis and clinical features are similar, form a group called Spondyloarthropathies (SpAs). The SpA group, more frequently AS, demonstrates a strong association with HLA-B27 positivity (3). The B27 allele assumes the majority of the hereditary burden in the development of SpA, especially in AS disease (4).

The prevalence of HLA-B27 varies by race in the general population. Its prevalence has been reported as 16-48% for Native Americans, 10-16% for Scandinavians, 9% for the United Kingdom, 2-9% for Europe, 8% for Caucasian races, 4% for North Africa, 0.1-0.5% for Japanese, and 6.8% for Turkey (3,5). The prevalence of

AS is known to range between 0.1% and 1.4% all over the world. AS is the most prevalent prototype of SpA, affecting about 0.3% of the adult US and about 0.2% of the adult European population. Particularly, its prevalence is even higher in North American populations and lower in Africa, similar to the prevalence of HLA-B27 (6). The incidence of HLA-B27 in AS varies in different racial and ethnic groups. Its incidence rates among patients with AS have been reported as 95% in Northern European countries, as 83% in Japan, and as 70% in Turkey (7). The cytochrome P450 (CYP) superfamily is the largest group of enzymes responsible for drug metabolism. It plays a role in the metabolism of many endogenous compounds such as eicosanoids, steroids, and exogenous substances such as xenobiotics and therapeutic drugs by inducing oxidation and reduction reactions (8).

Most of the CYP genes are polymorphic, and these allelic variants can cause significant changes in drug disposition or diseases by altering protein structure, expression, and activity (9). Genetic mutations play an important role in the differences of enzymatic activity of many CYPs. CYP2 members from the subfamily group of the CYP 450 enzyme family where polymorphisms are common, constitute approximately 30% of human variants (10). The CYP2D gene family consists of two nonfunctional pseudo genes (CYP2D8P and CYP2D7P) and the protein encoding CYP2D6 gene. Although it constitutes approximately 2-4% of all hepatic CYPs, it metabolizes 20% of the therapeutic drugs used and plays a role in the metabolization of environmental carcinogens and toxic substances (11).

It is known that CYP2D6 allele frequencies, which may cause differences in drug metabolism, vary according to ethnic origin (12). More than 100 different polymorphic variants have been reported in the CYP2D6 gene region (13). CYP2D6 alleles are divided into two groups as functional and nonfunctional alleles. Nonfunctional alleles are called slow metabolisers and cannot encode a functional protein product and therefore the CYP2D6 enzyme cannot show any activity. CYP2D6*4 is the most common variant allele in the Caucasians (allele frequency: 20%) (14,15) CYP2D6*4 allele frequency is at a very low level (nearly 0-2.8%) In Chinese (16), Korean (17), Malaysian (18) and Japanese (19) populations. The CYP2D6*4 frequency was found to be 7.6% in African Americans (20). If the CYP2D6*4 mutant allele is homozygous, the risk for AS disease is higher compared with the heterozygous mutant allele (21).

In this study, we aimed to determine the prevalence of HLA B27 and CYP2D6*4 polymorphisms in a normal healthy population in a province in northern Turkey.

Materials and Methods:

This study was carried out in a total of 12 urban and 58 rural areas, covering the province of Tokat with a population of approximately 600,000 in the East Black Sea region of Turkey. All urban areas and some rural areas selected by the cluster sampling method were included in the study. A total of 1063 individuals were included in our study and the HLAB27 and CYP2D6*4 polymorphisms were investigated. The healthy study population of 1063 subjects (524 males and 539 females) was randomly selected from 600,000 subjects. The study group consisted of male (49.3%), and female (50.7%) subjects with an overall mean age of 41.3±16.9 (min:18, max:95) years.

The individuals were informed about the research and their signed written informed consent was obtained using the "Clarified Approval document". For analysis, 2 mL of venous blood sample was taken into tubes containing anticoagulants and processed. DNA was isolated from venous blood with a ready-made genomic DNA isolation kit. HLA- B27 polymorphism was analyzed with the Genes / 4U test kit. CYP2D6*4 polymorphism was analyzed with the Tib / Molbiol test kit. Real-time polymerase chain reaction (RealTime-PCR) method was used to detect HLA- B27 and CYP2D6*4 polymorphisms. The testing phase was carried out with the LightCycler 480II Real Time PCR thermal cycler. DNA was amplified by RealTime-PCR method. The fluorescence of the product obtained was monitored in real time after each cycle. The presence and rate of polymorphisms were found by evaluating the melting curve of the PCR product.

Results

A total of 1063 samples were analyzed for HLAB27 and CYP2D6*4 polymorphisms. According

Table 1. HLA-B27 and CYP2D6*4 polymorphisms

Polymorphisms		Polymorphisms n/% (n = 1063)		
CYP2D6*4	Wild type 78.45 % (834)	Heterozygote 19.09 % (203)	Homozygote 2.44 % (26)	allele frequency 0.12
HLA-B27	Wild type 91.9% (977)	Positive 8.09 % (86)		

to the results, HLA-B27 polymorphism was positive in 86 (8.09%), CYP2D6*4 polymorphism was heterozygous in 203 (19.09%), homozygous in 26 (2.44%) and allele frequency 0.12 individuals. Twenty-nine (2.7%) individuals carried both HLA-B27 and CYP2D6*4 polymorphisms (Table 1).

Discussion

Although it is known that AS disease has a polygenic etiology, it has been stated that HLA-B27 positivity accounts for 40% of the genetic susceptibility to AS disease (22). Having HLA-B27 is strongly associated with the development of SpA. (3). Most reports describing the prevalence of HLA-B27, have confirmed the differences in its frequency and distribution in different populations and ethnic groups (23). The highest prevalence was found in the Pawaia tribe (53%) in Papua New Guinea. The prevalence of HLA-B27 is also fairly higher in Northern Scandinavia (14-16%). Its prevalence rates are 8-10% among Caucasians, 4.6% among Mexican Americans (4.6%), and 2.4% among African Americans. The prevalence of B-27 positivity is lower in the Chinese (2-9%) and Arab (2-5%) populations while it is extremely lower in Japanese (<1%) population (24).

Braun et al. investigated the relationship between HLA-B27 and spondylarthropathy; The researchers reported that individuals with HLA-B27 positivity had suffered from spondyloarthritis 20 times more frequently compared to the people without (25). In two separate studies investigating the relationship between AS and HLA-B27 positivity, the HLA positiveity rate in AS patients was found as high as 95%, and the researchers stated that AS developed in 20% of HLA-B27 positive individuals (26,27). The incidence of the disease is related to the prevalence of HLA-B27. It has been reported that, in the general population of Northwestern Europe the prevalence of HLA-B27 is 8%, and more than 90% of AS patients in this geographic region have this gene. Although HLA-B27 gene was detected in 50% of the patients with AS in a study conducted with African Americans, the rate of having this gene in healthy individuals was found to be 2-4 percent (2). In a study conducted in a healthy Turkish population, the prevalence of HLA-B27 gene was found to be 6.8% (28). In a study conducted in Turkish patients, 79 (70%) patients diagnosed with AS were HLA-B27 positive and 33 patients were identified as having wild-type alleles of this gene (7).

In our study, HLA-B27 positivity was detected at a rate of 8.09% (86 samples). We obtained results similar to the results of the study conducted by Gül et al. in a healthy population (28). In recent years, it has been reported that there is a relationship between the CYP2D6 phenotype or allele frequencies with Parkinson's, Alzheimer's, various types of cancer, epilepsy and bullous skin diseases. As an explanation of this relationship, it has been suggested that CYP2D6 may play a role in the conversion of procarcinogens into carcinogens in the metabolic pathway or in the conversion of protooncogenes into oncogenes by an as yet unknown mechanism (29). According to Ingelman-Sundberg et al. CYP2D6*4 with a dysfunctional allele is seen in 12-21% of Caucasians, and in 1-4% of Ethiopians and Saudi Arabians, while it is rarely seen in Asians (1-3%), and Japanese people (0.6%) (30). Köseler et al. reported that as the most common mutant allele in the Turkish population the frequency of the CYP2D6*4 allele ranges between 13.4% and 21 percent (31). Aydın M. et al. reported the frequency of CYP2D6*4 in Turkey as 15.4 percent (32).

In a screening study in Turkish population performed by Aynacıoğlu et al. the frequency of CYP2D6*4 polymorphism was reported as 11 percent (33). In

our study, CYP2D6*4 polymorphism was detected in 229 (21.53%) (incl. 203 [19.09%[heterozygous, and 26 [2.44%] homozygous polymorphisms) study participants We detected a CYP2D6*4 allele frequency of 0.12 in the healthy population. Our allele frequency and homozygous mutant gene results were comparable to those obtained by Aynacıoğlu et al. Considering the population size, although CYP2D6*4 allele frequencies we detected in our study was partially comparable to those reported by Aydın et al, we observed a higher proportion of mutant alleles (1%).

Conclusion

Investigating the prevalence of CYP enzyme and HLA gene families and their relationship with diseases in the field of molecular genetics will be able to provide valuable information to solve immunopathogenesis of relevant conditions.

Conflicts of Interest: The authors declare that they have no conflict of interest.

Funding Statement: "This study was supported by Tokat Gaziosmanpaşa University Scientific Research Projects Coordination Unit. Project Number: 20.13/63"

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