CYP2C9 and NAT2 Gene Polymorphisms in Patients with Drug Eruption

ILAÇ ERUPSİYONLU HASTALARDA CYP2C9 VE NAT2 GEN POLİMORFİZMİ

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Abstract

Objective: Interindividual variability in therapeutic drug response and drug toxicity is a major problem in clinical practice. Genetic polymorphisms of drug metabolizing enzymes play an important role in this field. The aim of the present study was to investigate differences in genotypes for polymorphic drug metabolizing enzymes such as CYP2C9 and NAT2, between adverse drug reaction in 36 cases and 104 control cases.

Material and Methods: Blood was collected in EDTA-containing tubes and DNA was extracted from the leucocytes by high pure template preparation kit. Polymorphism of NAT2 and CYP2C9 were detected by using LightCycler mutation detection kit by real time PCR with LightCycler instrument.

Results: In the cases group, the frequency of the NAT2*5A mutant genotype was higher in comparison with that of the control group and this increase was significant (odds ratio =37.47; 95% confidence interval= 4.98-282.10). Compared with the CYP2C9*3 wild allele, the variant allele was associated with increased risk of drug eruption (odds ratio=2.68; 95% confidence interval= 1.19-6.08).

Conclusion: Our results show that slow acetylator phenotypes of NAT2*5A and variant allele of CYP2C9*3 seem to be associated with the development of drug eruption.

Key Words: CYP2C9, drug eruption, NAT2 and polymorphism

Özet

Amaç: Terapötik ilaca karşı cevapta ve ilaç toksisitesinde bireylerarası çeşitlilik klinik uygulamada büyük bir problemdir. Bu alanda ilaç metabolize edici enzimlerdeki genetik polimorfizmler önemli bir role sahiptir. Çalışmamızın amacı, ilaca karşı reaksiyonu olan 36 hasta ve 104 kontrol arasında CYP2C9 ve NAT 2 gibi polimorfik ilaç metabolize edici enzimlerin genotipindeki farklılıkları saptamaktır.

Gereç ve Yöntemler: Kan EDTA içeren tüplerde toplandı ve DNA high pure template preparation kiti ile lökositlerden elde edildi. NAT2 ve CYP2C9 polimorfizmleri LightCycler mutasyon belirleme kiti kullanılarak LightCycler cihazında real time PCR ile saptandı.

Bulgular: Hasta grubu kontrol grubu ile karşılaştırıldığında NAT2*5A mutant genotip sıklığı daha yüksek bulundu ve bu artış istatiksel olarak anlamlıdır (odds ratio =37.47; %95 confidence interval= 4.98-282.10). Varyant allel CYP2C9*3 wild allel ile karşılaştırıldığında ilaç erupsiyon riskinin artmasıyla ilişkilidir. (odds ratio=2.68; %95 confidence interval=1.19-6.08).

Sonuç: Sonuçlarımız NAT2*5A yavaş asetilatör fenotipin ve CYP2C9*3 varyant allelin ilaç erupsiyonunun gelişmesi ile ilgili olduğunu gösteriyor.

Anahtar Kelimeler: CYP2C9, ilaç erupsiyonu, NAT2 ve polimorfizm

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Some therapeutic agents cause adverse reactions and these are very important problems in clinical practice. The spectrum of such reactions comprises; side effects, toxic reactions, drug interactions, idiosyncratic reactions, and immunologic reactions.[1] The most common allergic drug reactions are dermatological; thus the skin is of particular importance when adverse effects of drugs are to be considered. Almost any type of skin eruption can be secondary to medication.[2] For many types of drug eruptions, the precise mechanism by which cutaneous inflammation occurs is unknown. Several genetic influences on the expression of drug allergy have been reported.[3,4] Sustained immune responses to drugs are more likely in adults with specific Human Leukocyte Antigen (HLA) pheno-

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Table 1. Characteristics of the study population.

	Patients n, (%)	Controls n, (%)
	36 (100)	104 (100)
Age (years)	56.50 ± 10.20	58.00 ± 9.60
Sex		
Male	14 (55.7)	57 (55.0)
Female	22 (64.3)	47 (45.0)

types and drug metabolism propensities.[5] Specific HLA genes have been linked to the increased risk of some drug eruptions.[6,7] Slow N-acetylation phenotype and oxygenated amine detoxification phenotype appears to dispose to reactions to drugs.[8]

Most drug-metabolizing enzymes exhibit clinically relevant genetic polymorphism.[9] Polymorphisms are generated by mutations in genes for these enzymes, which causes decreased, increased, or absent enzyme expression or activity by multiple molecular mechanisms. Moreover, the variant alleles exist in the population at relatively high frequency.[10] Inter-subject variability in therapeutic drug response and drug toxicity is a major problem in clinical practice. In this field genetic polymorphisms of drug metabolizing enzymes play an important role.[11]

Cytochromes P450 (CYPs) are a super family of heme proteins which catalyze many types of reactions, predominantly hydroxylation. They participate in oxidative metabolism of a wide variety of structurally diverse compounds, including endogenously synthesized compounds such as steroids and fatty acids, as well as exogenous compounds such as drugs, carcinogens and environmental agents.[12] CYP2C gene subfamily represents a cluster of four genes on the chromosome 10q24, arranged in the sequential order CYP2C8, CYP2C9, CYP2C19, and CYP2C18.[13] Best known drug substrates for CYP2C9 are weak acids containing carboxylic acid group in their structure. Oral hypoglycemic agents (tolbutamide), some antiepileptic drugs (phentoin), oral anticoagulants (warfarin), a number of non-steroidal anti-inflammatory drugs (ibuprofen, diclofenac, piroxicam) and angiotensin II blockers (losartan) are principally metabolized by CYP2C9.[12-14]

Polymorphic N-acetyltransferase (NAT2) is involved in the metabolism of several compounds relevant in pharmacology or toxicology, with diverse clinical consequences.[11] Arylamine N-acetyltransferases (NATs) are polymorphic enzymes, well-known for their role in the metabolism of drugs and carcinogens. It is this genetic variation of drug metabolism that is one of the causes of inter-individual variation of the effect of a drug. Acetylation polymorphism relates to the metabolism of a number of arylamine and hydrazine drugs and carcinogens by cytosolic N-acetyltransferase-NAT2. In humans, NAT2 is responsible for the N-acetyltransferase activity.[15]

Detoxification features make it plausible to search for CYP and NAT polymorphism in patients with drug eruption. This made us decide to investigate the CYP and NAT polymorphism in patients with drug eruption. The aim of the present study was to look for evident differences in genotypes for polymorphic drug metabolizing enzymes between adverse drug reaction cases and controls.

Material and Methods

Subjects: This was a hospital-based case-control study conducted at the Hospital of Mersin University. The study was designed as a prospective study. **104 healthy control subjects (47 women, 57 men) who visited our hospital for an annual check up and hospital staff and 36 patients (22 women, 14 men) with drug eruption were participated in this study.** The patients and controls were from the same geographic region. Also, cases and controls are unrelated. Characteristics of the study population are given in Table 1. The diagnosis of drug eruption was based on the nature of the symptoms, the drug history and the temporal relationship between the institution of drug therapy and the onset of the reaction. Sometimes we confirmed a suspected allergic drug reaction by using in vivo testing such as patch or skin tests and provocative tests. All patients and control subjects were selected among people that had no history of cardiovascular disease, cancer, chronic degenerative neurological disease, chronic obstructive pulmonary disease, hepatitis, allergies in general or alcohol abuse. This

study was approved by the Ethics Committee of Mersin University, School of Medicine.

DNA extraction and genotyping of CYPC9 and NAT2: Blood was collected in EDTA-containing tubes and DNA was extracted from the leucocytes by high pure template preparation kit (Roche diagnostics, GmbH, Mannheim, Germany). NAT2*5A, NAT2*6A, NAT2*7A/B, NAT2*14A and CYP2C9*2, CYP2C9*3 allele were detected by using LightCycler NAT2 and CYP2C9 mutation detection kits by real time PCR with LightCycler instrument (Roche diagnostics, GmbH, Mannheim, Germany; catalog no: 3113914). The presence of mutations in both alleles of NAT2 was accepted as a slow acetylation phenotype. The wild types and heterozygous were termed as fast acetylators.

Statistical Analyses

Binary multiple logistic regression analysis was used to define possible risk factors and their odd's ratios with 95% confidence interval by SPSS 10.0 for Windows.

Results

Thirty six subjects with drug eruption and 104 healthy control subjects were enrolled in the study. The mean (± SD) age was 56.50 ± 10.20 in patients, and 58.00 ± 9.60 in control subjects and 55.7% of patients and 55.0% of controls were male (Table 1). **The clinical type of patients with drug eruption is shown in Table 2.**

Table 2. Clinical types of drug eruptions and using diagnostic tests.

Patient no.	Drug eruption type	Drug	Diagnostic test
1	Maculopapular eruption	Sodium valproate	Patch
2	Fixed drug eruption	Ampicillin/sulbactam and trimethoprim/sulfamethoxazole	Provocation
3	Maculopapular eruption	NSAID (naproxen)	Patch
4	Maculopapular eruption	Azithromycin and NSAID (nimesulide)	Patch
5	Maculopapular eruption	Acetaminophen	Patch
6	Erythema multiforme	Penicillin	Patch and provocation
7	Maculopapular eruption	Aspirin, acetaminophen and penicillin	Patch
8	Maculopapular eruption	NSAID (piroxicam), and penicillin	Patch
9	Maculopapular eruption	NSAID (ibuprofen)	Patch
10	Maculopapular eruption	NSAID (naproxen)	Patch
11	Maculopapular eruption	NSAID (diclofenac)	Patch
12	Fixed drug eruption	Penicillin	Provocation
13	Maculopapular eruption	NSAID (piroxicam)	Patch
14	Maculopapular eruption	Trimethoprim/sulfamethoxazole	Patch
15	Maculopapular eruption	Penicillin	Patch
16	Maculopapular eruption	NSAID (diclofenac)	Patch
17	Maculopapular eruption	NSAID (aspirin)	Patch
18	Urticarial eruption	Penicillin	Provocation
19	Maculopapular eruption	NSAID (naproxen)	Patch
20	Erythema multiforme	Sulphonamide	Provocation
21	Maculopapular eruption	Penicillin	Patch
22	Maculopapular eruption	NSAID (naproxen)	Patch
23	Fixed drug eruption	NSAID (naproxen), and opipramol	Provocation
24	Urticarial eruption	Sulphonamide	Provocation
25	Maculopapular eruption	Ampicillin	Patch
26	Urticarial eruption	Penicillin	Provocation
27	Maculopapular eruption	NSAID (aspirin)	Patch
28	Maculopapular eruption	NSAID (diclofenac)	Patch
29	Fixed drug eruption	NSAID (ibuprofen)	Provocation
30	Erythema multiforme	Penicillin	Patch and provocation
31	Maculopapular eruption	Risperidone	Patch
32	Maculopapular eruption	NSAID (naproxen)	Patch
33	Maculopapular eruption	NSAID (aspirin)	Patch
34	Fixed drug eruption	Tetracycline	Provocation
35	Urticarial eruption	Penicilin	Provocation
36	Maculopapular eruption	NSAID (aspirin)	Patch

Table 3. NAT2 and CYP2C9 genotypes and the risk of developing drug eruption.

	Cases (n= 36)	Control (n= 104)			
Variable	**n (%)**	**n (%)**	**OR ‡**	**95% CI**	**P value**
NAT2*5A Wild	4 (11.1)	41 (39.4)	1 (reference)	—	
Heterozygous	21 (58.3)	56 (53.8)	9.51	1.65-54.99	0.012
Mutant	11(30.6)	7 (6.7)	37.47	4.98-282.10	0.000
NAT2*6A Wild	19 (52.8)	60 (57.7)	1 (reference)	—	
Heterozygous	15 (41.7)	40 (38.5)	2.39	0.69-8.27	0.169
Mutant	2 (5.6)	4 (3.8)	3.61	0.24-52.38	.346-
NAT2*7A/B Wild	23 (63.9)	60 (57.7)	1 (reference)	—	
Heterozygous	13 (36.1)	41 (39.4)	1.20	0.30-4.71	0.797
Mutant[#]		3 (2.9)			
NAT2*14A Wild	26 (72.2)	57 (54.8)	1 (reference)	—	
Heterozygous	10 (27.8)	46 (44.2)	1.46	0.40-5.39	0.569
Mutant[#]		1 (1)			
CYP2C9*2 Wild	27 (75.0)	86 (82.7)	1 (reference)		
Variant	9 (25.0)	18 (17.3)	1.02	0.38-2.71	0.161
CYP2C9*3 Wild	16 (44.4)	72 (69.2)	1 (reference)		
Variant	20 (55.6)	32 (30.8)	2.68	1.19-6.08	0.018

‡ ORs (odds ratio); CI (confidence interval) from logistic regression. n; number of sample
Carriers of at least one intact allele are used as reference. p; values of significance with difference of each group.
[#]Odds ratio can not be calculated.

The frequencies of wild, heterozygous and mutant NAT2*5A acetylators were 11.1, 58.3 and 30.6% in patients and 39.4, 53.8 and 6.7% in healthy control cases respectively. In the cases group, the frequency of the NAT2*5A mutant genotype was higher in comparison with that of the control group and this increase was significant (OR= 37.47; 95% CI= 4.98-282.10). As shown in Table 3, NAT2*6A, NAT2*7A/B, NAT2*14A genotypes were not significant risk factors for drug eruption.

The frequencies of slow and fast NAT2*5A acetylators were 30.6 and 69.4% in cases and 6.7 and 93.3% in controls, respectively (Table 4). There was a significant difference in the distribution of the acetylator phenotype (p= 0.002) between cases and the controls. The odds ratio of drug eruption for the slow phenotype was 5.38 (95% CI= 1.84-15.71) compared with the fast type. The NAT2*6A slow and fast acetylator frequencies were 5.6 and 94.4% in cases and 3.8 and 96.2% in controls. No slow acetylator phenotypes for NAT2*7A/B and 14A in

Table 4. The distribution of the mutations NAT2*5A, NAT2*6A, NAT2*7A/B and NAT2*14A as phenotypes in-groups.

Acetylator type	**Patient group n, (%)**	**Control group n, (%)**	**OR (95% CI)**	**p values**
NAT2*5A Fast	25 (69,4)	97 (93.3)	1 (reference)	
Slow	11 (30,6)	7 (6.7)	5,38 (1,84-15,71)	0.002
NAT2*6A Fast	34 (94,4)	100 (96.2)	1 (reference)	
Slow	2 (5,6)	4 (3.8)	1,41 (0,16-12,19)	0.757
NAT2*7A/B Fast	36 (100)	101 (97.1)	1 (reference)	
Slow[#]		3 (2.9)		1.0
NAT2*14A Fast	36 (100)	103 (99.0)	1 (reference)	
Slow[#]		1 (1.0)		1.0

‡ ORs (odds ratio); CI (confidence interval) from logistic regression. n; number of sample
Carriers of at least one intact allele are used as reference. p; values of significance with difference of each group.
[#]Odds ratio can not be calculated.

cases group were found. In controls NAT2*7A/B and 14A slow acetylators frequencies were 2.9 and 1%, respectively, but there was no significant association with the NAT2*6A, NAT2*7A/B and NAT2*14A fast or slow acetylator status and developing drug eruption (Table 4).

The frequency for CYP2C9 mutated genes was higher in the cases analyzed, as compared with healthy controls. The variant alleles CYP2C9*2 (R144C) and *3 (I359L) produce slow-metabolizing enzymes. Compared with the CYP2C9*3 wild allele, the variant allele was associated with increased risk of drug eruption (odds ratio= 2.68; 95% confidence interval= 1.19-6.08) (Table 3).

Discussion

Interindividual variability in therapeutic drug response and drug toxicity is a major problem in clinical practice. Antimicrobial, antipyretic, anti-inflammatory and analgesic agents were the most common drugs that caused eruptions. Although the exact cause of drug eruption is still unknown, various immune mechanisms, genetic factors have been suspected.[2,16,17] Genetic polymorphisms of drug-metabolizing enzymes give rise to distinct subgroups in the population that differs in their ability to perform certain drug biotransformation reactions. Polymorphisms are generated by mutations in the genes for these enzymes, which cause decreased, increased, or absent enzyme expression or activity by multiple molecular mechanisms. Moreover, the variant alleles exist in the population at relatively high frequency. Genetic polymorphisms have been described for most drug metabolizing enzymes.[18,19]

In our study, the frequency of the NAT2*5A mutant genotype was higher in comparison with that of the control group and this increase was significant (OR= 37.47; 95% CI= 4.98-282.10). The frequencies for CYP2C9 mutated genes are higher in the cases analyzed, as compared with healthy controls. Compared with the CYP2C9*3 wild allele, the variant allele was associated with increased risk of drug eruption (odds ratio= 2.68; 95% confidence interval= 1.19-6.08)

The consequences of pharmacogenetic variation in NAT enzymes include; altered kinetics of specific drug substrates; drug-drug interactions resulting from altered kinetics; idiosyncratic adverse drug reactions. They have been extensively investigated for the arylamine-containing sulfonamide antimicrobial drugs. Individual differences in multiple metabolic pathways can increase the likelihood of covalent binding of reactive metabolites of the drugs to cell macromolecules with resultant cytotoxicity and immune response to neoantigens. This can result clinically in an idiosyncratic hypersensitivity reaction, manifested by fever, skin rash, and variable toxicity to organs including liver, bone marrow, kidney, lung, heart, and thyroid gland. Slow acetylation by NAT2 is a risk factor for such reactions to sulfonamides.[20] Variation in the regulation and expression of the human cytochrome P-450 enzyme system may play a key role in interindividual variations in sensitivity to drug toxicity and tissue-specific damage as well.[21] Anticonvulsants, many hypnotics, tricyclic antidepressants, anticoagulants and various anti-inflammatory and anxiolytic agents are eliminated by oxidation. For many drugs, oxidation rates vary as a continuous spectrum within population. Genetic differences in metabolism of sulphonamides may underlie idiosyncratic toxicity.[22] Oxidative metabolism of sulphonamides by cytochrome P-450 enzymes and N-acetylation yields a reactive hydroxylamine intermediate.[23]

Differences in rates of production of hydroxylamine metabolites of the drugs by cytochrome P450 (CYP2C9), myeloperoxidase, and thyroid peroxidase, along with an inherited abnormality in detoxification of the hydroxylamines are critically important in determining individual differences in adverse reaction risk. Intensive investigation of patients with these rare adverse reactions using a variety of tools from in vitro cell toxicity assays through molecular genetic analysis will help elucidate mechanisms of predisposition and ultimately lead to diagnostic tools to characterize individual risk and prevent idiosyncratic drug toxicity.[20]

The results of this preliminary study indicate that the slow-metabolizing enzymes do appear to play a role in the development of drug eruption.

Further studies on larger groups are needed to determine the prevalence of these polymorphism in patients with drug eruptions and to determine whether they constitute a major risk factor in the development of drug eruptions. We suggest that, clinicians should examine NAT2 and CYP2C9 polymorphisms of patients using especially non-steroid anti-inflammatory, antimicrobial, anticonvulsant drugs.

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