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CYP2C19*1 and CYP2C19*2 Polymorphism in Turkish Patients Being Diagnosed with Stable Coronary Artery Disease and Using Clopidogrel

Clopidogrel Kullanan, Koroner Arter Hastalığı Tanısı Mevcut Olan Türk Popülasyonunda CYP2C19*1 ve CYP2C19*2 Gen Polimorfizmi

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Abstract

Objective: The CYP2C19*1 has an entirely normal activity allele whose clopidogrel metabolism is normal. CYP2C19*2 called as non-functional alleles. In this study, we aimed to establish the CYP2C19*1 and CYP2C19*2 genotype frequencies both in Turkish patients with coronary artery disease (CAD), who used clopidogrel, and in healthy Turkish population as well as to present the differences in genotypes and alleles between both groups.

Method: One hundred healthy individuals and 200 patients diagnosed with CAD were included in the study. DNA was isolated and *CYP2C19* gene was amplified through the polymerase chain reaction method in the genomic DNAs obtained, and the polymorphic foci in these regions were specified.

Results: CYP2C19*1/1 genotype was identified in 132 patients (66%), CYP2C19*1/2 genotype in 62 patients (31%) and CYP2C19*2/2 genotype in 6 patients (3%) in the CAD group. In the control group, by contrast, 72 patients (72%) were identified with CYP2C19*1/1 genotype, 20 patients with CYP2C19*1/2 genotype and 8 patients with CYP2C19*2/2 genotype. There was a significant difference between the groups in terms of genotypes (p=0.034).

Conclusion: We found CYP2C19*1/2 and CYP2C19*2/2 genotype to be higher in the CAD patients than in the control group, highlighting the importance of checking *CYP2C19* gene polymorphism prior to the initiation of antiplatelet therapy in CAD patients.

Keywords: Clopidogrel metabolism, coronary artery disease, CYP2C19 polymorphism, Turkish population

Öz

Amaç: CYP2C19*1, klopidogrel metabolizması normal olan tamamen normal bir aktiviteye sahiptir. CYP2C19*2, işlevsel olmayan aleller olarak adlandırılır. Bu çalışmada, hem klopidogrel kullanan ve koroner arter hastalığı (KAH) olan Türk hastalarda hem de sağlıklı Türk popülasyonunda CYP2C19*1 ve CYP2C19*2 genotip frekanslarını belirlemeyi ve her iki grup arasındaki genotip ve alel farklılıklarını sunmayı amaçladık.

Yöntem: Çalışmaya 100 sağlıklı birey ve KAH tanısı almış 200 hasta dahil edildi. DNA izole edilerek ve *CYP2C19* geni polimeraz zincirleme reaksiyonu yöntemi ile amplifiye edilerek bu bölgelerdeki polimorfik odaklar belirlendi.

Bulgular: KAH grubunda 132 hastada (%66) CYP2C19*1/1 genotipi, 62 hastada (%31) CYP2C19*1/2 genotipi ve 6 hastada (%3) CYP2C19*2/2 genotipi tespit edildi. Kontrol grubunda ise 72 hasta (%72) CYP2C19*1/1 genotipi, 20 hasta CYP2C19*1/2 genotipi ve 8 hasta CYP2C19*2/2 genotipi ile tanımlanmıştır. Genotipler açısından gruplar arasında anlamlı fark vardı (p=0,034).

Sonuç: KAH hastalarında CYP2C19*1/2 ve CYP2C19*2/2 genotipinin kontrol grubuna göre daha yüksek olduğunu belirledik. Bu da koroner arter hastalarında antiplatelet tedaviye başlamadan önce *CYP2C19* gen polimorfizminin bakılmasının önemli olduğunu vurgulamaktadır.

Anahtar kelimeler: CYP2C19 polimorfizmi, klopidogrel metabolizması, koroner arter hastalığı, Türk popülasyonu

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Introduction

Irreversibly inhibiting the platelet P2Y12 receptor, thienopyridines are used as antithrombotic agents in the treatment of CAD, peripheral vascular and cerebrovascular diseases (1,2). Besides, clopidogrel is known as the most frequently used agent among thienopyridine drugs. Clopidogrel, which acts as a prodrug, proves an effective inhibitor of platelet aggregation due to the selective and irreversible blocking of the P2Y12 receptor in the platelet cell membrane. Moreover, its intestinal absorption is limited by a bowel effusion P-glycoprotein pump encoded by the *ABCB1* gene (1,2). Approximately 85% of this drug is hydrolyzed to an inactive metabolite by the esterases, while the remaining 15% is oxidized by the cytochrome P450 enzyme system and converted into active metabolite (thiol derivative-R-130964). As is well-known, the biotransformation of clopidogrel is made out of two steps. In addition, cytochrome P450 enzymes involved in clopidogrel biotransformation can be listed as CYP2C19, CYP2C9, CYP2B6, CYP3A4 and CYP1A2 (1,2).

Situated in the 10th chromosome (10q24.1-10q24.3), the genetic localization, where the CYP2C19 enzyme is coded, is 1.473 base pairs in length. This gene contains 9 exons and encodes a protein composed of 490 amino acids (1).

The Food and Drug Administration (FDA) describes the different alleles of CYP2C19 and their effects as follows: although the CYP2C19*1 allele is an ancestral type, it is a completely normal activity allele of clopidogrel, and also its clopidogrel metabolism is normal. Known as non-functional alleles of clopidogrel, CYP2C19*2 and CYP2C19*3 are termed as alleles deprived of a metabolism (clopidogrel resistance). The CYP2C19*4, CYP2C19*5, CYP2C19*6, CYP2C19*7 and CYP2C19*8 alleles may be deprived of clopidogrel metabolism or have low clopidogrel metabolism. With this warning, the FDA emphasizes that patients should be treated with clopidogrel by considering the genetic differences in CYP2C19 function (3).

The allele with normal activity is CYP2C19*1. On the other hand, the CYP2C19*2 polymorphism is the splicing damage mutation (rs4244285) resulting from the change of guanine adenine (G→A) at nucleotide position 681 of the cDNA sequence in exon 5. Altering the reading frame of the mRNA that starts with the amino acid 215, this change forms a non-functional protein by creating a stop code in the downstream region with 20 amino acids (1,2). The conversion of the prodrug clopidogrel into the active metabolite is inhibited, resulting in clopidogrel resistance.

To date, a considerable literature has grown up around the theme of the ethnic differences in the CYP2C19 genotype and its prevalence (4-15). It is now well established from a variety of studies that *CYP2C19* gene polymorphism is associated with clopidogrel resistance in patients using clopidogrel due to CAD. In addition, the existing body of research suggests that there are differences in CYP2C19 genotype in the group with CAD.

To this respect, the specific objective of this study is to establish the CYP2C19*1 and CYP2C19*2 allele frequencies both in Turkish patients with CAD, who thus use clopidogrel, and in healthy Turkish population as well as to present the differences in genotypes and alleles between both groups.

Materials and Methods

Having been approved by the Pamukkale University Ethics Committee, the study included 100 healthy volunteers and 200 patients diagnosed with CAD and using clopidogrel, all of whom lived in Denizli. A total of 300 individuals (100 controls and 200 patients) were informed about the informed consent of the Helsinki Declaration. All the individuals and patients consented, in writing, for the study after full explanation of what was involved. Written consent form was signed by all the participants (patients and control groups). This study is a prospective case-control study.

Data Collection

The data of the patients' age, gender and number of angiographic interventions were recorded in the data set.

Blood collection and DNA isolation

Initially, genomic DNA was isolated with standard phenol-chloroform method by taking blood into the anticoagulated (K3EDTA) vacuum tubes. Subsequently, the region specific to the *CYP2C19* gene was amplified through the polimerase chain reaction method in the genomic DNAs obtained, and the polymorphic foci in these regions were specified (4).

Statistical Analysis

SPSS statistical software version 23.0 was used for data analysis. Goodness of fit test was used for analyzing the distribution of the alleles and genotypes. Fisher's Exact and chi-square tests were used to compare the allele and genotype frequencies. Descriptive analysis was used to compare allele frequencies between the Turkish population and published data of other ethnic groups. A value of $p<0.05$ was considered as statistically significant.

Results

Table 1 presents the breakdown of data for the CAD group and the control group as well as the data for the Goodness of fit test according to the Hardy Weinber distribution. When the CAD patients and control group were evaluated together, CYP2C19*1/1 genotype was detected in 204 subjects (68%), followed by CYP2C19*1/2 genotype in 82 subjects (27.3%) and CYP2C19*2/2 genotype in 14 subjects (4.6%).

The CYP2C19*1 allele frequency was 490 (81.6%), whereas that of CYP2C19*2 was 110 (18.3%). As far as the genotype frequencies of the groups were concerned, CYP2C19*1/1 genotype was found in 132 patients (66%), CYP2C19*1/2 genotype in 62 patients (31%) and CYP2C19*2/2 genotype in 6 patients (3%) in the CAD group. On the other hand, there were 72 subjects (72%) with CYP2C19*1/1 genotype, 20 subjects with CYP2C19*1/2 genotype and 8 subjects with CYP2C19*2/2 genotype in the control group. A significant difference was found between the groups in terms of genotypes (p=0.034). The CYP2C19*1/1 genotype was found at higher percentages in the control group, whereas its CYP2C19*2/2 counterpart had a higher proportion in the CAD group (Table 2).

When it comes to the allele frequencies of the groups, the CYP2C19*1 allele frequency was 326 (81.5%) and that of CYP2C19*2 was 74 (19.5%) in the CAD group, while CYP2C19*1 allele frequency was 164 (82%) and that of CYP2C19*2 was 36 (18%) in the control group. No significant difference was found between the groups in terms of allele frequencies, which were similar in both groups (p=0.881) (Table 2).

Table 3-5 presents an overview of the data in other studies in which CYP2C19*1 and CYP2C19*2 genotype and alleles frequencies were investigated. The differences in these genotypes and alleles frequencies between our study and these studies can also be seen in these tables (4-23).

With respect to the relationship between the number of angiographic interventions and genotypes of the patients, 120 (90.9%) of the patients with CYP2C19*1/1 genotype underwent angiography only once, whereas 12 (9.9%) were exposed to angiography twice or more and to medical

Table 1. Non-parametric chi-square "goodness of fit" test

		CAD group n=200 subjects, n=400 alleles				
		Observed	**Expected**	**Expected proportion**	**Percentage deviation**	**Standardized residuals**
CYP2C19*2	*1/*1	132	132.85	0.66425	-0.64%	-0.07
	*1/*2	62	60.31	0.30155	+2.8%	+0.22
	*2/*2	6	6.85	0.03425	-12.41%	-0.32
		X^2=0.16, df=2, p=0.9231				
		Control group n=100 subjects, n=200 alleles				
		Observed	**Expected**	**Expected proportion**	**Percentage deviation**	**Standardized residuals**
CYP2C19*2	*1/*1	72	67.24	0.6724	+7.08%	+0.58
	*1/*2	20	29.52	0.2952	-32.25%	-1.75
	*2/*2	8	3.24	0.0324	+146.91%	+2.64
		X^2=10.4, df=2, **p=0.0055**				

CAD: Coronary artery disease

Table 2. Allele frequencies of CYP2C19*1 and *2 in CAD and control groups

CAD group n=200 subjects, n=400 alleles					**Control group n=100 n=200 alleles**				
Genotype	**n (%)**	**Allele frequency**		**p**	**Genotype**	**n (%)**	**Allele frequency**		**p**
*1/1	132 (66)	*1	81.5%	0.032	*1/1	72 (72)	* 1	82%	0.881
*1/2	62 (31)	*2	19.5%		*1/2	20 (20)	*2	18%	
*2/2	6 (3)				*2/2	8 (8)			

p-values are derived from Fisher's Exact test, CAD: Coronary artery disease

interventions during angiography. While 53 (77%) of the patients (CYP2C19*1/2 and CYP2C19*2/2) carrying the CYP2C19*2 allele underwent one angiography, 15 (23%) patients were subjected to 2 or more angiomas. Bearing the CYP2C19*2 allele heightened the risk of multiple angiographic interventions in the patient group by 2.83 times [p=0.16 and 95% confidence interval (CI) (1.24-6.45)] (Table 6).

Discussion

In 2017, the United States FDA issued some warnings on clopidogrel metabolism and the impact of the *CYP2C19* gene on this metabolism. In accordance with these warnings, it was reported that some patients might metabolize clopidogrel more poorly, and that clopidogrel activity would decrease in these patients (24).

In addition, FDA also warned that the CYP2C19*1 allele provides normal functional metabolism of clopidogrel, while CYP2C19*2 and CYP2C19*3 alleles cause dysfunction in the metabolism of clopidogrel. It was noted that if patients carry both of these non-functional alleles, they can be designated as poor metabolizers, whereas intermediate metabolizers are those who carry one copy of CYP2C19 encoding a non-functional allele, which might be either *1 or *2 (25).

There is a large body of literature that recognizes the variability of *CYP2C19* gene polymorphism in different ethnicities in healthy volunteers. As noted before, CYP2C19*1 allele frequency turns out to be 82%, while CYP2C19*2 allele frequency is 18% for healthy volunteers (control group) in our study. In contrast, CYP2C19*1 allele frequency is reported as 56%-74% and CYP2C19*2 allele frequency as 24% and 37% in the studies aimed at Chinese and Thai population (8-11,13). Our study is different from the Chinese and Thai populations in terms of *1 and *2 allele frequencies and healthy group population. For

Table 3. *CYP2C19* gene genotypes and allele frequencies of healthy group in different populations

Population	Subjects N	Alleles N	*1/1	*1/2	*2/2	*1	*2	Reference
Ethiopian	114	228	0.746	0.193	0.026	0.842	0.122	4
Zimbabwean	84	168	0.773	0.190	0.035	0.869	0.130	5
Chinese-Dai	193	286	0.419	0.440	0.046	0.663	0.303	6
Chinese-Han	101	202	0.316	0.465	N/E	0.56	0.37	7
Chinese-Li	100	200	0.48	0.49	0	0.740	0.245	8
Thai	1051	2102	0.407	0.351	0.073	0.63	0.27	9
Italian	360	720	0.794	0.189	0.017	0.889	0.111	10
Chinese-Hakka	6.686	13,372	0.417	0.396	0.097	0.64	0.31	11
Belgium	121	242	0.835	0.149	0.016	0.909	0.091	12
Beninese	111	222	0.739	0.261	0	0.87	0.13	12
Palestinian	100	200	0.81	0.19	0	0.905	0.095	13
Turkish	100	200	0.73	0.27	0	0.865	0.135	13
German	328	656	0.723	0.232	0.042	0.84	0.159	14
Turkish	404	804	0.76	0.223	0.099	0.875	0.121	14
Turkish	160	320	0.655	0.234	0.110	0.88	0.12	15
Turkish (present study)	**100**	**200**	**0.72**	**0.20**	**0.08**	**0.82**	**0.18**	**-**

Table 4. *CYP2C19* gene genotypes and allele frequencies in coronary artery disease population

Population	Subjects N	Alleles N	*1/1	*1/2	*2/2	*1	*2	Reference
Egyptian	230	460	75.2	23.1	1.7	86.7	13.3	16
Russian	81	162	84	16	0	92	8.0	17
Yakutsk (Russia)	268	536	65.67	33.58	0.075	82.46	17.54	18
Russian	143	286	83.92	15.38	0.070	91.60	8.40	18
Chinese	168	336	44.05	41.67	14.28	64.89	35.11	19
Chinese-Hakka	934	1868	40.36	40.26	9.42	63.17	31.64	11
Turkish	347	694	72.9	23.6	3.5	84.7	15.3	20
Turkish (present study)	**200**	**400**	**66.0**	**31.0**	**3.0**	**81.5**	**19.5**	**-**

instance, *1 allele frequency is higher than Chinese and Thai population, although that of *2 is lower in our study group. As far as the studies on African black race (Ethiopian, Zimbabwean, Beninese) are concerned, *1 allele frequency proves to be lower, whereas that of *2 is higher than our study (4,5,12). Previous research on European-Caucasian population (Italian, Belgium, German) establishes*1 allele frequency to be in the range of 84% -90.9%, while that of *2 is in the range of 9.1% -23% (10,12,14). *1 allele frequency reported in the existing study is lower than the percentages in Italian, Belgium and German population, but that of *2 is higher (10,12,14). Even though the allele frequencies in the healthy group are similar to those of the previous studies on the European, Turkish and Caucasian population, *2 allele frequencies turn out to be higher in our study (10,12-15).

With respect to the genotype frequencies in the studies on ethnic diversity, *1/1 genotype frequency is reported to be in the 31.6% -41.9% range, whereas *2/2 genotype frequency is in the range of 0% -9.7% in the Chinese population (6-8,11). In contrast to *1/1 genotype frequency in the current study which is noticeably different from the Chinese population,*2/2 genotype frequency seems to be in similar percentages both in our study's participants and in Chinese population (6-8). The last but not the least, *1/1 genotype frequency is considerably higher in our healthy volunteer group than in the Chinese population, while that of *1/2 is lower (6-8).

Research on the Caucasian population (Italian, Belgium, German) specifies *1/1 genotype frequencies to be between 70.6% and 83.5%.*1/1 genotype frequencies in our healthy volunteers are akin to the Caucasian population data unlike those of *2/2 which are higher in our healthy volunteers within our study than the aforementioned studies on the Caucasian population (10,12,14).

The earlier studies on the Turkish population have identified*1/1 genotype frequency as 65.5% -76%. On

Table 5. CAD and control groups in comparison studies

Population	Group (N)	Genotypes N (%)			Alleles (%)		p	Ref. no
		*1/1	*1/2	*2/2	*1	*2		
Russian	**CAD (561)**	448 (79.9)	106 (18.9)	7 (1.2)	89.3	10.7	0.086	21
	Control (694)	543 (78.2)	130 (18.7)	21 (3.0)	87.6	12.4		
Russian	**CAD (84)**	69 (82.1)	14 (16.7)	1 (1.2)	90.5	9.5	0.42	22
	Control (30)	23 (76.7)	6 (20.0)	1 (3.3)	86.7	13.3		
Kazakh Slav	**CAD (72)**	50 (69.4)	22 (30.6)	0.0	82.5	17.5	N/A	23
	Control (255)	179 (70.0)	71 (28.0)	5 (2.0)	84.7	15.3		
Turkish (present study)	**CAD (200)**	**132 (66.0)**	**62 (31.0)**	**6 (3.0)**	**81.5**	**19.5**	***0.034**	-
	Control (100)	**72 (72.0)**	**20 (20.0)**	**8 (8.0)**	**82.0**	**18.0**		

CAD: Coronary artery disease

Table 6. Genotypes and number of angiographic interventions

Number of intervention	Genotypes		p	95% CI
	CYP2C19*1/1 n (%)	CYP2C19*1/2 & CYP2C19 * 2/2 n (%)		
1	120 (90.9%)	53 (77%)	0.016	2.83 (1.24-6.4)
>1	12 (9.1%)	15 (23%)		

p-value is derived from chi-square test, CI: Confidence interval

the contrary, a study comparing Palestinian and Turkish populations reports no presence of genotype of *2/2 (13), though some findings in earlier studies establish*2/2 genotype frequency in healthy Turkish population to be around 10%. The results of healthy volunteers in our study seem to be consistent with the data of previous research with regard to both *1/1 and *2/2 genome frequencies (13-15).

When it comes to the CAD group within our study, *1 allele frequency is 81.5%, while that of *2 is 19.5%. Considering the allele frequencies concerning ethnicity, *2 allele frequency is lower in our study group than that reported by the previous studies on Chinese (11,19). In addition, *2 allele frequency in the Russian and Egyptian populations is reported as 8% and 13.3%, respectively (16,17). As can be noticed from the above-mentioned research, the percentages of *2 allele observed in this investigation are far above those observed by in these studies. However, earlier research on the Turkish CAD population has reported *2 allele frequency as 15.9%, consistent with the present data in our study (20).

With respect to genotype frequencies in CAD patients, *1/1 genotype frequency in the Chinese populations is lower than the patients in our study, whereas *2/2 genotype frequency is higher (11,19). In Russian and Russian-Yakutsk population, *2/2 genotype frequency is lower than our patient group (17,18). Moreover, genotype frequencies in our study are of similar nature to the previous study conducted on the Turkish population with CAD (20).

Studies comparing genotype and allele frequencies in individuals with CAD and healthy population report no difference in allele or genotype between control and CAD groups. However, a closer look into the pertaining studies will reveal the noticeable impact of the number of patients included in studies, and it is observed that as the number of patients increase, p value gets closer to the significance level (21-23). In our study, although no difference exists between the groups in terms of alleles in CAD group, there is a difference in genotypes in CAD group, which is mainly caused by the intermediate group. This case highlights the importance of checking *CYP2C19* gene polymorphism prior to the initiation of antiplatelet therapy in CAD patients.

As a review of current literature suggests, CYP2C19*2 allele is one of the overriding factors contributing to clopidogrel resistance (3). Having followed-up 1050 patients for cardiovascular diseases for 8 years, Rothenbacher et al. (26) concluded that the CYP2C19*2/2 genotype poses a risk for the development of cardiovascular diseases. Likewise, a meta-analysis by Singh et al. (27) revealed that CYP2C19*2 polymorphism might heighten the risk of stent thrombosis by 2.4 times (RR: 2.41, CI: 1.69-3.41, p<0.001). On the other hand, Nozari et al. (28) found that the risk of restenosis did not increase in individuals with CYP2C19*1/CYP2C19*2 genotype within one year.

Our study reveals that carrying CYP2C19*2 polymorphism (heterozygous or homozygous) may heighten the risk of secondary angiographic intervention. Although the number of our patients bearing homozygous polymorphism is highly low, our study may be considered as an important step towards unraveling the relationship between CYP2C19*2 polymorphism and increased risk of restenosis.

Study Limitations

Some limitations are inherent in our study. For example, we did not analyze alleles and genotype differences except CYP2C19*1 and *2 in our study. Further, we did not carry out the clinical follow-up of the patients in our study as well as not measuring clopidogrel metabolite.

Conclusion

As a conclusion, in our research, we studied *CYP2C19* gene polymorphism and CYP2C19*1 and CYP2C19*2 allele frequencies in the control group with 100 healthy volunteers and 200 CAD patients. In addition, the high frequency of percutaneous coronary intervention in individuals with CYP2C19*2 allele suggests that clopidogrel resistance is likely to pose an important challenge. CYP2C19*1/2 and *2/2 genotypes were found to be higher in CAD patients than the one in the control group, underlining how critical checking *CYP2C19* gene polymorphism is before setting out antiplatelet therapy in CAD patients.

Ethics

Ethics Committee Approval: Having been approved by the Pamukkale University Ethics Committee, the study included 100 healthy volunteers and 200 patients diagnosed with CAD and using clopidogrel, all of whom lived in Denizli.

Informed Consent: Written consent form was signed by all the participants

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: A.K., R.S., Design: A.K., İ.D.K., A.Y., Data Collection or Processing: A.Y., İ.D.K., A.K., Analysis or Interpretation: R.S., A.Y., İ.D.K., Literature Search: A.K., A.Y., Writing: R.S.

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