ISSN 1022-7954, Russian Journal of Genetics, 2025, Vol. 61, No. 10, pp. 1301–1312. © Pleiades Publishing, Inc., 2025.

HUMAN GENETICS

Genotype and Allele Frequencies of *AKR1C3* rs3763676 and rs12529 Variations in a Turkish Population: Novel PCR-RFLP Assays

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Received March 3, 2025; revised March 23, 2025; accepted April 1, 2025

Abstract—The impact of genetic mechanisms should be clearly understood before clinical practice of drugs since they could cause variations in biotransformation, clearance, and thus, the optimal dose of drugs. Naltrexone, an opioid antagonist metabolized by AKR isoenzymes, is used to manage opioid and alcohol use disorders. Although its high efficacy, adherence to treatment with naltrexone was different between patients due to most likely patients' genetic information. The aim of this study is to develop novel PCR-RFLP assays to genotype *AKR1C3* rs3763676 and rs12529 polymorphisms and to find out the allele and genotype frequencies of these polymorphisms in a Turkish population. Unrelated volunteers (n = 222) between 18–65 years were analyzed to determine genotype and allele frequencies of *AKR1C3* rs3763676 and rs12529 in a Turkish population with novel PCR-RFLP assays. The minor allele frequencies (MAF) of *AKR1C3* rs3763676 and rs12529 polymorphisms were 24.0% and 56.0%, respectively. MAF of *AKR1C3* rs12529 in Turkish population was higher than those of in African and in Taiwanese, respectively. The frequency of T (variant) allele of *AKR1C3* rs3763676 was higher in Korean as compared to Turkish. *AKR1C3* rs3763676 and rs12529 allele variants were not in linkage disequilibrium (D = 0.57, R^2 = 0.08). PCR-RFLP assays developed in the study and the documented allele frequencies will be useful for direct detection of *AKR1C3* rs3763676 and rs12529 variations in pharmacogenetic studies of naltrexone pharmacology.

Keywords: pharmacogenetics, *AKR1C3*, rs3763676, rs12529, PCR-RFLP

DOI: 10.1134/S1022795425700826

INTRODUCTION

Pharmacogenetics is a field of study examining the effects of genetic variations on drug toxicity or treatment response. Genetic variations such as single nucleotide polymorphism (SNP), insertion, deletion, or tandem repeats could cause changes in genes' products, which result in vulnerability to diseases or treatment failures [1, 2]. Pharmacokinetics has focused on genetic variations influencing the absorption, distribution, biotransformation and elimination of drugs, whereas pharmacodynamics studies have been interested in genes encoding transporters, receptors or channels [3]. For improved treatment outcomes, personalized treatment strategies according to pharmacogenetic test information should be clearly defined. Therefore, the role of genetic and epigenetic mechanisms should be clearly understood before clinical practice [4].

Naltrexone is an FDA-approved pharmacotherapy used to treat opioid (OUD) or alcohol use disorder (AUD) [5, 6]. Naltrexone and its major and long-acting metabolite 6-beta naltrexol are opioid-receptor antagonists. They bind competitively at opioid receptors (mu-, delta- and kappa-opioid receptors) and thus opioids could not bind to these receptors [7, 8]. Although naltrexonehydrochloride was first synthesized to treat opioid dependence [9], its clinical efficiency was not as expected. However, it is still used as an adjunctive medication in opioid detoxification. Naltrexone is also approved by FDA for the prevention of relapse in individuals with alcohol use disorder [6]. Preclinical studies demonstrated that naltrexone reduces alcohol consumption and euphoria in animals by antagonizing the opioid system related to the rewarding effect of alcohol [10, 11].

Recently, some studies have suggested that naltrexone could also be used in the management of behavioral addiction [12] and pathological gambling in Parkinson disease [13]. Although its high clinical efficacy is superior to the placebo in the treatment of AUD or OUD [14–17], adherence to treatment with naltrexone was different between patients [17] most likely due to patients' genetic background [1, 18–22]. Kranzler et al. [23] indicated that most of the patients (80%) discontinued naltrexone use by six months. A meta-analysis conducted by Chamorro and Marcos [19] demonstrated that relapse rate was found to be lower

in patients receiving naltrexone with G allele of *ORPM1* rs1799971 polymorphism compared to those with A allele. Polymorphisms on pharmacodynamic genes such as *OPRM1* can be used to predict individual response to drug treatments, while polymorphisms in genes encoding enzymes that metabolize drugs can guide the determination of drug doses specific to individuals [22].

Naltrexone is metabolized to its main metabolite, 6-beta-naltrexol, by hepatic reduction from its carbonyl moiety. This reduction is catalyzed by hepatic Aldo-keto reductase (AKR) enzymesfound in almost all living things [24, 25]. These monomeric NADPH-dependent oxidoreductase enzymes play role in phase I biotransformation of both endogenous and exogenous substrates by reducing ketones and aldehydes to their alcohols [25, 26]. In humans, 15 different AKR enzymes were identified. Amongst, AKR1C1–AKR1C4 function as hydroxysteroid dehydrogenases [27]. AKR1C3 and AKR1C4 enzymes are mostly found in liver. *AKR1C1–AKR1C4* genes found in chromosome 10 (p15–p14) encodes AKR1C1–AKR1C4 enzymes and have 12 exons [28].

Polymorphisms identified in *AKR1C* genes may cause different rates of metabolite formation between individuals, change in the overall duration of action of the drug, and therefore change clinical outcomes [29]. In a randomized trial, Krupitsky et al. [30] showed that 13.7% of the patients with naltrexone implant experienced 12 relapses between 6 and 8 weeks. They suggested that these relapses could be due to the rapid biotransformation of naltrexone. Porter and Somogyi [21] showed that there are interindividual variabilities between human liver samples in view of the enzymatic formation of 6-beta-naltrexol from naltrexone, which cause alterations in the efficacy of the treatment. Furthermore, Stancil et al. [31] demonstrated this variability in 6-beta-naltrexol formation between individuals and found that *AKR1C4* rs17134592 polymorphism has a greater effect on enzyme activity compared to the ages of human liver donors. Hitherto, to the best of our knowledge, there has been no study examining the effect of *AKR1C* polymorphisms on plasma and or urine naltrexone levels in patients receiving naltrexone treatment.

We are planning to determine this effect of *AKR1C3* polymorphisms on plasma levels of naltrexone and its metabolite 6-beta-naltrexol in individuals with opioid/alcohol use disorder in a near future. However, first, we aimed to optimize PCR-RFLP methods to genotype *AKR1C3* rs3763676 and rs12529 polymorphism since PCR-RFLP is still one of the common, easiest and most cost-effective methods used to genotype single nucleotide polymorphisms in research laboratories without high technology equipment and to find out genotype and allele frequencies of these polymorphisms in Turkish population in the current study.

MATERIALS AND METHODS

Case Selection

The current study comprised 222 unrelated Turkish volunteers attending to İbni Sina Hospital Blood Donation Center in Ankara. All participants were healthy, which is assessed by medical history. The study design was approved by Ethics Committee of Human Research at Ankara University (Approval no. 07-536-19 in 2019). The inclusion criteria were: (i) being between the ages 18 and 65, (ii) not having any clinical or psychiatric disease, (iii) being born in Turkey. All individuals who agreed to participate in the study signed the written informed consent and completed the questionnaire regarding the age, gender, marital, education and employment status. Four mL venous blood samples were collected from each participant for genetic analysis in accordance with the Declaration of Helsinki.

DNA Isolation

Genomic DNA was extracted from venous blood samples collected into tubes containing EDTA using the GeneJET Genomic DNA Purification Kit (Thermo Fisher Scientific, UK) according to the manufacturers' recommendations. Extracted DNA was stored at −20°C until PCR analysis.

Optimization of Polymerase Chain Reaction (PCR) Components and Conditions

The sequence data of *AKR1C3* gene region containing rs3763676 and rs12529 was obtained from NCBI database (NC_000010.11). The Primer-Blast software tool (NCBI) was used to design novel forward and reverse primers for amplifying regions containing rs3763676, and rs12529. The sequences of forward and reverse primer pairs, PCR components and conditions, and the length of PCR products were given in Table 1. PCR optimization is a multi-step process performed by changing the amount of PCR components and PCR conditions (temperature, time, etc.). In Table 1, both optimized amounts of PCR components and also PCR conditions was seen. It may be noted that same PCR products (480 bp) were amplified for *AKR1C3* rs3763676 and rs12529 polymorphisms, but their genotypes were determined with distinct restriction enzymes (Table 1). All PCR products were run on a 1% agarose gel containing ethidium bromide and visualized under UV transilluminator (SYNGENE, UK) (Fig. 1).

Optimization of Restriction Fragment Length Polymorphism (RFLP) Conditions

Restriction enzymes used to genotype *AKR1C3* rs3763676 and rs12529 variations were detected using NEB cutter tool (https://nc3.neb.com/NEBcutter/).

Table 1. List of *AKR1C3* SNPs with rs numbers, sequences of forward and reverse primers, PCR-RFLP components and conditions, sizes of undigested and digested PCR products (bp)

SNP (rs no.)	Primer sequences (5'–3')	PCR components	PCR conditions (Tm/Cy)	RFLP components	RFLP conditions	Undigested and digested PCR products, bp
AKR1C3 rs3763676	F: AAGGGGCATTATCACGGCAG R: CCTCTGGAGGTGCATAGGTG	10× buffer (2.5 μL), 10 mM dNTP (0.4 μL), 10 pmol F-primer (1 μL), 10 pmol R-primer (1 μL), 10 U Taq (0.125 μL), 17.975 μL water	Initial denaturation: 5 min, 94°C 35 cycles 30 s, 94°C 30 s, 60°C 45 s, 72°C Final extension: 10 min, 72°C	10× Tango buffer (1 μL), 10 U/μL *Hpy8I* (0.5 μL), 3.5 μL water	*Hpy8I* 37°C, 1 h	PCR: 480 A: 285 + 195 G: 260 + 195 + 25
AKR1C3 rs12529	F: AAGGGGCATTATCACGGCAG R: CCTCTGGAGGTGCATAGGTG			10× B buffer (1 μL), 10 U/μL *BsrI* (0.5 μL), 3.5 μL water	*BsrI* 65°C, 1 h	PCR: 480 C: 410 + 70 G: 480

The RFLP reactions were carried out in a volume of 10 μL containing 10× buffer, 5 U restriction enzyme and 5 μL distilled water. Temperatures for optimal digestion and the length of the digested PCR products were also given in Table 1. The digested PCR products were electrophoresed on a 2–3% agarose gel with ethidium bromide at 70 mA for 1.5 h and visualized under UV transilluminator (Fig. 2).

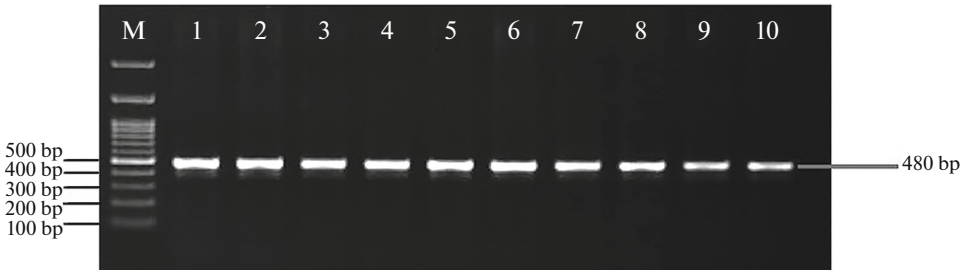

Fig. 1. Representative agarose gel (1%) images of PCR products of *AKR1C3* rs3763676 and rs12529 (480 bp) polymorphisms (M: 100 bp ladder, Lanes 1–10: PCR products).

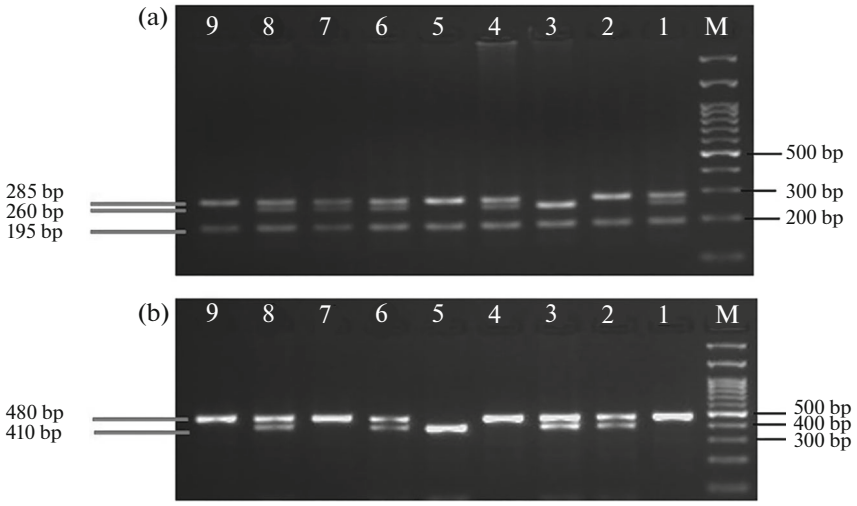

Fig. 2. Representative agarose gel (3%) images of digested PCR products for the (a) *AKR1C3* rs3763676, (b) *AKR1C3* rs12529 polymorphisms. (a) M: 100 bp ladder; lanes 2, 5 and 9: homozygous wild-type genotype (AA) (285 and 195 bp); lane 3: heterozygote genotype (AG) (285, 260, 195 and 25 bp); lanes 1, 4, 6, 7 and 8: homozygous variant genotype (GG) (260, 195 and 25 bp). (b) M: 100 bp ladder; lane 5: homozygous wild-type genotype (CC) (410 and 70 bp); lanes 2, 3, 6 and 8: heterozygote genotype (CG) (480, 410 and 70 bp); lanes 1, 4, 7 and 9: homozygous variant genotype (GG) (480 bp).

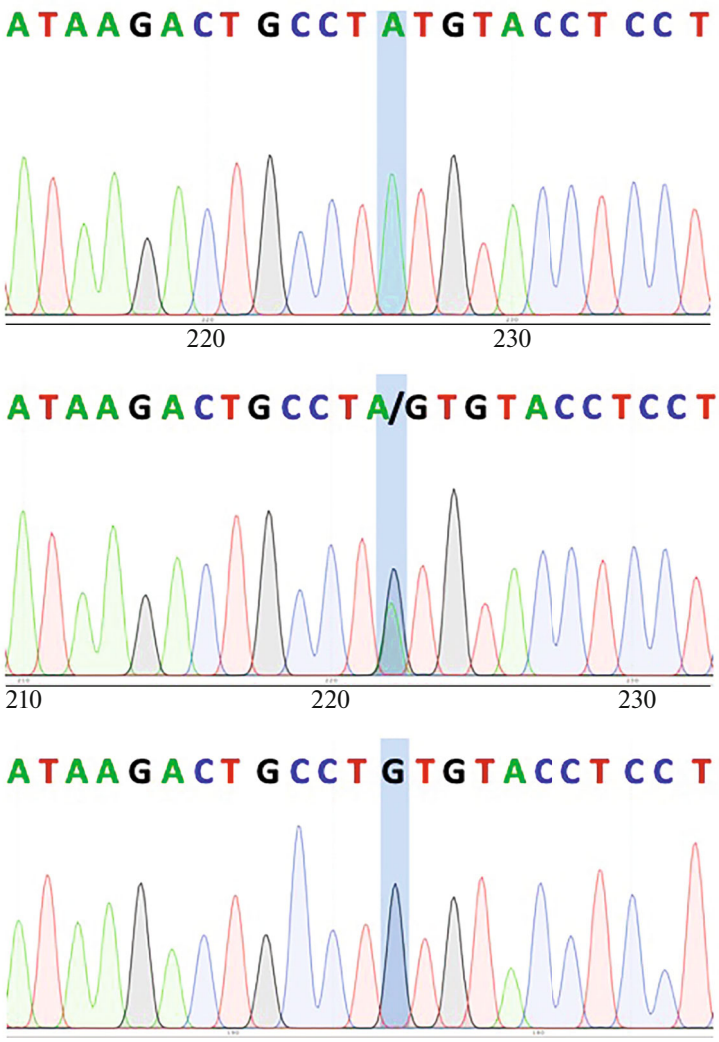

Fig. 3. Sequencing results for *AKR1C3* rs3763676, confirming the presence of wild type (presence of the "A" nucleotide only), heterozygote (presence of the "A" and "G" nucleotides) and variant (presence of the "G" nucleotide only) *AKR1C3* rs3763676 A>G.

Determination of the Reliability of the PCR-RFLP Assays

The reliabilities of the presented assays were assessed by direct sequencing (Applied Biosystems 3730xl DNA analyzer, USA). Wild type (n = 10), heterozygous (n = 10) and variant (n = 10) genotypes were verified using the same set of PCR primers (Figs. 3, 4).

Statistical Analysis

Allele and genotype frequencies were calculated using SPSS version 25.0. It was determined whether the genotype frequencies were in Hardy–Weinberg equilibrium or not by comparing them with the expected genotyped frequencies using the chi-square test. Demographic characteristics of participants and genotype and allele frequencies were given as percentages and 95% confidence interval. Linkage disequilibrium (LD) and haplotype analysis for *AKR1C3* rs3763676 and rs12529 were performed by SHEsis-Plus software [32]. Statistically significant p value was <0.05.

RESULTS

To genotype *AKR1C3* rs3763676 and rs12529 variations, novel PCR and RFLP methods were optimized in the current study. A 480 bp PCR product including the *AKR1C3* gene region containing both rs3763676 and rs12529 SNPs was amplified with same forward and reverse primers (Fig. 1). PCR products of *AKR1C3* rs3763676 and rs12529 variations were digested with *Hpy8I* and *BsrI* restriction enzymes, respectively. For *AKR1C3* rs3763676, the reference allele is "A" and the alt allele is "G." When the presence of A allele in this gene location, *Hpy8I* cuts DNA at only one recognition site and creates two fragments

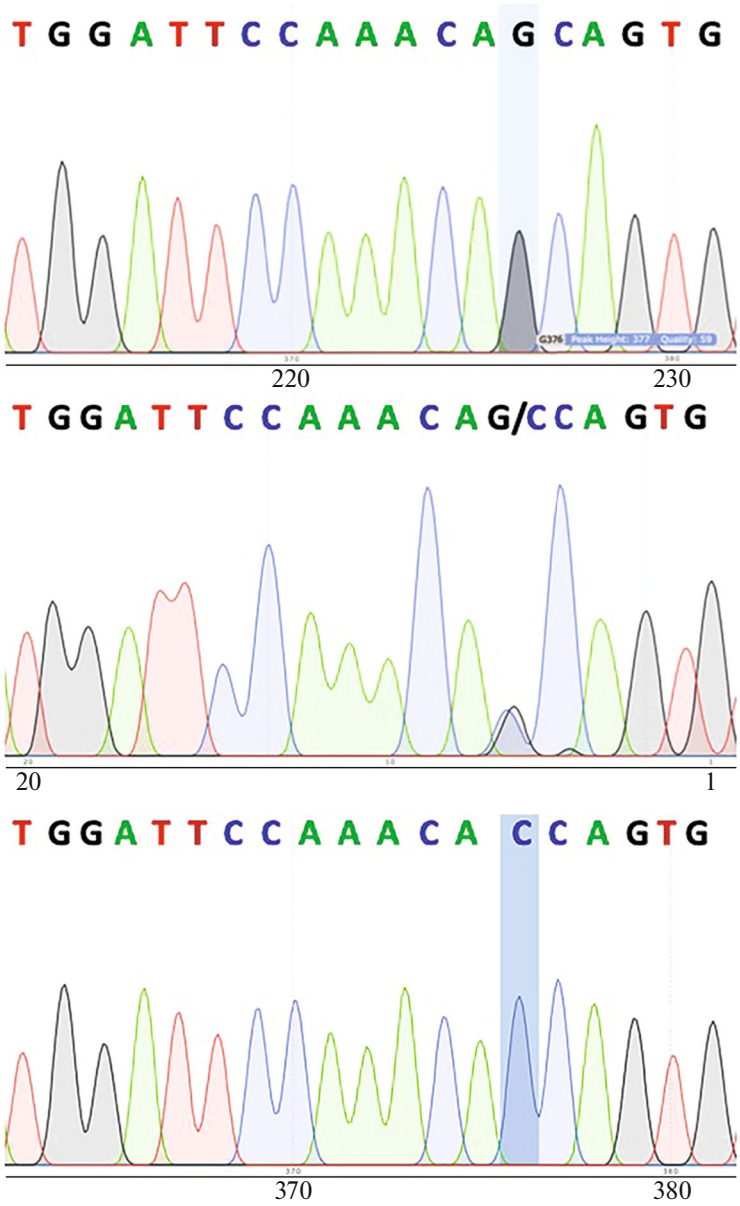

Fig. 4. Sequencing results for *AKR1C3* rs12529, confirming the presence of wild type (presence of the "G" nucleotide only), heterozygote (presence of the "G" and "C" nucleotides) and variant (presence of the "C" nucleotide only) *AKR1C3* rs12529 G>C.

(285 + 195 bp). The replacement of A (adenine) with G (guanine) of the *AKR1C3* rs3763676 causes an extra recognition site for *Hpy8I* and, thus, three fragments is created (260 + 195 + 25 bp) (Fig. 2a).

As for the determination of *AKR1C3* rs12529, the dominant allele is C (cytosine) and the recessive allele is G (guanine). *BsrI* has only one recognition site in the presence of C allele of the *AKR1C3* rs12529 polymorphism, and two fragments (410 + 70 bp) are created after digestion with *BsrI*. The C to G transversion leads to the loss of the *BsrI* cleavage site and the expected fragment (480 bp) is the same with the PCR product (Fig. 2b). In addition, Figs. 3 and 4 showed the confirmation of PCR-RFLP assays by direct sequencing.

After optimization and verification of PCR-RFLP assays, their efficacies were tried in blood samples of healthy individuals. A total of 222 Turkish volunteers (186 males and 39 females) were included in the current study. The median age was 37.0 years (IQR: 28.0–43.0 years). Demographic characteristics of them were shown in Table 2. Table 3 demonstrated the allele and genotype frequencies of *AKR1C3* rs3763676 and rs12529 polymorphisms with 95% confidence interval. The genotype frequencies of both polymorphisms

Table 2. Demographic characteristics of healthy volunteers ($n = 222$)

Parameter	Healthy volunteers ($n = 222$)	
Age (years) $\tilde{x}$ (IQR)	37.0 (28.0–43.0)	
Weight (kg) mean ± S.D. (min–max)	80.36 ± 14.15 (46–139)	
Height (cm) $\tilde{x}$ (IQR)	175.0 (168.5–180.0)	
	n	% frequency* (95%CI)
Gender		
Female	39	17.3 (12.4–22.2)
Male	186	82.7 (77.8–87.6)
Education		
Primary	26	11.6 (7.4–15.8)
Secondary	32	14.2 (9.6–18.8)
High School	95	42.2 (35.7–48.7)
Undergraduate	52	23.1 (17.6–28.6)
Graduate	10	4.4 (1.7–7.1)
Occupation		
Working	133	59.1 (52.7–65.5)
Not working	77	34.2 (28.0–40.4)
Marital status		
Single	84	37.3 (30.9–43.6)
Married	132	58.7 (52.3–65.1)

* Since demographic characteristics of a few volunteers could not be collected, total percentage were not 100%.

were consistent with Hardy–Weinberg equilibrium ($p > 0.05$).

Haplotype analysis using SHEsis-Plus software showed that *AKR1C3* rs3763676 and rs12529 allele variants were not in linkage disequilibrium ($D = 0.57$, $R^2 = 0.08$; Fig. 5). The haplotypes with frequencies >3% were A-C (4.0%), G-G (19.4%), A-G (35.9%) and G-C (4.5%). Allele frequencies of *AKR1C3* rs3763676 and rs12529 polymorphisms detected in different studies so far were also given in Table 4.

DISCUSSION

Various methods including PCR-sequencing, MassARRAY, TaqMan and tetraARMS PCR have been developed to genotype single nucleotide polymorphisms [33]. All these techniques require high technology equipment that are expensive [34]. However, PCR-RFLP is still common, inexpensive, sensitive and rapid method to determine SNPs for research laboratories. It also has high sensitivity and accuracy. Thus, we aimed to develop PCR-RFLP assays for genotyping of *AKR1C3* rs3763676 and rs12529 variations. As it can be seen in Table 4, these polymorphisms were detected by techniques such as TaqMan, SequenomMassArray and PCR-Sequencing so far. To the best of our knowledge, there have been no studies genotyping *AKR1C3* rs3763676 polymorphism by PCR-RFLP assays. Only Tiryakioglu and Tunali [35] developed a PCR-RFLP method to determine *AKR1C3* rs12529. However, the advantage of our new assay is that a primer pair can be used to amplify gene region covering two SNPs (*AKR1C3* rs3763676 and rs12529).

Thus, gene region containing *AKR1C3* rs3763676 and rs12529 SNPs could be amplified in one run. In addition, the lengths of digested PCR products of *AKR1C3* rs12529 were longer and, thus, they can be separated in a gel containing less agarose in a shorter time. The feasibility of all PCR-RFLP assays developed in the current study were also verified by applying them to human subjects. Therefore, they could be alternative assays to genotype *AKR1C3* rs3763676 and rs12529 polymorphisms for research laboratories with limited budgets.

In the current study, the genotype and allele frequencies of *AKR1C3* rs3763676 and rs12529 polymorphisms were obtained from 222 healthy Turkish volunteers. For, the minor allele frequencies (MAF) were 0.24 and 0.56 for *AKR1C3* rs3763676 and rs12529, respectively. Table 4 showed the minor allele frequencies of *AKR1C3* rs3763676 and rs12529 polymorphisms in other populations. AKR1C1–AKR1C4 enzymes act as the hydroxysteroid dehydrogenases metabolizing various substrates including progesterone, 5α-dihydrotestosterone, and naltrexone [25]. AKR1C3 enzymes also cause bioactivation of chemical carcinogens [36]. Thus, the effects of *AKR1C3* polymorphisms on diseases have been investigated mostly in cancer types such as prostate, bladder, breast, or leukemia (Table 4).

In some of these previous studies, healthy controls were also included. We compared the observed minor allele frequencies of the present study with those of healthy controls included previous case-control studies. For *AKR1C3* rs3763676, the minor allele (G) frequency was the highest for Korean (0.65), whereas it ranged from 0.22 (Greek) to 0.36 (Swedish) for Caucasians. It may be noted that the MAF in the Turkish population was closest to that of the Greeks. For *AKR1C3* rs12529, the MAF varied among different populations. It was the lowest (0.11) for Taiwanese and highest for Mexican (0.67). Our finding showing that the MAF of *AKR1C3* rs12529 polymorphism was 0.56 was consistent with Tiryakioglu and Tunali [35]. Previous studies, along with our study, indicated that the individual variabilities between treatment responses of

Table 3. Genotype and allele frequencies of *AKR1C3* rs3763676 and rs12529 polymorphisms

SNPs	Allele *n* (% frequency; 95%CI)		Genotype** *n* (% frequency; 95%CI)		
AKR1C3 rs3763676	A	G	AA	AG	GG
	184 (76.0; 70.6–1.4)	58 (24.0; 18.6–29.4)	68 (46.2; 37.3–55.1)	48 (21.3; 14.0–8.6)	5 (2.2; NA)
HWE *p*-value	$\chi^2 = 0.95, p = 0.33$				
AKR1C3 rs12529	C	G	CC	CG	GG
	195 (44.0; 37.7–0.3)	247 (56.0; 49.7–2.3)	41 (18.2; 13.1–3.3)	113 (50.2; 43.6–6.8)	67 (29.7; 23.7–5.7)
HWE *p*-value	$\chi^2 = 0.302; p = 0.58$				

N—size, CI—confidence interval, HWE—Hardy–Weinberg equilibrium, * NA—non-available due to $n < 5$, **—for each polymorphism, the number of genotyped samples were not equal due to the amount of restriction enzymes we had.

naltrexone could be explained by the interindividual differences in protein expression due to gene variations in *AKR1C3*.

Bains et al. [37] reported *AKR1C4* rs17134592 variation on exon 9 causes an amino acid exchange from leucin to valine and this change results in a 3–5 fold decreased enzyme activity. Jakobsson et al. [38] showed that *AKR1C3* rs3763676 variation in the promoter region cause lower promoter activity in lung, liver, and prostate cells. However, as mentioned in the introduction section, there is no data showing the impact of these *AKR1C3* polymorphisms on the naltrexone pharmacology in humans [31]. With the current study, we determined the minor allele frequencies of *AKR1C3* rs3763676 polymorphisms for Turkish population for the first time. In a near future, we are planning to examine the effect of these polymorphisms on plasma/urine naltrexone and 6-beta-naltrexol levels and adverse effects of naltrexone.

AKR1C3 rs3763676 and rs12529 allele variants were not in linkage disequilibrium (LD) ($D = 0.57$; $R^2 = 0.08$) in the current study. *AKR1C3* rs3763676 occurs in the promoter region and rs12529 is in intron 1 with allele frequencies of 24% and 56% respectively. In

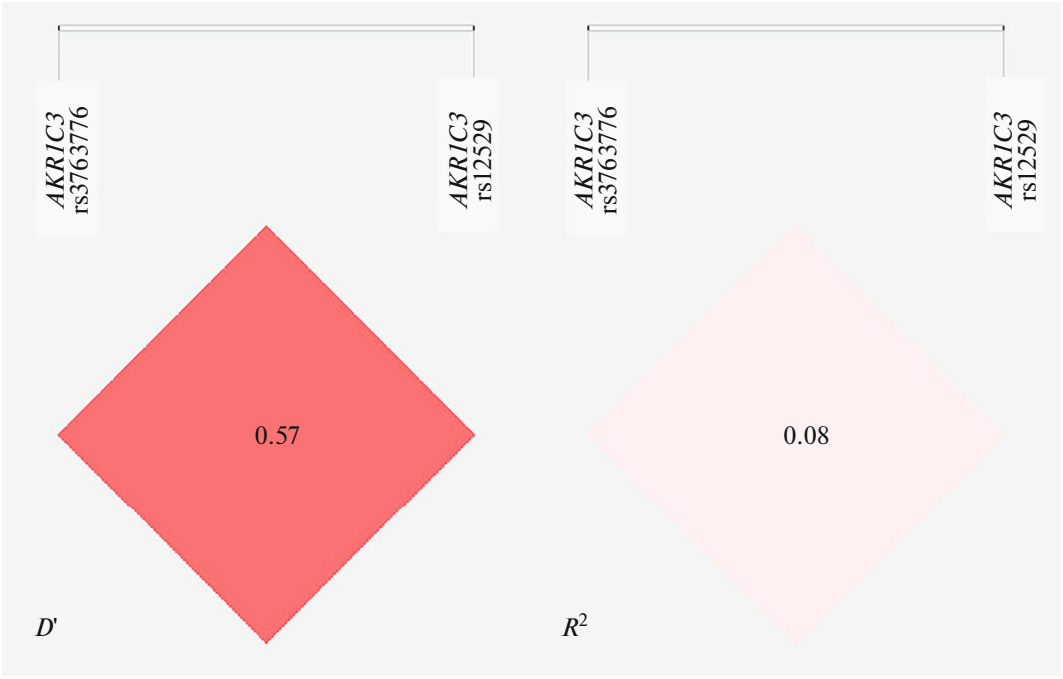

Fig. 5. Linkage disequilibrium (D') and correlation coefficient (R2) of *AKR1C3* rs3763676 and rs12529 polymorphisms.

Table 4. Differences in allele frequencies between study groups in literature

	Variations				
	study population and disease	minor allele and frequency	genotyping method	author–year	reference
AKR1C3 rs3763676	Turkish $n = 121$ healthy individuals	G allele 24.0%	PCR-RFLP	Present study	Present study
	Swedish ($n = 176$ patients, $n = 159$ controls) Prostate cancer	G allele 50% in cases 39% in controls	PCR-sequencing	Schulze et al., 2012	[40]
	Swedish ($n = 1045$) 157 Korean ($n = 157$)	G allele 65% in Koreans 34.4% in Swedes	PCR-sequencing	Jakobsson et al., 2007	[38]
	Caucasian $n =1086$ patients $n = 1032$ Bladder cancer	36% in cases 33% in controls	Golden Gate	Figueroa et al., 2008	[41]
	Swedish and Dutch ($n = 100$) Hypospadias	G allele 68.0% in cases 65.0% in controls	TaqMan	Soderhall et al., 2015	[39]
	Greek women with PCOS ($n = 150$) controls ($n = 51$)	G allele 22.0% in cases 20.0% in controls	PCR-sequencing	Marioli et al., 2009	[42]
	Caucasian, African-American, Hispanic, and Asian $n = 121$ (PCOS) $n = 128$ controls	G allele 32.2% in cases 22.3% in controls	PCR-sequencing	Qin et al., 2006	[43]
	Brazilian Females with external genitalia virilization ($n = 178$)	G allele 33.0%	PCR-sequencing	Kaupert et al., 2016	[44]
AKR1C3 rs12529	Turkish $n = 220$ Healthy individuals	G allele 56.0%	PCR-RFLP	Present study	Present study
	New Zealand Prostate cancer $n = 191$	G allele 46.6%	SequenomMassArray	Karunasinghe et al., 2016	[45]
	Swedish and Dutch ($n = 100$) Hypospadias	G allele 45.0% in cases 39.9% in controls	TaqMan	Soderhall et al., 2015	[39]
	Turkish $n = 250$ patients $n = 250$ controls Urinary bladder cancer	G allele 47.0% in cases 55.0% in controls	PCR-RFLP	Tiryakioğlu and Tunalı, 2016	[35]
	New Zealand $n = 516$ patients Prostate cancer	G allele 46.3%	SequenomMassArray	Karunasinghe et al., 2019	[36]
	Japanese $n = 104$ patients metastatic prostate cancer	G allele 89.0%	Taqman	Shiota et al., 2020	[46]

Table 4. (Contd.)

	Variations				
	study population and disease	minor allele and frequency	genotyping method	author–year	reference
AKR1C3 rs12529	Caucasians (n = 374) African Americans (AA) (n = 202) and European Americans (EA) (n = 232) Prostate cancer	G allele 46.0% in Caucasians 45.0% in AA 45.0% in EA	SequenomMassArray	Karunasinghe et al., 2018	[47]
	Japanese n = 231 psoriasis	G allele 86.6%	TaqMan	Nojiri et al., 2023	[48]
	Taiwanese n = 112 cases (mother and child pair) n = 211 control (mother and child pair) Childhood leukemia	G allele 17.0% in mother cases 11.0% control mothers 18.0% in child cases 11.0% in control children	TaqMan	Liu et al., 2008	[49]
	Swedish Women (n = 46)	G allele 17.0%	Allelic discrimination assay	Ekström et al., 2022	[50]
	New Zealand (NZ) n = 515 cases n = 572 control US n = 976 cases n = 1034 control Taiwan n = 645 Prostate cancer	G allele 38.0% in NZ controls 84.0–88.0% NZ and Taiwan cases	SequenomMassArray	Karunasinghe et al., 2022	[51]
	Mexican n = 140 men Healthy	G allele 67.0%	TaqMan	Barron-Vivanco et al., 2013	[52]

contrast to our study, Söderhäll et al. [39] reported that haplotype analysis using the Haploview 4.0 software demonstrated that *AKR1C3* for rs3763676, rs12529 and rs7741 were in one LD block.

In conclusion, novel PCR-RFLP assays were developed in the current study for analysis of *AKR1C3* rs3763676 and rs12529 polymorphisms, which can beeasily used in research laboratories not having special equipment. The observed minor allele frequencies of these two variations indicated that *AKR1C3* genes are polymorphic in Turkish population. Therefore, variable treatment responses and adverse effects of naltrexone between Turkish individuals could beexplain with *AKR1C3* rs3763676 and rs12529 polymorphisms. After demonstrating the effects of these polymorphisms on plasma/urine naltrexone levels with further studies, personalized pharmacotherapy could be improved especially in individuals with AUD and OUD.

AUTHOR CONTRIBUTION

Dilek Kaya-Akyüzlü developed the study concept, performed the statistical analysis, and wrote the manuscript. Selin Özkan-Kotiloğlu and Mukaddes Asena Yıldırım performed genetic analysis. All authors read and approved the final manuscript.

FUNDING

No funding was received for conducting this study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study design was approved by Ethics Committee of Human Research at Ankara University (Approval no. 07-536-19 in 2019).

CONFLICT OF INTEREST

The authors of this work declare that they have no conflicts of interest.

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