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The role of mdr1 gene polymorphisms in type 2 Diabetes Mellitus

Tip 2 diyabette mdr1 gen polimorfizmlerinin rolü

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ABSTRACT

Objective: The ABCB1/MDR1 is a 170-kDa transporter protein called P-glycoprotein (P-gp) which has been associated with the transport of cellular lipids and drugs. Recent studies focused on MDR1, and its effects on lipid transport, show that the constitutive expression of P-gp transporter in normal tissues plays an important role in drug disposition and response. In addition, it is known that some polymorphisms on the MDR1 gene alter the expression of the P-gp. This study aims to investigate the effects of the MDR1 C3435T and the C1236T gene polymorphisms on the dyslipidemia of diabetic patients.

Material and methods: The PCR-RFLP method has been used on 77 patients and 75 controls in order to determine the MDR1 genotype.

Results: There are no statistical differences for the MDR1 C3435T and the C1236T genotype ranges among between our inspected groups. However, the C1236T mutant type T allele ratio increases statistically in the patient group (p = 0.026 OR: 1.679 % 95 CI: 1.062 – 2.652). A weak connection has been observed between the MDR1 C3435T C and the C1236T C alleles, according to the linkage disequilibrium analysis.

Conclusion: This study is one of the preceding studies, which examines the relationship between MDR1 polymorphisms and type 2 diabetes. Studies on MDR1 genotypes, and their effects on lipid levels are new in literature, hence our study proves to be unique when it comes to both the C3435T and the C1236T polymorphisms in type 2 diabetes patients; however, further research is still needed for the confirmation of our findings.

Key Words: Diabetes Mellitus, MDR1, C3435T, C1236T, polymorphism, lipid, risk

Conflict of Interest*:* Authors have no conflict of interest

ÖZET

Amaç: ABCB1/MDR1 170 kDa'luk transporter protein olup P-glikoprotein (P-gp) olarak ta bilinir ve hücresel lipid ve ilaç taşınması ile ilişkilidir. Son yıllarda MDR1 ve lipid taşınması üzerine etkisine odaklanan çalışmalarda P-gp taşıyıcı proteininin normal dokulardaki ekspresyonunun ilaç düzenlemesi ve yanıtında önemli bir rol oynadığı gösterilmiştir. Ayrıca, MDR1 genindeki bazı polimorfizmlerin P-gp ekspresyonunu değştirdiği bilinmektedir. Çalışmamız MDR1 C3435T ve C1236T gen polimorfizmlerinin diyabetik hastalarda dislipidemi üzerindeki etkisini incelemeyi amaçlamaktadır.

Yöntemler: Amacımız doğrultusunda MDR1 genotiplerinin tayini için 77 hasta ve 75 kontrolde PCR-RFLP metodu kullanıldı.

Bulgular: Çalışma gruplarında MDR1 C3435T ve C1236T gen polimorfizmleri açısından anlamlı bir bulguya rastlamadık. Ancak C136T mutant T alleli taşıma oranı hasta grubunda anlamlı olarak artmış olarak saptanmıştır (p = 0.026 OR: 1.679 % 95 CI: 1.062 – 2.652). Ayrıca MDR1 C3435T C ve C1236T C allelleri arasında zayıf ilişki gösterilmiştir.

Tartışma: Çalışmamız bu alanda yapılan öncü çalışmalardan biridir. MDR1 genotipleri ve bunların lipid düzeyleri üzerine olan etkileri literatüre yeni girmiş olup bizim çalışmamıza ait bulguların desteklenmesi için daha ileri çalışmalara ihtiyaç bulunmaktadır.

Anahtar Kelimeler: Diabetes Mellitus, MDR1, C3435T, C1236T, polimorfizm, lipid, risk

Çıkar Çatışması: Yazarların çıkar çatışması bulunmamaktadır.

Introduction

The ATP-binding cassette transporter (ABC) family is the largest among the transmembrane protein families and is responsible for transporting a variety of molecules, including lipids, sterols, metabolic products, and drugs. The MDR1/ABCB1 gene, which is a member of the ABC transporter family, encodes the 170 kDa ATP dependent transmembrane protein called P-glycoprotein. As an efflux transporter, P-glycoprotein actively pumps toxic substances, endogenic molecules, xenobiotics, and anti-cancer drugs out of the cell, and causes the development of drug resistance by decreasing the drug concentration in the cell.

In an experimental study, the P-gp has played a role in cholesterol reabsorption from the intestine [1]. An another study showed that the inhibition of P-gp activity in cultured cell lines prevents cholesterol biosynthesis [2]. Also, the genetic polymorphism of the ABCB1 gene has been shown to be associated with plasma lipid levels and correlated with the response to the statin therapy in both men and women [3-6].

The polymorphism (C3435T) located in the exon 26 of the MDR1 gene decreases the P-glycoprotein expression, and results as expression differences among people. Studies on ABCB1 polymorphisms in healthy people indicate that the plasma levels of the apolipoprotein A1 are considerably high in subjects who have at least one 3434T allele [3]. It is also determined that these plasma levels change depending on sex, total cholesterol, and apolipoprotein. The B levels are lower in women who have T-76 or the 1236T allele.

Single nucleotide polymorphisms, such as the A61G, G1199A, A2956G, T3421A, and the C1236T [8] polymorphisms, are determined in the MDR1 gene [7]. Some studies show that the C3435T (exon 26), G2677T (exon 21), and the T129C (exon 1b) polymorphisms are related to the low expression of P-gp in normal tissues [9,10]. Likewise, Marzolini et al. have claimed that the C3435T (rs1045642) polymorphism alters the P-gp expression, therefore, this may be related to substrat-drug pharmacokinetics [11].

It is considered that the C3435T polymorphism is associated with the changes in the MDR1 gene regions, which control expressions such as mRNA processing, promotor, or enhencer regions, affect post-transcriptional modifications and alter the translational control of the mRNA [7,8].

A study performed on hypercholesterolemic individuals among the Brazilian population showed that the C3435T and the G3677T/A polymorphisms are potential indicators of drug regulation and effects [4]. It is observed that total cholesterol and LDL cholesterol decrease significantly in individuals carrying the non-homozygous 1236T allele due to the use of simvastatin [12].

There are no previous studies on MDR1 genotypes and plasma lipid levels in type 2 diabetic patients. Therefore, in this study, the aim has been to investigate the effects of the MDR1 gene, the C3435T, and the C1236T polymorphisms in lipid profiles of Type 2 diabetes patients.

Material and Methods

Sample Selection: Medically diagnosed 77 type 2 diabetes patients (52 female and 25 male), and 75 healthy people (24 female and 51 male) were examined in this study. The diagnoses of the patients were made by the Department of Internal Medicine, Haseki Educational and Research Hospital. The control group members were selected randomly out of healthy individuals who do not have any first-degree relations with a diabetes patient. This study was approved by the Ethical Committee of Istanbul University, The Istanbul Faculty of Medicine (no:2168).

DNA Isolation: Blood specimens were collected in tubes containing EDTA, and DNA samples were isolated from whole blood via the salting out procedure [13].

The Determination of the MDR1 C3435T Gene Polymorphism: The genotyping of the C3435T SNP was performed by a polymerase chain reaction (PCR-RFLP techniques). The 231bp strand of genomic DNA containing the site of the C3435T SNP was amplified by the use of the following oligonucleotides: the sense 5-ACT CTT GTT TTC AGC, and the antisense 5-AGA GAC TTA CAT TAG GCA GTG ACT C-3. The PCR reaction was performed in a final volume of 25 µl with 100 ng genomic DNA, 10 mM tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 250 µM each dNTPs, 13 pmol's each primer, and 1 U Taq DNA polymerase. The PCR conditions were as follows: a denaturation step at 94°C for 5 min, followed by 33 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 30 s, and a final incubation at 72°C for 5 min. A 10-µl aliquot of the PCR product was digested with 5U of MboI endonuclease at 37°C for 4 h. Products were analyzed by 2% agarose gel electrophoresis after ethidium bromide staining. The C3435T SNP was identified through the loss of the MboI restriction site; the C allele bands were 163 and 68 base-pair fragments, and the T allele band was a single uncut 231 base-pair fragment [14].

The Determination of the MDR1 C1236T Gene Polymorphism: The PCR reaction was performed in a final volume of 25 µl with 100 ng genomic DNA, 10 mM tris-HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM each dNTPs, 20 pmol each primer (Sense: 5-TTCACTTCAGTTACCCATC-3, Anti-sense: 5-CATAGAGCCTCTGCATCA-3), and 1 U Taq DNA polymerase. Cycling was performed in a PCR system as follows: initial denaturation at 94°C for 10 min, and 34 cycles of 94°C for 45 sec, 54°C for 45 sec, and a final step at 72°C for 10 min. The PCR product was 314bp. The enzymatic digestion mixture including 1 U Hae III was performed at 37°C overnight. Products were analyzed by 3% agarose gel electrophoresis after ethidium bromide staining. Three possible genotypes

are defined by 3 distinct banding patterns: CC 252 and 62bp fragments, CT 314bp, 252bp, 62bp, 35bp, and TT 217bp, 62bp and 35bp fragments [15].

Statistical Analysis: The Statistical analysis was done with the use of SPSS v16 (SPSS Inc, Chicago, Illinois), and PLINK v.1.07 software [16]. All data was presented as mean ± standard deviation (SD) or as proportions. A double-sided P value of 0.05 was accepted as the threshold for defining the statistical significance. Categorical variables such as genotypes and alleles were compared by using the Chi-Square test. Continuous variables were compared with Student's t-test and one-way ANOVA.

Results

The demographic data of the study group was as demonstrated in Table 1. According to our findings, the triglyceride ($p<0.01$, %95 Cl: 8.46 - 49.63), the LDL-cholesterol ($p<0.01$, %95 Cl: 10.43 – 39.84), the total cholesterol ($p<0.001$, %95 Cl: 18.72 – 49. 28), the VLDL-cholesterol ($p<0.01$, %95 Cl: 2.37 – 10.49),the fasting blood glucose ($p<0.001$, %95 Cl: 57.89 – 121.52) levels, and also the diastolic ($p<0.001$, %95 Cl: 7.52 – 15.41) and systolic ($p<0.001$, %95 Cl:10.87 – 21.67) blood pressures in the patient group were drastically higher than in healthy subjects.

Each of the cases and control groups were confirmed with the chi-square goodness of fit test for the Hardy-Weinberg equilibrium.

There is not any significant difference in between the genotypic distribution of MDR1 C3435T and C1236T. On the other hand, there was found a statistical difference of the T allele on the C1236T polymorphism between patients and control groups. There is an increase of carrying the T allele in diabetes type 2 patients for C1236T ($p = 0.026$ OR: 1.679 % 95 CI: 1.062 – 2.652).

In the linkage disequilibrium analyses, a weak association is determined between the MDR1 C3435T and the C1236T alleles (D': 0.437, r^2: 0.128).

In addition, we compared each of the C3453T and the C1236T polymorphisms with biochemical parameters. There was no correlation between the MDR genotypes and biochemical data in the patient group. However, the BMI of the subjects in the control group who have a TT genotype increased relatively to ones who have CT (p: 0.041, %95 CI: 0.08-3.77) for the C3435T polymorphism (Table 3).

Discussion

Diabetes Mellitus (DM) is a chronic and progressive disorder. It comes into being with the relative absence or resistance of insulin, characterized with hyperglycemia it advances with the malfunction of carbohydrates, proteins, and lipid metabolism. The increasing incidence and complications result from common health problems, which are becoming epidemic [17]. P-gp brings multi drug resistance in cancer cells, and indicates many drugs (e.g. steroids, immunosupressors, antilipidemic etc.) in normal tissues [18]. The fact that polymorphisms in the MDR1 gene play an important role in its function by affecting its activity is considered.

It is claimed that the C3435T polymorphism can interact with other polymorphisms on the MDR1 gene (such as on promoter/enhancer region, T129C or G2677T/A. Moreover, it is considered that C3435T polymorphism affects posttranscriptional modifications or it associates with a number of sequences related to mRNA processing [19].

Nakamura et al conducted a study on the effect of the 3435C>T genotype of the ABCB1 gene on cortisol and aldesteron serum levels in Japanese women who have normal menstrual cycles. It shows that there is an increase in cortisol levels and a decrease in aldosteron levels of individuals who have the 3435CT and the 3435TT genotype relative to those who have the 3435CC genotype [20]. Evidence from studies on P-gp, the product of MDR1, shows it is effective on cellular cholesterol levels in different stages. These are summarized as: (i) Expression can be regulated by cholesterol levels, (ii) It is localized on cholesterol-rich cell membrane, (iii) It has a role in the reformation of proteoliposomes, and (iv) has high basal activity in the absence of cholesterol [21 -24]. Taegtmeyer et al. studied MDR1 polymorphisms in adult heart transplant patients with advanced heart failure, and they found that there is an association between the ABCB1 haplotype and the fasting plasma LDL cholesterol concentration [25]. Fiegenbaum et al. examined the relationship between lipid levels and C3435T/C1236T polymorphisms on hypercholesterolemic patients treated with simvastatin, and they reported that there is a significant decrease in the levels of total cholesterol and LDL cholesterol of individuals carrying non-homozygous 1236T variant allele [12].

In this study, we aimed to investigate the distributions of the C3435T and the C1236T polymorphisms, and their effects on lipid profiles of Type 2 diabetes patients. Although we did not find any statistical importance when we compared the genotype distributions for C1236T, we did determine that there is a significant difference between the allelic distributions of cases and control groups ($p = 0.026$ OR: 1.679 % 95 CI: 1.062 – 2.652). However, there is no correlation between lipid parameters and the C1236T genotype distributions for both patients and control groups.

Although the C3435T polymorphism is a silent mutation, research on this polymorphism shows that the P-glycoprotein expression decreases in some normal tissues of the homozygous TT individuals [7, 26-29]. In contrast, a study on the Japanese population reported an increased expression of P-glycoprotein in patients of T allele [30,31]. A study on the Turkish population reported allele frequencies of C3435T as 40% C and 60% T [21]. Similarly, we found allelic frequencies to be 40.8%

Table 1. Demographic data of the study group

GROUP	**PATIENT (n=77)**	**CONTROL (n=75)**
Sex (Female / Male)	52/25	24/51
Age (Years)	57.62±14.40	54.45±11.37
BMI (kg/m²)	26.87±6.42	25.72±3.39
Smoking (%)	19.5	12
Triglyceride (mg/dl)	171.14±70,63*	142.09±56.89
Total cholesterol (mg/dl)	229.35±52.54**	195.34±42.08
HDL-cholesterol (mg/dl)	38.66±9.31	36.23±9.29
LDL-cholesterol (mg/dl)	156.40±50.77*	131.27±40.22
VLDL-cholesterol (mg/dl)	34.29±14.17*	27.85±10.90
Fasting blood glucose (mg/dl)	171.29±54.96**	81.58±12.57
Total cholesterol/ HDL-cholesterol (mg/dl)	6.30±2.24	5.72±2.018
SBP (mmHg)	135.69±21.42**	119.41±10.16
DBP (mmHg)	84.80±15,90**	73.33±7.08
Creatinine (mg/dl)	0.96±0.27	-

n: number of individual, SBP: systolic blood pressure, DBP: Diastolic blood pressure, BMI: Body-mass index
Differences in groups were compared with Chi-square and Student t test. *$p < 0.01$, **$p < 0.001$.

Table 2. Genetic and allelic distributions of C1236T and C3435T

	Patient (n:77)	Control (n:75)
C1236T		
Genotypes		
CC	22 (%28.6)	32 (%42.7)
TT	22 (%28.6)	13 (%17.3)
CT	33 (%42.8)	30 (%40.0)
Alleles		
C	77 (%50)	94 (%62.6)
T	77 (%50)*	56 (%37.4)
C3435T		
Genotypes		
CC	18 (%23.4)	18 (%24)
TT	27 (%35.1)	20 (%26.7)
CT	32 (%41.6)	37 (%49.3)
Alleles		
C	68 (%44.15)	73 (%40.8)
T	86 (%55.85)	77 (%59.2)

n: number of individuals, Compared with chi-square test.

Table 3. Distribution of MDR1 C3435T genotypes for biochemical data

C3435T	PATIENT (n:77)			CONTROL (n:75)		
	CC n:18	TT n: 27	CT n:32	CC n:18	TT n:20	CT n:37
Triglyceride (mg/dl)	194.72± 85.27	165.11± 55.92	162.97± 72.09	157.33± 72.60	145.85± 60.77	132.65± 44.72
Total Cholesterol (mg/dl)	240.44± 50.58	229.81± 43.87	222.72± 60.27	196.62± 56.96	202.08± 38.88	191.08± 35.57
HDL-Cholesterol (mg/dl)	37.39± 8.28	41.00± 11.38	37.41± 7.69	37.28± 8.30	33.25± 8.81	37.32± 9.86
VLDL-Cholesterol (mg/dl)	39.17± 17.21	33.04± 11.11	32.59± 14.42	31.18± 14.48	29.08± 12.13	25.57± 7.47
LDL-Cholesterol (mg/dl)	163.89± 48.80	155.78± 44.90	152.72± 57.23	128.17± 50.39	139.75± 37.60	128.19± 36.41
Systolic Blood Pressure (mmHg)	127.39± 13.60	138.70± 23.96	137.81± 22.14	118.89± 6.31	119.55± 12.84	119.59± 10.29
Diastolic Blood Pressure (mmHg)	79.16± 6.24	86.66± 20.66	86.40± 14.71	74.16± 7.52	71.25± 6.46	74.05± 7.15
Total Cholesterol / HDL-Cholesterol (mg/dl)	6.84± 2.50	5.95± 1.81	6.29± 2.43	5.42± 1.47	6.47± 2.34	5.47± 2.00
Body Mass Index (Kg/m^2)	25.66± 3.50	28.91± 9.55	25.84± 6.42	25.87± 3.09	26.39± 3.10*	25.00± 3.56

Values are given as X ± SD in the table. One-way ANOVA test was used for in group comparison
* p:0.041, %95Cl:0.08-3.77

Table 4. Distribution of MDR1 C1236T genotypes for biochemical data

C1236T	PATIENT (n:77)			CONTROL (n:75)		
	CC n:22	TT n: 22	CT n:33	CC n:32	TT n:13	CT n:30
Triglyceride (mg/dl)	171.23± 84.38	181.64± 71.0	164.09± 61.10	135.31± 62.39	152.92± 53.56	144.63± 52.92
Total Cholesterol (mg/dl)	235.36± 60.46	219.15± 47.54	232.14± 50.69	198.55± 50.01	192.11± 23.21	193.33± 40.03
HDL-Cholesterol (mg/dl)	39.82± 11.48	40.36± 11.09	36.76± 5.68	38.38± 11.46	34.46± 7.17	34.70± 7.07
VLDL-Cholesterol (mg/dl)	34.27± 16.90	37.15± 14.31	32.38± 12.07	26.77± 12.39	30.34± 10.80	27.93± 9.31
LDL-Cholesterol (mg/dl)	161.27± 61.11	141.64± 42.2	163.00± 47.85	133.41± 46.46	127.31± 24.87	130.70± 39.39
Systolic Blood Pressure (mmHg)	130.14± 18.21	141.14± 21.87	135.76± 22.74	118.91± 5.92	123.08± 15.48	118.37± 10.92
Diastolic Blood Pressure (mmHg)	80.45± 5.54	86.36± 14.57	86.66± 20.56	72.81± 6.59	75.38± 9.67	73.00± 6.37
Total Cholesterol / HDL-Cholesterol (mg/dl)	6.59± 3.13	5.73± 1.76	6.48± 1.79	5.59± 2.19	5.74± 1.12	5.86± 2.16
Body Mass Index (Kg/m^2)	26.15± 4.42	29.13± 9.88	25.86± 4.01	25.21± 3.30	26.89± 4.63	25.75± 5.80

Values are given as X ± SD in the table. One-way ANOVA test was used for in-group comparison

of C and 59.2% of T, and additionally we noticed genotype frequencies of 23.4% CC, 41.6% CT, 35.1% TT for the patients, and 24% CC, 49.3% CT, 26.7% TT for healthy subjects. Jeannesson et.al [3] determined that the apolipoprotein A1 levels of individuals who have at least a 3435 T allele are significantly high, and this shows, as well, that these levels vary dependent on sex. Other studies have claimed that the absorption of digoxin is higher in individuals who have a TT3435 genotype compared to those who have a CC3435 genotype [7, 32-34].

Through their study, Sai Babu M et al have designed a new drug molecule based on synonymous and nonsynonymous SNPs of the MDR1 gene, which is not a substrate to P-gp and acts directly on the ßhydroxy methylglutaryl coenzyme A reductase (HMG-CoA), as a target site for statins. They suggested that this new drug does not efflux out by P-gp, therefore, the individuals with any P-gp genotype will respond equally to this new drug. In addition to this, our new drug molecule is a better inhibitor of the HMG-CoA in comparison to other statins [35].

In our study, we did not observe any statistical differences between the patient group and the healthy subject group regarding the C3434T genotype distributions, or between these distributions and lipid levels. In addition, we did not determine any divergence distributions of genotypes dependent on sex.

Our study is one of the preceding studies, which has examined the relationship between the MDR1 polymorphisms and type 2 diabetes. Studies especially on MDR1 genotypes and their effects on lipid levels are new in literature; hence, our study, in this respect, proves to be unique, upon consideration of both the C3435T and the C1236T polymorphisms in type 2 diabetes patients. Although we did not find any strong correlation between lipid levels in diabetes patients and the C3435T, or the C1236T polymorphisms directly, there may be complex effects of the MDR1 polymorphisms due to the usage of drugs, which are taken regularly in type 2 diabetes patients. However, further research is needed in order to confirm of our findings.

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Conflict of Interest*:* Authors have no conflict of interest

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