RESEARCH ARTICLE

Effect of ATP-binding Cassette Transporter A1 (ABCA1) Gene Polymorphisms on Plasma Lipid Variables and Common Demographic Parameters in Greek Nurses

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Abstract:

Objective:

The present study is on line with our previous studies evaluating the influence of ATP-binding cassette transporter A1 (*ABCA1*) gene polymorphisms on the lipid variables of Greek student-nurses. The current study was undertaken to (1) estimate the influence of variant(s) such as rs2066715 (V825I), R219K, R1587K, I883M of *ABCA1* gene on lipid variables and (2) evaluate the effect of all four *ABCA1* polymorphisms on common demographic parameters.

Methods:

The study population involved 432 unrelated nurses (86 men) who were genotyped for *ABCA1* polymorphisms and correlated according to lipid variables [total cholesterol (TC), triglycerides (TGs), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and apolipoprotein (apo) A] and demographic parameters (age, gender, BMI, waist circumference).

Results:

According to lipid variables concentration there was no difference between genotypes and alleles of V825I, R219K and I883M polymorphisms. The LDL-C concentration was 13% lower in RR compared with RK genotype (100.7 *vs*. 113.9 mg/dl, p=0.013) of R1587K gene polymorphism. In regression analysis the effects of age, gender and only R1587K gene polymorphism on LDL-C concentrations were proved significant. Additionally, LDL-C was increased (by 1.29 mg/dl on average) by every year of increase of age. Moreover, females had lower LDL-C concentrations as compared with males.

Conclusion:

Findings suggested that only R1587K polymorphism of *ABCA1* gene was associated with lipid variables, age, and gender of Greek nurses. These findings may be helpful in assessing the risk factors for premature coronary heart disease and distinct individuals with lower/higher atherosclerotic burden.

Keywords: ATP-binding cassette transporter A1, ABCA1 gene polymorphisms, V825I, R219K, R1587K, I883M.

1. INTRODUCTION

    Genes that influence protein function, which mediate cholesterol metabolism, are the most likely to influence the

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plasma lipid and lipoprotein concentrations. The ATP-binding cassette transporter A1 (ABCA1) protein belongs to ABC proteins family, which is the ingredient of biological membranes and acts as a transfer-vehicle for cellular cholesterol efflux [1, 2]. ABCA1 protein plays a critical role in the inhibition of macrophage foam cell creation and atherosclerosis by mediating the dynamic transport of intracellular cholesterol and phospholipids to apolipoprotein (apo) A1, the major lipoprotein of the high density lipoprotein (HDL) particle. The ABCA1 protein is encoded by *ABCA1* gene, which is located in the chromosome 9. Mutations in the genes encoding ABC proteins are associated with several human disorders including Tangier disease [1, 3]. Also, numerous *ABCA1* gene polymorphisms have been already evaluated, such as R219K, R1587K and I883M (rs2230806, rs2230808 and rs4149313, respectively) [4, 5]. In this study we evaluated (1) the effect of V825I (rs2066715) gene polymorphism (additionally to already reported R219K, R1587K and I883M) on lipid variables total cholesterol (TC), triglyceride (TG), HDL-C, low density lipoprotein cholesterol (LDL-C) and apolipoprotein (apo) A1 and analysed the possibly correlations between them and (2) the demographic parameters such as age, gender, BMI and waist circumference) in Greek student-nurses.

2. MATERIALS AND METHODS

2.1. Subjects

The genotyping of 432 Greek students median (range) age 22 (18-57) years who were attended to the University of Nursing of Technological and Educational Institution was performed. All students had no personal history of coronary heart disease (CHD) and were not taking any drugs. Also, exclusion criteria were diabetes mellitus, thyroid and liver disease, high alcohol consumption, professional athleticism and any chronic disease.

All subjects were attended to the University every day and were staying for 8-10 hours. Individuals were eating at the school canteen, which served typical Mediterranean food. Only one (evening) meal daily was most likely to be different in each student.

The University of Nursing of Technological and Educational Institution ethics committee approved the protocol of this study. All subjects signed an informed consent form.

2.2. Blood Chemistry

Plasma TC, TGs, HDL-C and apo A1 were measured using enzymatic colorimetric methods on Roche Integra Biochemical analyzer with commercially available kits (Roche). The serum LDL-C concentration was calculated using the Friedewald formula only in patients with TGs concentration < 400 mg/dl.

2.3. DNA Analysis and Determination of Blood Lipids

All *ABCA1* gene polymorphisms (R219K, R1587K, I883M and V825I) were detected using polymerase chain reaction (PCR) and restricted fragment length polymorphism analysis (RFLP's). The PCR was performed by using Taq polymerase KAPATaq. For R219K polymorphism the oligonucleotide primers, which were used are AAAGA-CTTCAAGGACCCAGCTT and CCTCACATTCCGAAA-GCATTA [5]. PCR was subjected to 95 $^{\circ}$C for 5 min, thirty cycles of 95 $^{\circ}$C for 30 s, 55 $^{\circ}$C for 30 s and 72 $^{\circ}$C for 30s and final extension to 72 $^{\circ}$C for 7 min, producing a fragment of 309 bp. This fragment was subsequently cleaved by *EcoNI*, creating fragments for R allele 309 bp and for K allele 184 bp and 125bp, which were subjected to electrophoresis on an agarose gel 3% and visualized with ethidium bromide.

For R1587K polymorphism the oligonucleotide primers which were used are AAGATTTATGACAGGACTGGA-CACGA and TGAATGCCCCTGCCAACTTTAC [6]. PCR was subjected to 95 °C for 5 min, thirty cycles of 95 °C for 30 s, 60 °C for 30 s and 72 °C for 30s and final extension to 72 °C for 7 min, producing a fragment of 139 bp. This fragment was subsequently cleaved by *BssSI*, creating fragments for R allele 117 bp and 22 bp and for K allele 139 bp, which were subjected to electrophoresis on an agarose gel 3% and visualized with ethidium bromide.

For V825I polymorphism the oligonucleotide primers which were used are TTCTGCACCTTATGATTGATCC and AGCACAAAGAAAGGACATCAGC [5]. PCR was subjected to 95 °C for 5 min, thirty cycles of 95 °C for 30 s, 56 °C for 30 s and 72 °C for 30s and final extension to 72 °C for 7 min, producing a fragment of 392 bp. This fragment was subsequently cleaved by *BsaI*, creating fragments for V allele 265 bp and 127bp and for I allele 392 bp, which were subjected to electrophoresis on an agarose gel 3% and visualized with ethidium bromide.

2.4. Statistical Analysis

All data except for TC and LDL-C deviated from normality (Kolmogorov-Smirnov test). Non-normally distributed continuous variables are shown as median and interquartile range (25th, 75th percentile), while TC concentrations are presented as mean±SD. All categorical variables are presented as relative (percentage) frequencies.

The variables that deviated from normality (encoding TG, HDL-C and apo A1) underwent a logarithmic transformation. After logarithmic transformation the pre-mentioned variables, except apo A1, followed normal distribution. Thus regression analysis was applied. Non-parametric procedures were used for the analysis of data regarding apo A1.

Firstly, Univariate analysis was conducted in order to examine the effect of each parameter age, gender, BMI (body mass index), age, waist circumference, genotypes (R219, R1587K, I883M and V825I) as well as their alleles on TC, TG, HDL-C, LDL-C and apo A1 concentrations. One Way Anova with Bonferroni correction for multiple comparisons or Kruskall Wallis test where applied where appropriate in order to compare the continuous variables among the 3 genotype groups. The Mann-Whitney U test or the Student's T-Test, where appropriate, was used to compare the continuous variables between the 2 groups of carriers.

Multivariable Linear regression models were constructed where TC, LDL-C and the logarithmic transformed HDL-C and TG were used as dependent variables. The variables, which had significant effects in univariable analysis were used as independent factors for the construction of the models.

All tests were 2-sided at a significance level of $p < 0.05$. Data were analyzed using SPSS™ (Version 21, Chicago IL, USA).

3. RESULTS

3.1. Clinical and Laboratory Parameters

Demographic data, clinical characteristics and lipid variables of the study cohort are shown in Table **1**. Genotype frequencies are shown in Table **2**.

Table 1. Demographic data and lipid variable of the study cohort.

Variable	Mean±SD/Median(Range)
Age (years)	22 (18-57)
Height (cm)	166 (150-207)
Weight (Kg)	62 (40-132)
BMI (cm/Kg2)	22 (16-44)
Waist Circumference (cm)	88 (48-142)
Gender	**N(%)**
Male	86(20)
Female	346(80)
Total Cholesterol (mg/dl)	202±66
LDL-C (mg/dl)	107±46
HDL-C (mg/dl)	65 (16-200)
TGs (mg/dl)	98 (12-523)
Apo A1(mg/l)	1.5 (0.4-3)

BMI = body mass index, TGs = triglycerides, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, Apo = apolipoprotein

Table 2. Genotype and allele frequencies in the Whole Cohort.

	R219K		R1587K		I883M		V825I
	N(%)		**N(%)**		**N(%)**		**N(%)**
Genotypes							
RR	218(50.5)	RR	207(47.9)	II	298(69.0)	II	4(0.9)
RK	185(42.8)	RK	183(42.4)	IM	126(29.2)	IV	56(13.0)
KK	29(6.7)	KK	42(9.7)	MM	8(1.9)	VV	372(86.1)

(Table 4) contd.....

R219K		R1587K		I883M		V825I	
	N(%)		N(%)		N(%)		N(%)
Alleles							
R	71.9	R	69.1	I	83.6	I	7.4
K	28.1	K	30.9	M	16.4	V	92.6

3.2. ABCA1 Gene Polymorphisms

According to lipid variables there was no difference in the genotypic and allelic frequencies of V825I, R219K and I883M polymorphisms, (Table **3**).

Table 3. Lipid concentrations (TC, TG, HDL and LDL) in mg/dl and ApoA1 in mg/l among the different genotypes.

		TC Mean±SD	TG Median(IQR)	HDL-C Median(IQR)	LDL-C (Mean±SD)	Apo A1 Median(IQR)
R219K	RR	201±64.8	99 (12-523)	66 (25-200)	106±46	1.4(0.5-3.1)
	RK	204±68	99(18-481)	64(16-191)	106±46	1.5(0.4-3.0)
	KK	207±65	97(37-306)	61(41-111)	116±45	1.5(0.8-2.8)
	p	0.868	0.496	0.718	0.509	0.278
R1587K	RR	197±68	95(12-481)	65(24-200)	101±45	1.5(0.4-3.0)
	RK	209±61	104(12-441)	66(16-156)	114±43	1.5(0.4-3.0)
	KK	200±78	87(25-523)	60(30-155)	107±54	1.4(0.7-2.9)
	p	0.202	0.263	0.885	0.017	0.703
I883M	II	201±68	100(12-523)	65(16-200)	106±48	1.4(0.4-3.0)
	IM	206±63	93(30-472)	66(33-155)	110±42	1.5(0.6-3.0)
	MM	212±63	170(42-321)	69(41-122)	103±38	1.5(0.9-2.5)
	p	0.696	0.894	0.720	0.604	0.347
V825I	II	198±86	170(42-321)	65(41-122)	89±40	1.4(0.9-2.3)
	IV	200±55	93(33-328)	67(33-128)	108±40	1.4(0.6-2.6)
	VV	203±67	99(12-523)	65(16-200)	107±47	1.5(0.4-3.0)
		0.951	0.335	0.976	0.737	0.836

P = p value, TC = total cholesterol, TG = triglycerides, HDL-C = high density lipoprotein cholesterol, LDL-C = low density lipoprotein cholesterol, Apo = apolipoprotein

3.3. R1587K Genotypes

As it is shown in the Table **3** there were significant alterations in LDL-C concentrations among the three R1587K genotypes (p=0.017). Specifically, the LDL-C concentration was 13% lower in RR compared with RK genotype (100.7 *vs.* 113.9 mg/dl, p=0.013).

3.4. Regression Analysis and Four ABCA1 Gene Polymorphisms

The effects of age, gender and R1587K polymorphism on LDL-C concentrations were proved significant. Specifically, LDL-C was higher (by 1.29 mg/ml on average) by every year of increase of age (CI95% 0.451-2.130, p=0.003). Additionally, females had lower LDL-C concentrations (B coefficient -16.476, CI95% -27.508-(-5.444), p=0.004) as compared with males. Also, subjects with RK genotype had significantly higher LDL-C concentration compared with RR genotype (B coefficient 7.107, CI95% 0.254-13.960, p=0.042).

4. DISCUSSION

We examined the probable impact of the *ABCA1* polymorphisms as a genetic influence on lipid variables and demographic parameters in Greek young nurses. Findings suggested that only R1587K polymorphism out of four studied *ABCA1* gene polymorphisms was associated with lipid variables, age and gender of studied cohort.

Particularly, the R219K (rs2230806) gene polymorphism, which has been related to CHD risk [7], ischemic stroke [8], TGs and HDL-C concentrations [9] was evaluated by Liu *et al.* [10] in meta-analysis that included 13 studies with 11,678 individuals. Significant association between R219K gene polymorphism and increased CHD risk was found in total population analyses in all 4 genetic comparison models. In analyses based on ethnicity, the association was still significant in Asians, but not in Caucasians. According to our previous [11] study from the young Greek women-nurses (living and working in the similar conditions) we did not find any association of R219K with demographic (smoking, waist circumference, BMI) or lipid parameters. Similarly in this study we have not observed any association of R219K

gene polymorphism and demographic and lipid parameters.

According to I883M (rs4149313) gene polymorphism, Jiang *et al.* [12] investigated the relationship of I883M polymorphism in a meta-analysis of 14,040 cases and 28,607 controls from 31 published case-control studies, with CHD. They found no significant results for I883M polymorphism of ABCA1 in all genetic models. Porchay-Baldérelli *et al.* [13] reported association of *M* allele of *I883M* with higher HDL-C concentrations. Slatter *et al.* [14] found that the I883M SNP was over-represented in high-HDL individuals. Marvaki *et al.* [15] in their study reported that Logistic Regression revealed that subjects with RK genotype of R1587K polymorphism had 69% higher risk on having LDL-C above normal limits (>160 mg/dl) as compared with those with RR genotype but no differences were found between high and low levels of HDL-C.

According to V825I (rs2066715) gene polymorphism, Yin *et al.* has not found association with BMI [16] although they found association with TC and apo A1 [17] in normal weight subjects and LDL-C and apo B in overweight. Cao *et al.* [18] reported that the V825I polymorphism in the *ABCA1* gene is associated with HDL-C and apo A1 concentrations in males Han, and serum TC concentrations in the Bai Ku Yao populations. They suggested that difference in the association of V825I polymorphism and serum lipid levels between the two ethnic groups might partly result from different *ABCA1* gene-environmental interactions. Slatter *et al.* [14] found that the V825I SNP was over-represented in high-HDL individuals. In our study we have not found any association of V821I and demographic and lipid parameters.

According to R1587K (rs2230808) gene polymorphism Frikke-Schmidt *et al.* [4], Clee *et al.* [9] and Slatter *et al.* [14] found that this polymorphism is overexpresed in individuals with low HDL-C concentrations. Tregouet *et al.* [19] stated that R1587K has impact on the apo A1 concentration. Pasdar *et al.* [20] found the association of R1587K polymorphism and apo A concentration. However, Tupitsina *et al.* [6] observed that in patients with CHD the R1587K polymorphism did not affect lipids concentrations. Wang *et al.* [21] did not found any association of R1587K with lipid variable concentrations in patients with type 2 diabetes mellitus. In our study, in regression analysis, was found that the R1587K polymorphism had the effect of LDL-C concentration, age and BMI. Specifically, LDL-C was higher (by 1.29 mg/ml on average) by every year of increase of age and subjects with *RK* genotype had significantly higher LDL-C concentration compared with *RR* genotype.

The possible mechanisms of genetic variations and demographic and lipid parameters are still not well defined. This occurs because every study is evaluated various cohorts (race, age, smoking status, dietary traditions, BMI, healthy or with all kind of diseases and others). Therefore, in our study the environmental influence was somewhat moderated. The advantage of our study was that the study cohort was practically homogenous, since the nurses most of the time were following the same day-to-day program and were eating in the same school canteen. Thus the influence of diet or physical activities was unlikely, which may partially explain the lack of association of R219K, R1587K I883M and V825I of *ABCA1* gene polymorphisms on HDL-C concentrations.

Findings suggested that only R1587K polymorphism out of 4 studied *ABCA1* gene polymorphisms was associated with lipid variable, age and gender of studied cohort.

Subjects with RK genotype had higher LDL-C concentration compared to RR genotype. These findings may be helpful in assessing the risk factors for premature CHD and distinct individuals with lower/higher atherosclerotic burden. Nevertheless, this is only a clinical observation. Hopefully in near future we will be able to identify high risk subjects through genetic testing also. However, the genetic assessment has disadvantages yet; is still expensive, needs time for evaluation and the replication is problematic. Nevertheless, these restrictions may become less significant as technology advances.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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REFERENCES

[1]   Kolovou GD, Mikhailidis DP, Anagnostopoulou KK, Daskalopoulou SS, Cokkinos DV. Tangier disease four decades of research: a reflection of the importance of HDL. Curr Med Chem 2006; 13(7): 771-82.

[http://dx.doi.org/10.2174/092986706776055580] [PMID: 16611066]

[2] Dean M, Hamon Y, Chimini G. The human ATP-binding cassette (ABC) transporter superfamily. J Lipid Res 2001; 42(7): 1007-17.
[PMID: 11441126]

[3] Kolovou GD, Wade DP, Sengupta R, Cokkinos DV. Tangier disease with unusual clinical manifestations. Clin Genet 2003; 63(4): 323-4.
[http://dx.doi.org/10.1034/j.1399-0004.2003.00056.x] [PMID: 12702168]

[4] Frikke-Schmidt R, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Genetic variation in ABC transporter A1 contributes to HDL cholesterol in the general population. J Clin Invest 2004; 114(9): 1343-53.
[http://dx.doi.org/10.1172/JCI200420361] [PMID: 15520867]

[5] Saleheen D, Khanum S, Haider SR, et al. A novel haplotype in ABCA1 gene effects plasma HDL-C concentration. Int J Cardiol 2007; 115(1): 7-13.
[http://dx.doi.org/10.1016/j.ijcard.2005.12.020] [PMID: 16806540]

[6] Tupitsina TV, Slominsky PA, Yefereva YM, et al. Linkage analysis by a transmission/ Disequilibrium of Russian sibling pairs with coronary artery disease. Balkan J Med Genet 2007; 10: 43-54.
[http://dx.doi.org/10.2478/v10034-008-0006-0]

[7] Benton JL, Ding J, Tsai MY, et al. Associations between two common polymorphisms in the ABCA1 gene and subclinical atherosclerosis: Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis 2007; 193(2): 352-60.
[http://dx.doi.org/10.1016/j.atherosclerosis.2006.06.024] [PMID: 16879828]

[8] Hou R, Zhu X, Pan X, Guo R, Ma T, Xu X. ATP-binding cassette transporter A1 R219K polymorphism and ischemic stroke risk in the Chinese population: a meta-analysis. J Neurol Sci 2014; 336(1-2): 57-61.
[http://dx.doi.org/10.1016/j.jns.2013.10.006] [PMID: 24157307]

[9] Clee SM, Zwinderman AH, Engert JC, et al. Common genetic variation in ABCA1 is associated with altered lipoprotein levels and a modified risk for coronary artery disease. Circulation 2001; 103(9): 1198-205.
[http://dx.doi.org/10.1161/01.CIR.103.9.1198] [PMID: 11238261]

[10] Liu N, Hou M, Ren W, Cao J, Wu H, Zhou W. The R219K polymorphism on ATP-binding cassette transporter A1 gene is associated with coronary heart disease risk in Asia population: evidence from a meta-analysis. Cell Biochem Biophys 2015; 71(1): 49-55.
[http://dx.doi.org/10.1007/s12013-014-0161-8] [PMID: 25104170]

[11] Kolovou V, Marvaki A, Karakosta A, et al. Association of gender, ABCA1 gene polymorphisms and lipid profile in Greek young nurses. Lipids Health Dis 2012; 11: 62.
[http://dx.doi.org/10.1186/1476-511X-11-62] [PMID: 22668585]

[12] Jiang Z, Zhou R, Xu C, Feng G, Zhou Y. Genetic variation of the ATP-binding cassette transporter A1 and susceptibility to coronary heart disease. Mol Genet Metab 2011; 103(1): 81-8.
[http://dx.doi.org/10.1016/j.ymgme.2011.01.005] [PMID: 21300560]

[13] Porchay-Baldérelli I, Péan F, Emery N, et al. Relationships between common polymorphisms of adenosine triphosphate-binding cassette transporter A1 and high-density lipoprotein cholesterol and coronary heart disease in a population with type 2 diabetes mellitus. Metabolism 2009; 58(1): 74-9.
[http://dx.doi.org/10.1016/j.metabol.2008.08.009] [PMID: 19059534]

[14] Slatter TL, Jones GT, Williams MJ, van Rij AM, McCormick SP. Novel rare mutations and promoter haplotypes in ABCA1 contribute to low-HDL-C levels. Clin Genet 2008; 73(2): 179-84.
[http://dx.doi.org/10.1111/j.1399-0004.2007.00940.x] [PMID: 18199144]

[15] Marvaki A, Kolovou V, Katsiki N, et al. Impact of 3 Common ABCA1 Gene Polymorphisms on Optimal *vs*. Non-Optimal Lipid Profile in Greek Young Nurses. Open Cardiovasc Med J 2014; 8: 83-7.
[http://dx.doi.org/10.2174/1874192401408010083] [PMID: 25279016]

[16] Yin RX, Wu DF, Aung LH, et al. Several lipid-related gene polymorphisms interact with overweight/obesity to modulate blood pressure levels. Int J Mol Sci 2012; 13(9): 12062-81.
[http://dx.doi.org/10.3390/ijms130912062] [PMID: 23109900]

[17] Yin RX, Wu DF, Miao L, et al. Several genetic polymorphisms interact with overweight/obesity to influence serum lipid levels. Cardiovasc Diabetol 2012; 11: 123.
[http://dx.doi.org/10.1186/1475-2840-11-123] [PMID: 23039238]

[18] Cao XL, Yin RX, Wu DF, et al. Genetic variant of V825I in the ATP-binding cassette transporter A1 gene and serum lipid levels in the Guangxi Bai Ku Yao and Han populations. Lipids Health Dis 2011; 10: 14.
[http://dx.doi.org/10.1186/1476-511X-10-14] [PMID: 21247457]

[19] Tregouet DA, Ricard S, Nicaud V, et al. In-depth haplotype analysis of ABCA1 gene polymorphisms in relation to plasma ApoA1 levels and myocardial infarction. Arterioscler Thromb Vasc Biol 2004; 24(4): 775-81.
[http://dx.doi.org/10.1161/01.ATV.0000121573.29550.1a] [PMID: 14962947]

[20] Pasdar A, Yadegarfar G, Cumming A, Whalley L, St Clair D, MacLeod MJ. The effect of ABCA1 gene polymorphisms on ischaemic stroke risk and relationship with lipid profile. BMC Med Genet 2007; 8: 30.
[http://dx.doi.org/10.1186/1471-2350-8-30] [PMID: 17553166]

[21]   Wang J, Bao YQ, Hu C, *et al.* Effects of ABCA1 variants on rosiglitazone monotherapy in newly diagnosed type 2 diabetes patients. Acta Pharmacol Sin 2008; 29(2): 252-8.
[http://dx.doi.org/10.1111/j.1745-7254.2008.00744.x] [PMID: 18215356]