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

Vana Kolovou¹, Apostolia Marvaki¹, Maria Boutsikou², Georgios Vasilopoulos³, Dimitrios Degiannis¹, Christina Marvaki³ and Genovefa Kolovou²*

¹Molecular Immunology Laboratory, Onassis Cardiac Surgery Center, Athens, Greece
²Cardiology Department, Onassis Cardiac Surgery Center, Athens, Greece
³Department of Nursing, A' Technological Educational Institute of Athens, Greece

Received: August 22, 2015 Revised: September 20, 2015 Accepted: October 22, 2015

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
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 \textit{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 \textit{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 \textit{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 AAAGACTCTTAAGGACCCACCTT and CCTCACATTCCGAAA-GCATTA [5]. PCR was subjected to 95 °C for 5 min, thirty cycles of 95 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s and final extension to 72 °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 184 bp and 125bp, 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                        |                       |
| 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(%)    |
| 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)|
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.

| Alleles | 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)      |
| p       | 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 overexpressed 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 A1 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.

ACKNOWLEDGEMENTS

Declared none.

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.
Kolovou et al.

Yin RX, Wu DF, Miao L, Porchay-Baldérelli I, Péan F, Emery N, 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. Cell Biochem Biophys 2015; 71(1): 49-55.

Frikke-Schmidt R, Nordestgaard BG, Jensen GB, Tybjaerg-Hansen A. Genetic variation in ATP-binding cassette transporter A1 contributes to HDL cholesterol in the general population. J Clin Invest 2004; 114(9): 1343-53.

Benton JL, Ding J, Tsai MY, Kolovou V, Marvaki A, Karakosta A, 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.

Saleheen D, Khanum S, Haider SR, et al. The effect of ABCA1 gene polymorphisms on ischaemic stroke risk and relationship with lipid profile. BMC Med Genet 2007; 8: 30.

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.

[PMID: 16611066]

[PMID: 11441126]

[PMID: 12702168]

[PMID: 15520867]

[PMID: 16806540]

[PMID: 10.1161/j.jncard.2005.12.020]

[PMID: 10.1186/1476-511X-10-14]

[PMID: 10.2174/09298670676055580]

[PMID: 11238261]

[PMID: 25104170]

[PMID: 21300560]

[PMID: 11253070]

[PMID: 10.1161/01.CIR.1039.1198]

[PMID: 25157307]

[PMID: 10.1007/s12013-014-0161-8]

[PMID: 10.1007/s10118-008-0868-3]

[PMID: 11238261]

[PMID: 1874192401408010083]

[PMID: 10.1111/j.1399-0004.2007.00940.x]

[PMID: 10.1186/1476-511X-11-62]

[PMID: 10.1016/j.mge.2006.06.024]

[PMID: 21300560]

[PMID: 21247457]

[PMID: 19059534]

[PMID: 21300560]

[PMID: 25279016]

[PMID: 10.1034/j.1399-0004.2003.00056.x]

[PMID: 10.1186/1399-0004.2007.00056.x]

[PMID: 10.1016/j.metabol.2008.08.009]

[PMID: 15879828]

[PMID: 21247457]

[PMID: 12702168]

[PMID: 23109900]

[PMID: 23039238]

[PMID: 21251970]

[PMID: 19059534]

[PMID: 18199144]

[PMID: 21300560]

[PMID: 25279016]

[PMID: 16879828]
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]