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ORIGINAL ARTICLE

APOL1 polymorphisms are not influencing acute coronary syndrome risk in Czech males

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Abstract

Background: The highest mortality and morbidity worldwide is associated with atherosclerotic cardiovascular disease (ASCVD), which has in background both environmental and genetic risk factors. Apolipoprotein L1 (*APOL1*) variability influences the risk of ASCVD in Africans, but little is known about the *APOL1* and ASCVD in other ethnic groups.

Methods: To investigate the role of *APOL1* and ASCVD, we have genotyped four (rs13056427, rs136147, rs10854688 and rs9610473) *APOL1* polymorphisms in a group of 1541 male patients with acute coronary syndrome (ACS) and 1338 male controls.

Results: Individual *APOL1* polymorphisms were not associated with traditional CVD risk factors such as smoking, hypertension or diabetes prevalence, with BMI values or plasma lipid levels. Neither individual polymorphisms nor haplotypes were associated with an increased risk of ACS nor did they predict total or cardiovascular mortality over the 10.2 ± 3.9 years of follow-up.

Conclusions: We conclude that *APOL1* genetic variability has no major effect on risk of ACS in Caucasians.

KEYWORDS
apolipoprotein L1, cardiovascular disease, Caucasians, mortality, polymorphism

1 | BACKGROUND

Atherosclerotic cardiovascular disease (ASCVD) is the leading cause of morbidity and mortality worldwide and is associated with a substantial health, emotional and financial burden on society.

In addition to the well-known environmental risk factors resulting from the unhealthy lifestyles, such as smoking, obesity, dyslipidaemia, hypertension and type 2 diabetes mellitus, genetic factors and gene–environment interactions play an important role in the development of ASCVD. Although there are some exceptions (Vrablik et al., 2020), ASCVD is polygenic and a long list of common variants have been found to be associated with ASCVD, mostly identified by the genome-wide analyses approach (Nikpay et al., 2015; Vrablik et al., 2021).

It is interesting to note that there are existing significant CVD-associated variants that are not associated with traditional ASCVD risk factors (such as the variants at the 9p21/ANRIL and 2q36.3 loci). The effect of some others on ASCVD is far greater than expected from the ODDs on traditional risk factors (this is particularly true for the variants within the *FTO* gene) (Hubacek et al., 2016; Palomaki et al., 2010).

One of such candidate for ASCVD risk prediction that has recently attracted interest is the gene for apolipoprotein L1 (*APOL1*; OMIM accs. No. 603743; gene ID: 8542; HGNC ID:618) (Estrella & Parekh, 2017). A primary role of APOL1 is the immune clearance of *Trypanosoma brucei*, resulting in protection against the African sleeping sickness (Pays & Vanhollebeke, 2009; Vanhamme et al., 2003). Two variants [G1 – rs73885319 (Ser342 → Gly) and rs60910145 (Ile384 → Met), which are in complete linkage disequilibrium; and G2 (rs71785313), insertion/deletion of two amino acids Asp388Tyr389] are associated with an approximately twofold increased risk of myocardial infarction (Friedman, 2017; Young et al., 2017), but they occur exclusively in black Africans.

Nevertheless, the entire variability of the *APOL1* gene has been studied in detail by Peng et al. (2017). and hundreds of SNPs (about 100 with a minor allele frequency of at least 1%) have been detected. Based on this screening, we selected the *APOL1* variants rs13056427, rs136147, rs10854688 and rs9610473, which are common in Caucasians.

Six different APOL genes/proteins are known (APOL1-APOL6) (Page et al., 2001). The exact role of each APOL family protein is unclear, but all act as ion channels (Thomson & Finkelstein, 2015; Vanhollebeke & Pays, 2006). The APOL1 is mainly produced in the liver, but low levels of *APOL1* expression have been detected in almost all human tissues (Duchateau et al., 2001). APOL1 is the only member of the family to be detected in plasma, where it is associated with the HDL-3 particle subfraction (Duchateau et al., 2000). The concentration is very low (only about 3–15 μg/mL) compared to the major plasma apolipoproteins such as apolipoprotein A1 (1–2 mg/mL) or apolipoprotein B (0.5–1.5 mg/mL) (Duchateau et al., 2000).

Based on abovementioned facts, we hypothesise that common variants within the *APOL1* gene could contribute to the development of ASCVD in the Czech/Caucasian population.

2 | MATERIALS AND METHODS

2.1 | Ethical compliance

The study protocol was approved by the IKEM's Ethics Committee and complies with the 1964 Declaration of Helsinki and its subsequent amendments. All participants signed an informed consent form.

2.2 | Subjects

Adult males under the age of 65 who had a first coronary attack were included. Patients with acute coronary syndrome (ACS) were enrolled between April 2006 and April 2017 at the Department of Cardiology of Institute for Clinical and Experimental Medicine as previously described in detail (Hubacek et al., 2010; Staněk et al., 2017). The DNA bank of male subjects examined as the Czech branch (Cífková et al., 2010) of the post-MONICA study (Tunstall-Pedoe, 2003) was used as a control. A total of 1541 patients and 1338 controls were included in the study.

For patients, mortality data were collected until 12/2020. Mortality information was obtained from the Institute of Health, Informatics and Statistics (Ministry of Health, Czech Republic), where all death certificates are analysed (Hubacek et al., 2015).

All participating subjects were self-identified Caucasians.

2.3 | Genetic analysis

DNA samples were isolated from whole uncoagulated EDTA blood according to Miller et al. (1988) with a slight modifications.

Four SNPs within the *APOL1* gene (GenBank reference sequence: NG_023228.1) were analysed. For genotyping of rs13056427 (NC_000022.11:36253755:C:T) and rs9610473 (NC_000022.11:36266330:T:C) polymorphisms, PCR tests were performed on the MJ Research DYAD Disciple PCR device and PCR-RFLP was applied. PCR chemicals were provided by Fermentas International (Burlington, Ontario, Canada). rs136147 (NC_000022.11:36256842:G:T; Assay ID: C_26581261_10) and rs10854688 (NC_000022.11:36257807:C:T; Assay ID: C_2958212_10) were analysed using pre-designed TaqMan assays (Applied Biosystems) and the 7300 Real-Time PCR instrument. For further details, see Hubacek et al. (2022).

2.4 | Statistical analysis

Odds ratios (95% CI) and corresponding chi-squared tests were calculated using the freely available statistical software package 'Social Science Statistics' (https://www.socscistatistics.com; accessed August 2023). The procedures are fully compatible with SPSS software. ANOVA (for potential analysis of plasma lipids, blood pressure and BMI

values) or chi-square (diabetes and smoking status) was used to calculate potential associations between individual SNPs and traditional ASCVD risk factors. As there was no association between individual SNPs and traditional risk factors for ASCVD, no further adjustment was made.

Multivariate regression analysis was used to analyse the mortality between patients. The following factors have been included into the analysis: age, smoking status, BMI, diabetes, hypertension, diagnosis, LDL cholesterol, number of affected arteries and all four *APOL1* polymorphisms (rs13056427, rs9610473, rs136147 and rs10854688).

3 | RESULTS

General descriptions of the patients included are summarised in Table 1. As expected, the prevalence of most ASCVD risk factors was increased in the patient group.

The minimum call rates for individual genotypes were 98.5% in patients and 99% in controls. In controls, the distributions of all SNPs were within the Hardy–Weinberg equilibrium (p values between 0.16 and 0.77) and similar to those in other Caucasian populations.

In the general population sample, we found no significant associations (not shown in detail) between SNPs and traditional ASCVD risk factors such as plasma lipids (total, LDL- and HDL- cholesterol, triglycerides), BMI (both analysed as continuous traits), smoking (self-reported ever smoking), hypertension (SBP/DBP above 140/90 mmHg or self-reported treatment) and diabetes (self-reported presence of the disease).

We have not detected significant differences in the frequencies of *APOL1* genotypes or alleles between patients and controls (Table 2), except for rs13056427 where heterozygotes were slightly more overrepresented among patients. However, for the minor homozygotes there was an opposite trend, and as the comparison CC versus +T allele shows no significant differences (OR 1.13; 95% CI 0.97–1.32; $p = 0.11$), we assume that this represents a false-positive finding.

Among the 15 haplotypes with a population frequency above 1%, only TC/GG/CC/TT (rs13056427/rs136147/rs10854688/rs9610473) was significantly associated with an increased risk of ACVD with OR [(95%CI) 1.42 (1.05–1.93); $p < 0.05$] (Table 3). This difference disappeared after Bonferroni correction for multiple testing.

Regarding mortality (there were 274 deaths among ACS subjects within 10.2 ± 3.9 years of follow-up), neither total nor cardiovascular mortality was influenced by *APOL1* variability (see Table 4 and Supplementary Tables for total mortality data). Multivariate regression analysis did not significantly change the results, and *APOL1* polymorphisms were not associated with mortality. Out of the other parameters, only increased age, presence of diabetes and number of affected arteries were associated with increased risk of mortality (all $p < 0.01$).

TABLE 1 Basic characteristics of the groups analysed.

	Patients	Controls	*p*
N	1528	1338	
Age (years)	55.3 ± 8.42	48.9 ± 10.8	0.005
Total cholesterol (mmol/L)	5.31 ± 1.17	5.70 ± 1.01	0.01
Triglycerides (mmol/L)	1.89 ± 1.35	1.95 ± 1.26	n.s
LDL cholesterol (mmol/L)	3.59 ± 1.00	3.45 ± 0.99	0.05
Hypertension (%)	49	41	0.0001
Diabetes (%)	19	9	0.00001
Smoking (%)	80	58	0.00001
Obesity (%)	34	30	0.05
Dyslipidaemic treatment (%)	17	n.a.	
Hypertension treatment (%)	36	n.a.	
Anticoagulant treatment (%)	17	n.a.	
Number of stents	1.2 ± 0.7	n.a.	
Q/non-Q (%)	50/50	n.a.	
Unstable angina pectoris (%)	0.4	n.a.	
Affected arteries (*N*)	2.9 ± 0.9	n.a.	

Note: Continuous variables are presented as mean ± SD.

4 | DISCUSSION

Our study is the first one, which focused on the potential associations between common *APOL1* polymorphisms and the risk of cardiovascular disease in a Central European Caucasian population. In general, we found no association between the *APOL1* gene variability and the risk of disease prevalence or mortality. Detected small differences in haplotype frequencies need to be unconditionally confirmed by subsequent studies in independent populations, as they were not significant after correction for multiple comparisons and could represent a false-positive finding.

The primary role of APOL1 is in immune regulation of protection against sleeping sickness. Two functional variants, originally named as G1 and G2 (Cooper et al., 2016), have spread in the black Africans as a consequence of the selective pressure and have not been detected in other ethnic groups – for example, we have not detected any carriers of the minor G1 or G2 allele in a group of 564 unrelated Caucasian subjects (Hubacek et al., 2022). In the case that G1 and/or G2 alleles are present, APOL1 inserts into the parasitic membrane and creates ion channel with subsequent ion influx leading to the cell death.

TABLE 2 Genotype frequencies of *APOL1* polymorphisms in patients and healthy controls.

	Controls		Patients		OR		
	N	%	*N*	%	Crude	*p*	*p*[a]
rs13056427							
CC	853	64.1	931	61.3	1.00		0.24[&]
CT	416	31.3	536	35.3	1.18 (1.01–1.38)	0.04	0.04[#]
TT	61	4.6	53	3.5	0.79 (0.55–1.16)	0.24	0.14[§]
rs136147							
GG	414	31.2	480	31.7	1.00		0.73[&]
GT	640	48.2	729	48.2	0.97 (0.82–1.15)	0.84	0.89[#]
TT	275	20.7	303	20.0	0.95 (0.77–1.17)	0.63	0.67[§]
rs10854688							
TT	581	43.8	639	42.1	1.00		0.50[&]
TC	576	43.4	650	42.8	1.03 (0.88–1.21)	0.75	0.20[#]
CC	169	12.7	228	15.0	1.23 (0.98–1.54)	0.08	0.08[§]
rs9610473							
TT	931	70.3	1091	71.8	1.00		0.36[&]
TC	361	27.2	390	25.7	0.92 (0.78–1.09)	0.34	0.64[#]
CC	33	2.5	38	2.5	0.98 (0.61–1.58)	0.94	0.76[§]

Note: *APOL1* gene (GenBank reference sequence: NG_023228.1).

[a]*p* is calculated for dominant[&], co-dominant[#] and recessive[§] models of comparisons.

TABLE 3 Distribution of individual *APOL1* SNP combinations in controls (post-MONICA population) and ACS patients (in the following order: rs13056427/rs136147/rs10854688/rs9610473).

	post-MONICA (1322)		ACS (1496)			
Haplotypes	**N**	**%**	**N**	**%**	**OR (95% CI)**	***p***
CC/TT/TT/TT	230	17.4	269	18.0	1.04 (0.86–1.26)	0.68
TC/GT/CT/TT	198	15.0	252	16,8	1.15 (0.94–1.41)	0.18
CC/GT/TT/TC	168	12.7	183	12.2	0.96 (0.77–1.20)	0.70
CC/GT/CT/TT	163	12.3	172	11.5	0.92 (0.74–1.10)	0.50
CC/GT/TT/TT	72	5.5	66	4.4	0.80 (0.57–1.13)	0.20
TC/GG/CC/TT	**72**	**5.5**	**113**	**7.6**	**1.42 (1.05–1.93)**	**0.02**
TC/GG/CT/TC	67	5.1	87	5.8	1.16 (0.83–1.61)	0.38
CC/GG/CT/TC	51	3.9	54	3.6	0.93 (0.63–1.38)	0.78
TT/GG/CC/TT	48	3.6	45	3.0	0.82 (0.54–1.24)	0.36
CC/GG/TT/TC	39	3.0	29	1.9	0.65 (0.40–1.06)	0.08
CC/GG/TT/CC	30	2.3	34	2.3	1.00 (0.61–1.65)	0.99
CC/GG/CC/TT	29	2.2	30	2.0	0.91 (0.54–1.53)	0.73
TC/GG/CT/TT	27	2.0	29	1.9	0.95 (0.56–1.61)	0.84
CC/GG/CT/TT	27	2.0	30	2.0	0.98 (0.58–1.66)	0.94
TC/TT/TT/TT	20	1.5	18	1.2	0.79 (0.42–1.51)	0.48
Other haplotypes	81	6.1	85	5.7	0.92 (0.67–1.26)	0.62

Note: *p* and OR (95% CI) are calculated based on 'the individual haplotype frequency' versus 'cumulative frequency of all other haplotypes'. *APOL1* gene (GenBank reference sequence: NG_023228.1). Bold values indicate $p < 0.05$ considered as statistically significant.

On the other hand, these variants are an extreme susceptibility factor for renal failure (even 7–10 times increased risk of disease) (Young et al., 2017) as well as they are known to be associated with an increased risk of CVD (estimated doubling of risk, after correction for traditional ASCVD risk factors). There are several lines of evidence suggesting that APOL1 could contribute to play an important role in ASCVD pathology in general. For example, the

TABLE 4 Genotype frequencies of *APOL1* polymorphisms in patients, survivors and non-survivors.

	Survivors		Non-survivors		OR		
	N	%	*N*	%	Crude	*p*	*p*[a]
rs13056427							
CC	682	60.7	177	64.8	1.00		0.34[&]
CT	399	35.5	89	32.6	0.85 (0.65–1.14)	0.29	0.37[#]
TT	42	3.8	7	2.6	0.64 (0.28–1.45)	0.28	0.21[§]
rs136147							
GG	352	31.6	91	33.5	1.00		0.11[&]
GT	549	49.2	117	43.0	0.82 (0.61–1.12)	0.21	0.13[#]
TT	214	19.2	64	23.5	1.16 (0.80–1.66)	0.43	0.55[§]
rs10854688							
TT	471	42.0	122	45.0	1.00		0.66[&]
TC	484	43.2	106	39.1	0.85 (0.63–1.13)	0.26	0.48[#]
CC	166	14.8	43	15.9	1.00 (0.68–1.47)	1.00	0.37[§]
rs9610473							
TT	796	71.1	198	72.5	1.00		0.69[&]
TC	295	26.4	67	24.5	0.91 (0.67–1.24)	0.56	0.78[#]
CC	28	2.5	8	2.9	1.15 (0.52–2.56)	0.73	0.65[§]

Note: M—major allele. m—minor allele. *APOL1* gene (GenBank reference sequence: NG_023228.1).
[a]*p* is calculated for +M versus mm[&], MM versus Mm versus mm[#] and MM versus +m[§] models of comparisons.

vascular endothelium has been shown to be an important source of circulating APOL1 (Monajemi et al., 2002). In plasma (APOL1 is the only one secreted protein from the APOL family), APOL1 is associated with HDL particles (Duchateau et al., 2001) and it has been suggested that it may be involved in some way in cholesterol transport or metabolism (Shin & McCullough, 2014). Plasma levels of APOL1 correlate with both plasma cholesterol and plasma triglyceride levels (Duchateau et al., 2000). In patients with familial hypercholesterolaemia, who are at increased risk of premature ASCVD, plasma levels of APOL1 were significantly lower in patients who suffered a cardiac event (Cubedo et al., 2016) compared to healthy subjects with familial hypercholesterolaemia. Albeit the results are not unambiguous, carriers of G1 and G2 alleles seem to be also under increased risk of obesity (Nadkarni et al., 2021) and have 2- to 5-year decrease in hypertension diagnosis (Nadkarni et al., 2017), thus increasing prevalence of two traditional ASCVD risk factors. Finally, in an animal model, G1 and G2 transgenic mice have impaired reverse cholesterol transport (Ryu et al., 2019).

In general, most of these studies have examined plasma levels of APOL1. As we do not have data on the plasma concentration of APOL1 in our subjects (samples were analysed in a retrospective design and no samples, stored in Biobank, were available for this type of study), we cannot analyse whether the four SNPs examined have a potential effect on plasma APOL1 concentration.

To date, there is little information on the potential effect of other (non-G1 and non-G2) *APOL1* variants on the development of ASCVD in non-black African ethnicities. Consistent with our findings, variants within the *APOL1* have not been reported as potential targets associated with CVD in genome-wide association studies (Kessler & Schunkert, 2021). We have confirmed these findings in a population that has never been screened by GWAS for ASCVD risk and have shown that *APOL1* haplotype analysis is also rather unlikely to be informative in efforts to identify subjects at increased risk of premature ASCVD. On the other hand, because we have selected only four common polymorphisms and did not perform in-depth DNA sequencing of the *APOL1* region in our samples, we cannot exclude that some rare variants may also play an important role in CVD pathology also in Caucasians. Finally, we have not included females in our study. Thus, we cannot exclude some sex-specific effect of examined variants.

In conclusion, our study suggests that common variants within the *APOL1* gene are unlikely to be important genetic determinants of ASCVD or traditional cardiovascular disease risk factors in white Caucasians.

AUTHOR CONTRIBUTIONS

Conception and the design: JAH, VA, VL, VS, MG, JKe, JKa and JP. Data collection and handling: VA, VS, JM, MG, JKe, JKa and JP. DNA analyses: JAH. Analysis and interpretation of the data: JAH, VL, JP and VA. Funding:

JAH, JP, VA and JKa. JAH and JP wrote the original draft, and all authors read, corrected and approved the final manuscript version and agreed to be accountable for all aspects of the work. JAH, JM, VL and JP confirm the authenticity of all the raw data.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

For ethical standards, data are not publicly available. For scientific purposes, raw data are available upon reasonable request from corresponding author.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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