Variants within HNF1α and ANGPTL4 genes and acute coronary syndrome in Czech population. The GENDEMIP study

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Abstract

BACKGROUND: Atherosclerosis is a complex arterial disease involving interactions between multiple genetic and environmental factors. A large number of genetic polymorphisms associated with atherosclerotic diseases have been identified in recent years. We investigated the possible association between hepatic nuclear factor (*HNF1-α*) and angiopoietin-like 4 (*ANGPTL4*) single nucleotide polymorphisms and the risk of acute coronary syndrome (ACS) in the Czech population.

MATERIALS AND METHODS: A total of 1,182 patients with ACS (835 males and 347 females) and 1,200 healthy controls (827 males and 373 females) were included in the study. All patients were younger than 65 years of age. rs7310409 (A>G within the *HNF1-α* gene) and rs116843064 (G>A within the *ANGPTL4* gene) were genotyped using TaqMan genotyping assays.

RESULTS: The frequencies of the genotypes in patients with ACS did not significantly differ from the control group for the rs7310409 polymorphism (AA=17.1%, AG=46.6%, GG=36.2% vs. AA=14.4%, AG=50.3%, GG=35.3%, respectively; p=0.12) or the rs116843064 polymorphism (AA=0.1%, AG=3.5%, GG=96.4% vs. AA=0.1%, AG=4.2%, GG=95.7%, respectively; p=0.69). There was no interaction with gender. In addition, gene variants were not associated with common cardiovascular risk factors (dyslipidaemia, hypertension, smoking, obesity and diabetes).

CONCLUSIONS: No association was observed between polymorphisms within the *HNF1-α* and *ANGPTL4* genes and the risk of ACS in the Czech population.

Abbreviations:

HNF1-α	- hepatic nuclear factor
ANGPTL4	- angiopoietin-like 4
CRP	- C-reactive protein
CVD	- cardiovascular disease
ACS	- acute coronary syndrome
SNP	- single nucleotide polymorphism
PCR	- polymerase chain reaction

ORIGINAL ARTICLE

INTRODUCTION

Atherosclerosis is a complex arterial disease that can result in multiple cardiovascular disorders. Elevated blood lipoprotein levels are associated with the initiation and progression of atherosclerosis. However, inflammation also plays an important role in the development of atherosclerosis. Circulating inflammatory parameters are considered possible indicators of an increased risk of cardiovascular disease (CVD), and C-reactive protein receives the most attention (Roy *et al.* 2009). However, higher CRP levels are associated with more than one hundred different factors (Kushner *et al.* 2006; Hubacek *et al.* 2011).

In recent years, some genome-wide association studies have identified single nucleotide polymorphisms associated with plasma CRP levels and triglycerides.

One such polymorphism was identified in a gene coding for hepatic nuclear factor 1-α (HNF1-α, also known as TCF1 and MIM 142410), a transcription factor that plays an important role in diverse metabolic functions in the liver (cholesterol, bile acid, and lipoprotein metabolism), kidneys, and intestines (Ridker *et al.* 2008; Reiner *et al.* 2008). HNF-1α regulates the transcription of numerous genes in miscellaneous tissues, including genes expressed exclusively in the liver. The human CRP gene promoter contains two functional HNF1-α-binding sites (Toniatti *et al.* 1990), and the rs7310409 (A>G in the third intron, position 121424861 on chromosome 12) variant within the *HNF1-α* gene is of potential functional significance.

The second polymorphism we evaluated is located in the angiopoietin-like 4 gene (*ANGPTL4*, MIM 605910). Angptl4 is an inhibitor of lipoprotein lipase (LPL) activity, and it influences plasma triglycerides (Smart-Halajko *et al.* 2010). The Glu40Lys (E40K) variant (rs116843064, G>A exchange within the first exon, position 8335323 on chromosome 19) prevents Angptl4 oligomer formation, which is essential in Angptl4-mediated inhibition of LPL. Carriers of the K40 allele have significantly lower triglycerides (TG) levels compared to E40 homozygotes (Romeo *et al.* 2007; Talmud *et al.* 2008).

In our study, we focused on the potential effects of the *HNF1-A* (rs7310409) and *ANGPTL4* (rs116843064) polymorphisms on the risk of acute coronary syndrome development in the Czech Slavonic population.

MATERIALS AND METHODS

We analysed 1,182 patients with ACS (835 males and 347 females, aged under 65 years) who were consecutively collected as previously described in detail (GENDEMIP study, Pitha *et al.* 2007; Hubacek *et al.* 2010a). As a control, we genotyped a portion of the subjects included in the Czech post-MONICA study. A total of 1,200 control individuals (827 males and 373 females, aged less than 65 years) were selected from the general population according the WHO MONICA Project protocol (Tunstall-Pedoe 2003). All examined individuals were Caucasian. All included patients signed the informed consent, which was approved with the study protocol by the institutional ethics committee.

Genomic DNA was extracted from peripheral blood white cells. *HNF1-α* polymorphism rs7310409 was genotyped using a TaqMan SNP assay (C_26991788_10, Applied Biosystems). *ANGPTL4* variant rs116843064 was genotyped using a fluorogenic 5'-nucleotidase assay with the custom order TaqMan assay system (no.1084905, Applied Biosystems). SNP analyses were performed according to the AB protocol on a 7300 Real-Time PCR instrument with reagents purchased from Applied Biosystems.

The Hardy-Weinberg test (http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls) was applied to confirm the independent segregation of the alleles. Chi-square tests and odds ratios (95% CI) were calculated according to http://www.physics.csbsju.edu/cgi-bin/stats/contingency_form.sh?nrow=2&ncolumn=3 and http://www.

Tab. 1. Basic characteristics of the analyzed individuals.

	Males			Females		
	Controls	**ACS patients**	***p*-value**	**Controls**	**ACS patients**	***p*-value**
N	827	835	-	373	347	-
Age (years)	49.0 (10.8)	55.2 (7.5)	0.001	48.6 (10.6)	62.6 (8.5)	0.001
Cholesterol (mmol/L)	5.76 (1.06)	5.22 (1.15)	0.001	5.80 (1.15)	5.40 (1.26)	0.001
Triglycerides (mmol/L)	1.97 (1.28)	2.05 (1.46)	ns	1.47 (0.82)	1.86 (1.16)	0.001
BMI (kg/m^2)	28.2 (4.0)	28.5 (4.3)	ns	27.6 (5.5)	28.8 (5.6)	0.001
Never smokers (%)	67.3	12.9	0.001	78.5	64.5	0.001
Diabetes prevalence (%)	8.9	34.7	0.001	6.8	50.4	0.001
Hypertension prevalence (%)	40.7	52.8	0.001	33.1	62.7	0.001

Values are expressed as mean (SD).

hutchon.net/ConfidOR.htm. Individuals carrying the risk factors are expressed as a percentage of the total sample. The mean ± SD are reported for risk score, and *p*-values less than 0.05 were considered to be significant. ANOVA was used to analyse the associations between SNPs and quantitative traits.

RESULTS

For controls, the call rates were 88.9% for rs7310409 and 98.7% for rs116843064. For patients, the call rates were 98.7% for rs7310409 and 98.8% for rs116843064. In the Czech population, the genotype distributions were within the expected Hardy-Weinberg equilibrium (p=0.27 for rs7310409 and p=0.33 for rs116843064; males and females analysed together).

The basic characteristics of the patients and controls are summarised in Table 1. The patient groups were older and had a higher prevalence of smoking, diabetes mellitus type 2 and hypertension. Male patients and controls did not differ in BMI. Plasma cholesterol was lower in the patient groups, likely because more patients were being treated with statins (20%) in comparison to the controls (7%).

The frequency of genotypes in patients with ACS did not significantly differ (codominant model of analysis) for rs7310409 within the *HNF1-α* gene (p=0.12) or rs116843064 within the *ANGPTL4* gene (p=0.69). More detail and the distributions of individual genotypes between genders are presented in Tables 2 and 3.

The patients with acute MI had higher levels of plasma CRP and were typically not fasting, which elevated their plasma triglyceride levels. Thus, these patients were excluded from the association analysis. We found no correlation between rs116843064 and plasma triglycerides or rs7310409 and plasma CRP in controls (Table 4).

Finally, the gene variants under study were not associated with the primary cardiovascular risk factors (dyslipidaemia, hypertension, smoking, obesity and diabetes, results not shown in details).

Tab. 2. The frequencies of the *HNF1-α* rs7310409 genotypes in controls and in acute coronary syndrome (ACS) patients.

Males	Controls		ACS patients		OR	*p*-value	**p*-value
	N	%	N	%	Crude		
GG	241	34.2	298	35.9	1		
AG	358	50.7	385	46.4	0.87 (0.69–1.09)	0.22	0.19
AA	106	15.0	146	17.6	1.11 (0.82–1.51)	0.48	
Females	**Controls**		**ACS patients**		**OR**	***p*-value**	***p-value**
	N	**%**	**N**	**%**	**Crude**		
GG	136	37.5	125	37.0	1		
AG	179	49.3	159	47.0	0.97 (0.70–1.33)	0.84	0.57
AA	48	13.2	54	16.0	1.22 (0.77–1.94)	0.39	

**p*-value for codominant model of analysis is given.

Tab. 3. The frequencies of the *ANGPTL4* rs116843064 genotypes in controls and in acute coronary syndrome (ACS) patients.

Males	Controls		ACS patients		OR	*p*-value	**p*-value
	N	%	N	%	Crude		
GG	743	95.9	798	96.5	1		
AG	31	4.0	28	3.4	0.84 (0.50–1.42)	0.51	0.81
AA	1	0.1	1	0.1	0.93 (0.06–14.9)	0.96	
Females	**Controls**		**ACS patients**		**OR**	***p*-value**	***p-value**
	N	**%**	**N**	**%**	**Crude**		
GG	350	95.4	328	96.2	1		
AG	17	4.6	13	3.8	0.82 (0.39–1.70)	0.59	0.59
AA	0	0	0	0	–	–	

**p*-value for codominant model of analysis is given.

Tab. 4. Comparison of CRP plasma levels and plasma triglycerides in control group of healthy individuals (MONICA) depended on genotype. P value is for the entire population, adjusted for sex.

	males	females	*p*-value
HNF1-α	**CRP (mg/L)**		
GG	1.76 (2.12)	1.86 (2.32)	
AG	1.73 (2.11)	1.95 (2.25)	0.69
AA	1.62 (2.23)	1.91 (2.49)	
***ANGPTL* (E40K)**	**Triglycerides (mmol/L)**		
GG	2.04 (1.35)	1.49 (0.88)	
AG+AA	1.74 (0.68)	1.43 (1.02)	0.84

Values are expressed as mean (SD). For numbers of analysed individuals see Table 3.

DISCUSSION

Cardiovascular disease undoubtedly has an associated genetic background, and the wide spectrum of candidate gene variants include those with potential effects on plasma lipids and inflammation. Although the role of plasma lipids in CVD development has been shown to be causal (Triglyceride Coronary Disease Genetics Consortium 2010), the extent of specific or direct CRP involvement in the pathogenesis of CVD remains unclear.

A number of polymorphisms within the *CRP* gene or its promoter have been described (Kleber *et al.* 2010), and some were used to prove the causality between CRP and CVD. A recent study by Wensley *et al.* (2011) demonstrated a clear link between CRP SNPs and plasma CRP levels and between CRP levels and CVD. However,

the risk ratios for CVD per each additional copy of an allele associated with increased CRP were almost identical. This result indicates that the CRP concentration itself is very unlikely to be a causal factor of CVD. This finding could at least partially explain why we found no association between the *HNF1-α* polymorphism and an elevated risk of ACS.

Although CRP is transcriptionally activated through its interaction with HNF1-α and the examined variant rs7310409 was strongly associated with plasma CRP in WGHS (Ridker 2008), we failed to confirm these findings in the Czech population. Additionally, the second variant that we evaluated, E40K within the *ANGPTL4* gene, did not show any association with ACS (or plasma TG levels), as suggested previously (Talmud *et al.* 2008). However, when analysing the association between SNPs and plasma CRP and triglycerides, only data from the control group were used because the values from patients with ACS are biased.

In addition to the variants identified in the "association studies era," many variants potentially associated with CVD were detected using genome-wide screenings (Swerdlow *et al.* 2012) or deep sequencing of particular genes in recent years. The first analyses were typically performed using individuals from the USA and Western Europe and were not further confirmed.

In our study, we focused on the possible influence of the *HNF1-α* and *ANGPTL4* genes on the development of ACS in the Czech Slavonic population. Previously, we were able to confirm the importance of some (Poledne *et al.* 2010; Hubacek *et al.* 2010a; 2012) but not all (Vrablik *et al.* 2008, Hubacek *et al.* 2010b and 2010c) of the variants detected in genome-wide studies. This further underlines the importance of confirmatory studies in different populations.

In our study, we did not detect an association between the common variants within the genes for *HNF1-α* and *ANGPTL4* and the development of ACS in the Czech Slavonic population.

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