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Association of the novel single-nucleotide polymorphism which increases oxidized low-density lipoprotein levels with cerebrovascular disease events

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ABSTRACT

Background and purpose: Patients with genetic background for high circulating oxidized low-density lipoprotein (oxLDL) levels might be at an increased risk of cerebrovascular disease (CVD).
Methods: The association of oxLDL-variant rs676210 with CVD events was studied in patients undergoing coronary angiography (study A; $N = 2913$ [271 cases]). We sought to replicate the results in a large genome-wide association study meta-analysis of ischaemic stroke (study B; $N = 3548$ cases, 5972 controls).
Results: In study A, the prevalence of hypertension, diabetes and >50% carotid stenosis as well as the levels of LDL cholesterol differed significantly between cases and controls. In a logistic regression model adjusted for the significant covariates, rs676210 associated with CVD events ($p = 0.030$; odds ratio = 1.29 [95% confidence interval 1.03–1.63] for risk allele G). In study B, rs676210 did not associate with the history of ischaemic stroke.
Conclusions: The oxLDL levels increasing variant rs676210 associates with CVD events in patients undergoing coronary angiography.

1. Introduction

In our earlier genome-wide association study (GWAS) on oxidized low-density lipoprotein (oxLDL), we found an apolipoprotein-B (apoB) Pro2739Leu missense mutation (rs676210) associating with oxLDL but not with history of coronary artery disease (CAD) or myocardial infarction (MI) [1]. Since there is some

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evidence that oxLDL associates with ischaemic stroke [2–4], rs676210 may also associate with ischaemic stroke.

Ischaemic stroke can be classified into five subtypes according to Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [5]; 1) large-artery atherosclerosis (LAA), 2) small-vessel disease (SVD), 3) cardioembolic stroke (CE), 4) other aetiology, or 5) unknown aetiology. One would expect oxLDL to associate especially with LAA since oxLDL is considered to be essential in the atherosclerotic process [6]. There is also some evidence that oxLDL could bind to thrombocytes and induce platelet adhesion to the vascular wall in acute coronary syndromes [7], which could also increase the risk of LAA subtype of stroke. However, only few studies indicate an association of circulating oxLDL with LAA [4], and, furthermore, we did not find an association between rs676210 and the history of CAD in the previous study [1].

Since rs676210 associates strongly with oxLDL in both the young healthy population and elderly angiography patients [1], it could be used to substitute the effect of the lifetime risk of increased oxLDL levels on ischaemic stroke. Therefore, we first studied the association of rs676210 with the history of cerebrovascular disease (CVD) events (transient ischaemic attack [TIA], or stroke) in patients undergoing coronary angiography (the Ludwigshafen Risk and Cardiovascular Health [LURIC] study [8]; $N = 2918$ [271 cases]). In the second phase, we sought to replicate the results in a large GWAS meta-analysis of history of ischaemic stroke and its pathophysiological subtypes (Wellcome Trust Case Control Consortium 2 [WTCCC2] ischaemic stroke GWAS [9]; total $N = 3548$ cases, 5972.controls).

2. Materials and methods

For all cohorts, the recruitment of patients was approved by the relevant local ethics committees, and studies were conducted in accordance with the Declaration of Helsinki.

The Ludwigshafen Risk and Cardiovascular Health (LURIC) study consists of 3316 Caucasian patients who were referred for coronary angiography because of chest pain at a tertiary care centre in Southwest Germany between 1997 and 2000 [8]. For the 2913 LURIC participants, all necessary covariate and endpoint data were available and they were included in the present study. Previous CVD events were defined as a documented history of a foregoing transient ischaemic attack (TIA), prolonged ischaemic deficit, or cerebral infarction with or without a remaining neurologic deficit [8].

Discovery stroke cohorts in WTCCC2 ischaemic stroke GWAS included samples from the UK (a–c) and Germany (d), with a total of 3548 cases and 5972 controls [9]. Cases were phenotyped and classified into mutually exclusive aetiologic subtypes according to the TOAST classification [5]. The control data set for the British discovery samples was the WTCCC2 common control set, which includes healthy blood donors from the United Kingdom Blood Service's (UKBS) collection and individuals from the 1958 Birth Cohort dataset (58C). The control data set for the German cases was taken from the MONICA/KORA Augsburg Study's population-based controls from the same region in Germany.

2.1. Genotyping and quality control (QC) in the different cohorts

In LURIC, Metabochip and in WTCCC2 Illumina chips were used for genotyping the studied single-nucleotide polymorphism (SNP), rs676210. The SNP was directly genotyped in all studies and met all quality control criteria.

Statistics for the LURIC study were performed using logistic regression in the R Statistical package v. 2.15.2 (http://www.r-project.org). In the WTCCC2, analysis was performed with logistic regression using PLINK [10] on the separate groups; meta-analysis using an inverse-variance-weighted approach was performed using METAL [11].

3. Results

The general characteristics of the LURIC cohort and the difference between CVD event cases and controls are displayed in Supplementary Table 1. More than 60% of the LURIC population was diagnosed with CAD, and CAD and MI were more prevalent among CVD event cases. Out of the known ischaemic stroke risk factors, hypertension, diabetes, carotid stenosis, atrial fibrillation and LDL cholesterol were associated with history of CVD events. OxLDL did not associate with CVD events in LURIC ($p = 0.955$).

In the logistic regression model with no covariates, rs676210 associated with CVD events ($p = 0.030$, odds ratio [OR] = 1.28 [95% confidence interval, CI = 1.03–1.60] for risk allele G). In the logistic regression model adjusted for the significant risk factors (hypertension, diabetes, carotid stenosis, atrial fibrillation and LDL cholesterol), rs676210 remained significantly associated with CVD events ($p = 0.030$, OR = 1.29 [1.03–1.63] for risk allele G). The studied LURIC variables according to rs676210 genotype are displayed in Table 1. In addition to oxLDL and CVD events, only BMI and smoking were borderline-significantly associated with rs676210.

We attempted to replicate the association of rs676210 with CVD events in LURIC in the meta-analysis of WTCCC2 ischaemic stroke cohorts. The general characteristics as well as number of cases and controls in WTCCC2 are displayed in Supplementary Table 2. Logistic regression without adjustments was used as in LURIC. There was no significant association of rs676210 with all types of ischaemic stroke ($p = 0.81$, OR = 1.00 [0.93–1.09]), LAA ($p = 0.85$, OR = 0.99 [0.87–1.12]), CE ($p = 0.66$, OR = 1.03 [0.90–1.18]), or SVD ($p = 0.65$, OR = 0.97 [0.83–1.13]).

Table 1
The association of the apolipoprotein-B Pro2739Leu missense mutation rs676210 with cerebrovascular disease (CVD) events and its risk factors in LURIC.

Variables	rs676210 genotype			*p*-value
	AA	AG	GG	
	($N = 165$)	($N = 987$)	($N = 1761$)	
CVD event (yes)	11 (6.67%)	80 (8.11%)	180 (10.2%)	0.030
oxLDL (U/l)	58.9 (52.6)	68.7 (26.2)	80.1 (24.5)	<<0.001
Sex (female)	46 (27.9%)	310 (31.4%)	537 (30.5%)	0.643
Hypertension (yes)	95 (57.6%)	562 (56.9%)	1049 (59.6%)	0.392
Diabetes (yes)	52 (31.5%)	327 (33.1%)	562 (31.9%)	0.787
Carotid stenosis (yes)	12 (7.27%)	42 (4.26%)	80 (4.54%)	0.229
Atrial fibrillation (yes)	17 (10.3%)	127 (12.9%)	218 (12.4%)	0.633
CAD (yes)	119 (72.1%)	662 (67.1%)	1183 (67.2%)	0.469
MI (yes)	62 (37.6%)	423 (42.9%)	722 (41%)	0.373
Smoking (yes, ever)	116 (70.3%)	607 (61.5%)	1133 (64.3%)	0.0643
BMI (kg/m^2)	26.9 (3.44)	27.3 (4.12)	27.5 (4.1)	0.0501
LDL cholesterol (mg/dl)	119 (33.8)	116 (34.9)	115 (33.5)	0.111

Abbreviations: LURIC, Ludwigshafen Risk and Cardiovascular Health study; CAD, coronary artery disease (over 50% stenosis); MI, myocardial infarction; BMI, body-mass index; LDL, low-density lipoprotein; oxLDL, oxidized LDL.
Statistics: Values are numbers (percentages) in cases of categorical data and means (standard deviations) in cases of continuous data; *p* values (difference between rs676210 genotype groups) calculated with chi-square test for categorical data, with logistic regression for CVD events and with analysis of variance (ANOVA) for other continuous data. The CVD event model is adjusted for hypertension, diabetes, carotid stenosis, atrial fibrillation and low-density lipoprotein cholesterol.