Gene 494 (2012) 190–195

Contents lists available at SciVerse ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Significant associations of the rs2943634 (2q36.3) genetic polymorphism with adiponectin, high density lipoprotein cholesterol and ischemic stroke

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ARTICLE INFO

Article history:
Accepted 6 December 2011
Available online 20 December 2011

Keywords:
Cardiovascular diseases
SNP
Risk factor

ABSTRACT

Background: rs2943634 C/A single nucleotide polymorphism (SNP), located in a non coding region of chromosome 2q36.3, has been associated with coronary artery disease in two genome wide association studies. Our goal was to investigate its relation with myocardial infarction (MI) and ischemic stroke (IS), as well as with 12 intermediate risk phenotypes, in a population-based prospective cohort study.
Methods: rs2943634 was genotyped in a case–cohort study including a random sample of 1891 individuals (subcohort) and all incident MI (n = 211) and IS (n = 144) cases during a mean follow-up of 8.2 ± 2.2 years, nested within the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort comprising 27,548 middle-aged men and women.
Results: rs2943634 minor allele (A) was associated in an additive fashion with lower risk of IS but not with MI [hazard ratio (HR) = 0.66; 95% confidence interval (CI): 0.50–0.87; P = 0.003; HR = 1.02; 95% CI: 0.82–1.28; P = 0.83 respectively, for the age and sex adjusted model]. Furthermore, it was related to slightly higher levels of plasma adiponectin [CC 6.94, CA 7.27, AA 7.86 μg/ml, P = 0.0002] and high density lipoprotein (HDL)-cholesterol (CC 52.08, CA 53.05 and AA 55.27 mg/dl, P = 0.002), based on additive models. Adjustment for adiponectin and HDL-cholesterol did not attenuate the association between the SNP and IS risk. In contrast, adjustment for adiponectin abolished the association between the SNP and HDL-cholesterol and adjustment for HDL-cholesterol attenuated the association between the SNP and adiponectin.
Conclusions: Our findings suggest that rs2943634 is associated with IS risk and with plasma levels of HDL-cholesterol and adiponectin in this German population. Further investigations are needed to confirm these results and to clarify the mechanisms underlying the association.

1. Introduction

rs2943634 C/A single nucleotide polymorphism (SNP) is located in a non coding region on chromosome 2 at 2q36.3, however the Welcome Trust Case Control Consortium (WTCCC) Study, which enrolled 1926 coronary artery disease (CAD) cases and 2938 controls of white European origin, imputed an association between its most frequent allele (C) and CAD (Samani et al., 2007). Authors could reproduce the association in the German myocardial family study which involved 875 myocardial infarction (MI) cases and 1644 controls (Samani et al., 2007), however, further replication studies have not been able to confirm this association (Bressler et al., 2010; Ghazouani et al., 2010; Karvanen et al., 2009; Muendlein et al., 2009; Samani et al., 2009; Wang et al., 2011). Ischemic stroke (IS) shares common physiopathological mechanisms with MI due to atherosclerotic disease (Faxon et al., 2004). Thus rs2943634 has also been investigated on its relationship with IS (Karvanen et al., 2009)

Abbreviations: DIfE, German Institute of Human Nutrition; SNP, Single nucleotide polymorphism; MI, Myocardial infarction; IS, Ischemic stroke; EPIC, European Prospective Investigation into Cancer and Nutrition; A, Adenine; C, Cytosine; HR, Hazard ratio; CI, Confidence interval; HDL, High density lipoprotein; WTCCC, Welcome Trust Case Control Consortium; CAD, Coronary artery disease; CVD, Cardiovascular diseases; BMI, Body mass index; LDL, Low density lipoprotein; WC, Waist circumference; SBP, Systolic blood pressure; DBP, Diastolic blood pressure; TG, Triglycerides; HbA1c, Glycated hemoglobin; hs-CRP, High-sensitivity C-reactive protein; MDC, Max Delbrück Center for Molecular Medicine; HWE, Hardy–Weinberg equilibrium; SD, Standard deviation; SEM, Means and standard error; apoA-I, Apolipoprotein A-I; ABCA1, ATP-binding cassette transporter; CETP, Cholesteryl ester transfer protein; LPL, Lipoprotein lipase; PI3K, Phosphatidylinositol-3-kinase.

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doi:10.1016/j.gene.2011.12.009

and carotid artery intima-media thickness, a subclinical marker of atherosclerosis associated with stroke (Cunnington et al., 2009), with no significant results. Association data between rs2943634 and intermediate risk phenotypes of cardiovascular diseases (CVD) is scarce and inconsistent (Cunnington et al., 2009; Karvanen et al., 2009; Samani et al., 2007), although significant associations have been reported with body mass index (BMI) (Samani et al., 2007), blood pressure (Karvanen et al., 2009), low density lipoprotein (LDL)-cholesterol (Samani et al., 2007) and high density lipoprotein (HDL)-cholesterol (Karvanen et al., 2009). In the present study our goal was to investigate the association of rs2943634 with MI, IS, both CVD combined, and with intermediate risk phenotypes that may implicate causal pathways, in a middle-aged population.

2. Methods

2.1. Study population

The European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam cohort comprises 27,548 men and women from the general population of the Potsdam area in Germany, mainly aged 35–65 years at recruitment (1994–1998) (Boeing et al., 1999). The associations of rs2943634 with risk of MI, IS and CVD were analyzed using a case–cohort design. With this type of study design, the results are expected to be generalizable to the entire cohort (Kulathinal et al., 2007; Prentice and Self, 1988). The study population included a random sample of 2500 individuals (subcohort) and all newly occurred MI and IS cases from the EPIC-Potsdam cohort verified during a mean follow-up of 8.2 ± 2.2 years. Identification and verification of incident cases have been described in detail before (Weikert et al., 2008). Of individuals who had both, MI and IS, we considered only the first event. After exclusion of individuals with a history of MI or IS at baseline, and those without fully obtained follow-up data or with missing biomarkers, covariates or genotype data, the final study population comprised a subcohort of 1891 participants and a total of 211 MI cases and 144 IS cases (21 MI cases and 20 IS cases belonging to the subcohort). Participants were not required to be fasted at the baseline assessment, however, 655 individuals did not eat for at least 8 h before the blood drawing. The subcohort was used in a cross-sectional study to investigate the association between rs2943634 alleles and the following cardiovascular intermediate risk phenotypes: BMI, waist circumference (WC), systolic blood pressure (SBP), diastolic blood pressure (DBP), total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides (TG), glucose, glycated hemoglobin (HbA1c), high-sensitivity C-reactive protein (hs-CRP), adiponectin and creatinine. Informed consent was obtained from all study participants, and approval was given by the Ethical Committee of the State of Brandenburg, Germany.

2.2. Biochemical and genetic analyses

At baseline 30 ml of venous blood was collected from all the study participants, it was fractionated into serum, plasma, buffy coat and erythrocytes and stored in liquid nitrogen until the time of analysis. All biomarkers were analyzed between 2007 and 2008 at the Internal Medicine Department of the University of Tübingen, Germany. Plasma levels of total cholesterol, HDL-cholesterol, TG, hs-CRP, glucose and creatinine were determined with the automatic ADVIA 1650 analyzer (Siemens Medical Solutions, Erlangen, Germany). LDL-cholesterol was determined using the Friedewald formula (Friedewald et al., 1972). Plasma total adiponectin was determined with an enzyme-linked immunosorbent assay (Linco Research, St Charles, Mo). Genotyping of whole genome amplified DNA samples was performed in 2008 with the TaqMan® System (Applied Biosystems, Foster City, CA, USA) at the Max Delbrück Center for Molecular Medicine (MDC), Berlin, Germany. The genotyping success rate was 99%.

2.3. Statistical analysis

Hardy–Weinberg equilibrium (HWE) of the SNP was tested using the χ^2 test. Baseline characteristics of the study populations were described as means ± standard deviation (SD) for continuous traits, medians and 25th and 75th percentiles for the skewed variables, and % for categorical variables. Analysis of covariance considering the additive genetic model was used to assess the association between rs2943634 genotypes (independent variables) and BMI, WC, SBP, DBP, total cholesterol, HDL-cholesterol, LDL-cholesterol, TG, blood glucose, HbA1c, hs-CRP, creatinine, and adiponectin (dependent variables). P for trend was calculated with a linear regression model. Data is reported as means and standard error (SEM), except for TG, hs-CRP, creatinine, and adiponectin which were used log-transformed in order to normalize their distributions, and data is reported as geometric means and 95% CI. Also to better reach normality of their distributions, glucose was used square root transformed and HbA1c inverse transformed, data for them is respectively reported as squared and inverse means and 95% CI. Normality of variables was tested by estimating their skewness and kurtosis, by comparing their means and median values and by plotting their distribution in histograms. Association analysis between rs2943634 and fasting depending variables were performed only in participants who were fasting at the time of blood drawing. Association between adiponectin and HDL-cholesterol in the subcohort was examined by means of Spearman partial correlation coefficient.

Association of the SNP with risk of MI, IS and CVD, was calculated as hazard ratios (HR) with 95% confidence interval (95% CI) using Cox proportional-hazard regression, modified according to the Prentice method (Prentice and Self, 1988) to account for the case–cohort design, using a robust estimator for CI and considering the additive and co-dominant genetic models. Age was the underlying time variable in the counting processes, with entry defined as the individuals' age at the time of recruitment and exit defined as age at the diagnosis of CVD, or censoring.

Statistical models were adjusted for sex and age (model 1). The second model further included the significantly associated intermediate traits HDL cholesterol and adiponectin. The third model additionally included known cardiovascular risk factors: smoking status (never smoker, former smoker, current smoker<20 cigarettes per day, current smoker≥20 cigarettes per day), sports activity (<2 h/wk versus ≥2 h/wk), educational attainment (vocational school or less, technical school, university), BMI (continuous), WC (continuous), alcohol consumption (men: =0 g/d, >0 to 12 g/d, >12 to 24 g/d; >24 g/d; women: =0 g/d, >0 to 6 g/d, >6 to 12 g/d; >12 g/d), HbA1c, systolic blood pressure, antihypertensive medication, total cholesterol and hs-CRP. Effect modification by sex was evaluated by modeling the product term sex × genotype along with main effects (in the age-adjusted linear regression analysis).

All statistical analyses were performed using the SAS Version 9.2 (SAS Institute, Cary, NC). The detectable odds ratio of the SNP with CVD, MI and IS was calculated with Quanto (Gauderman and Morrison, 2006) concerning a log-additive model, a desirable power >80%, $\alpha = 0.05$, a minor allele frequency of 36% and a baseline overall disease prevalence in the EPIC-Potsdam population of 3.1% for CVD (n = 847), 2.0% for MI (n = 544) and 1.1% for IS (n = 303). Our estimates suggested that the detectable odds ratio was 1.26 for CVD, 1.34 for MI and 1.41 for IS when considering a possible harmful effect of the SNP, and 0.79, 0.75 and 0.71 respectively when considering a possible protective effect.

3. Results

3.1. Characteristics of participants

Demographic, biochemical, clinical and lifestyle characteristics of the subcohort and incident IS and MI cases are given in Table 1. The