Mutation Research 778 (2015) 18–25

Contents lists available at ScienceDirect

Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis

journal homepage: www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Genetic determinants of quantitative traits associated with cardiovascular disease risk

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ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form 30 April 2015
Accepted 5 May 2015
Available online 15 May 2015

Keywords:
Cardiovascular disease
Candidate gene
Risk factor
Genetic susceptibility
Quantitative trait

ABSTRACT

Established risk factors for cardiovascular diseases (CVD) may be moderated by genetic variants. In 2403 unrelated individuals from general practice (mean age 40.5 years), we evaluated the influence of 15 variants in 12 candidate genes on quantitative traits (QT) associated with CVD (body mass index, abdominal obesity, glucose, serum lipids, and blood pressure). Prior to multiple testing correction, univariate analysis associated *APOE* rs429358, rs7412 and *ATG16L1* rs2241880 variants with serum lipid levels, while *LEPR* rs1137100 and *ATG16L1* rs2241880 variants were linked to obesity related QTs. After taking into account confounding factors and correcting for multiple comparisons only *APOE* rs429358 and rs7412 variants remained significantly associated with risk of dyslipidemia. *APOE* rs429358 variant almost tripled the risk in homozygous subjects (OR = 2.97; 95% CI 1.09–8.10, $p < 0.03$) and had a lesser but still highly significant association also in heterozygous individuals (OR = 1.67; 95% CI 1.24–2.10; $p < 0.001$). Associations with hypertension, diabetes mellitus, and metabolic syndrome were not significant after Bonferroni correction. The influence of genetic variation is more evident in dyslipidemia than in other analyzed QTs. These results may contribute to strategic research aimed at including genetic variation in the set of data required to identify subjects at high risk of CVD.

1. Introduction

Cardiovascular-related quantitative traits (QT) represent intermediate risk factors that, if not prevented, can lead to cardiovascular disease (CVD). Determination of relevant candidate genes and combinations of susceptibility variants can therefore play an important role in primary and secondary prevention. Knowledge of these "susceptibility" biomarkers can also provide novel insights into pathways involved in the pathophysiology of disease and the new possibilities of pharmacological interventions [1].

Obesity can be considered an underlying risk factor, especially for atherosclerotic CVD, because of its clustering of cardiovascular risk factors (e.g., dyslipidemia, hypertension and hyperglycemia). Visceral fat promotes insulin resistance that leads to increased hepatic glucose production and decreased muscle glucose uptake

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http://dx.doi.org/10.1016/j.mrfmmm.2015.05.005
0027-5107/

and oxidation [2,3]. Elevations in fasting glucose levels may be significantly related to coronary heart diseases morbidity and mortality [4]. Visceral fat serves also as a source of inflammatory responses in obesity and CVD [5]. Central obesity (the accumulation of abdominal fat) is strongly associated with abnormalities in blood lipid levels, including low density lipoprotein (LDL) and high density lipoprotein (HDL) cholesterol and triglycerides. Cardiovascular-related QTs are also known to be affected by multiple epidemiological risk factors such as body composition, lifestyle, positive family history of premature CVD and aging. Interactions between them have been frequently studied [6–8]. Extensive evidence is also available about the paramount role of gene-environment interaction in the etiology and pathogenesis of CVD and other non-communicable disease [9]. Moreover the hormonal environment and tissue-specific gene expression differ between sexes and can lead to sex-specific susceptibility to the disease [10].

Despite the impressive effort to identify susceptibility variants and the number of uncovered genetic associations for CVD [11–13], we are still far from understanding how genetic and environmental factors interact and affect CVD susceptibility. Robust results on the role of genetic background, besides mechanistic implications, may have application in clinics as susceptibility biomarkers. They also may provide possible therapeutic targets, and contribute to disentangling the relative role of different pathways leading to CVD.

The aim of this study was to evaluate the extent of genetic susceptibility by targeting candidate SNPs in pathways leading to CVD, rather than in existing CVD, whose etiology is difficult to assess. The idea was to anticipate the identification of individuals genetically at risk, stratifying population on the basis of QTs rather than CVD. To provide biological consistency, best candidate SNPs have been selected, a rigorous Bonferroni correction has been applied (despite the use of candidate SNPs), and a large population homogeneous by age, clinical conditions, and ethnicity has been carefully selected.

2. Methods

The current cross-sectional study enrolled 2403 participants from 7 Slovak cities (Bratislava, Nitra, Zvolen, Žilina, Poprad, Prešov, Košice) and their surrounding areas. Selection of participants was based on the preventive clinical examination of 40 year old subjects at general practice clinics, between October 2006 and the end of 2006. Age distribution in the study ranged from 39.1 to 45.3 years with the mean of 40.5 ± 0.74 years. All participants were Caucasian of Slovak origin; no persons of other ethnicity living in Slovakia were included in the study. Sampling for biomarker analysis and DNA extraction was carried out during medical examination. Biochemical analyses were performed in regional accredited medical diagnostic laboratories. LDL cholesterol was calculated using the Friedewald formula in all subjects with triglycerides <4.5 mmol/l and also directly measured LDL was available for 1169 subjects. Fasting venous blood samples were collected in serum and EDTA monovettes (Sarstedt, Germany) and transported to our laboratory for DNA extraction and genotyping. Anthropometric measures and blood pressure were collected by trained nurses. Body mass index (BMI) was calculated as the weight (kg) divided by square of height (m). Waist was measured as the minimum circumference at the natural waistline between the lower rib margins and iliac crest, or at the level of the umbilicus if there was no natural waistline. All subjects completed a questionnaire, with questions about their personal and family history of premature CVD, lifestyle and environmental risk factors. Personal case history questions consisted of information about previous myocardial infarction, ischemic stroke, present ischemic heart disease or peripheral arterial disease. As part of the assessment, atherosclerosis risk factors were collected (see Table 1), including: dyslipidemia, hypertension, type 1 or 2 diabetes, and the family history of CVD (myocardial infarction and/or stroke among first degree relatives before the age of 60). The combination of risk factors was determined following recommendations of the Cholesterol Education Program's Adult Treatment Panel III [14]. The study was approved by the ethical committee of the Slovak Medical University and all participants gave written informed consent. Samples were coded and all analyses were performed blind.

2.1. Selection of genetic variants

Selection of variants was based on their previous association with CVD or related traits, including obesity, metabolic abnormalities, lipid and folate metabolism. Some of them, mostly those located outside annotated genes, were findings from genome-wide association analyses [15]. With few exceptions (*9p21.3* rs1333049, *FTO* rs9939609 and *MTHFD1L* rs6922269) they were all coding, non-synonymous single nucleotide polymorphisms (SNPs) with minor allele frequencies (MAF) higher than 0.05. The full list and basic characteristics of the selected variants are provided in Table 2.

2.2. DNA extraction and genotyping

DNA was extracted using a modified salting-out method [16]. We implemented several quality control measures to minimize errors associated with DNA sample handling and DNA quality. The DNA concentration was measured by spectrophotometry at 260 nm, and DNA purity was determined by the A260/A280 ratio. Genotyping was performed using 5' nuclease assay chemistry with TaqMan® MGB probes (Applied Biosystems, Warrington, UK). Genotyping reactions were carried out in 10 μl 96-well formats, using SNP genotyping Assays-on-Demand™, TaqMan® Genotyping Master Mix and 10ng of genomic DNA, according to supplier's recommendations. ABI PRISM® 7500 Sequence Detection Systems (Applied Biosystems) was used for end-point detection and allele calling.

2.3. Validation

Genotyping results were validated by direct sequencing of 92 random samples for each SNP. DNA fragments containing variants were amplified by PCR using appropriate primers (Supplementary Table 1). Sequencing reactions were performed using a BigDye® Terminator 3.1 Cycle Sequencing Kit (Applied Biosystems) and analyzed by SeqScape® v 2.5 Software. Only if 100% concordance was achieved was genotyping continued with 96 well formats.

2.4. Statistical analysis

Analysis of quantitative traits was performed in 2372 individuals without a personal history of CVD. Individuals using lipid-lowering medication ($n = 60$) were excluded from the analyses of lipid variables, and individuals using drugs for hypertension ($n = 194$) were excluded from the analysis of blood pressure. Studied variables were BMI, waist circumference, glucose, mean blood pressure (systolic and diastolic blood pressure, each value based on two subsequent measurements), and lipid variables: total, HDL and LDL cholesterol and triglycerides. Univariate analysis was performed using statistical software SPSS 16.0. Normality of distribution was tested by the Kolmogorov–Smirnoff test. If normally distributed, sample means were tested by Student *t*-test or analysis of variance (ANOVA) with Bonferroni's or Tamhane's tests for multiple comparisons depending on homogeneity of variance. Non-parametric Mann–Whitney *U* or Kruskal–Wallis *H* test were used for non-normally distributed variables. Pearson's or Spearman's correlations (depending on departure from normality) were used