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Original Research Article

Classical rather than genetic risk factors account for high cardiovascular disease prevalence in Lithuania: A cross-sectional population study

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ABSTRACT

Purpose: Cardiovascular disease (CVD) mortality accounts for 54% of all deaths in Lithuania, making it the highest among all of the European Union countries. We evaluated the prevalence of several CVD risk factors, including lifestyle, blood biochemistry and genetic predisposition to determine the reasons behind significantly increased CVD prevalence in Lithuania.

Materials and methods: In total 435 volunteers of Lithuanian ethnicity and stable geographic settlement for 3 generations, had their anthropometric, biochemical and behavioural risk factors measured. A randomly selected sample of 166 volunteers had their 60 CVD risk alleles genotyped. The prevalence of risk alleles and cumulative CVD genetic risk score were compared with population of North-West European origin (CEU) using data from the phase 3 HapMap project.

Results: CVD was present in 33.8% of study volunteers, 84% of participants consumed alcohol, 21% were current smokers and only 30% of participants engaged in higher levels of physical activity. Also, the average BMI (males 28.3 ± 4.3 kg/m^2, females 27.3 ± 5.0 kg/m^2), total cholesterol (males 6.1 ± 1.2 mmol/L, females 6.2 ± 1.0 mmol/L) and LDL-cholesterol (males 4.1 ± 1.1 mmol/L, females 4.1 ± 1.0 mmol/L) were above the normal values. The cumulative genetic susceptibility to develop CVD in Lithuanians was only 1.4% higher than in CEU population.

Conclusions: High BMI and poor population plasma lipid profile are the major contributing factors to high CVD mortality and morbidity in Lithuania. Smoking, alcohol consumption and preliminary genetic predisposition results do not explain the difference in CVD mortality between the Lithuanian and wider European populations. CVD prevention programmes in Lithuania should primarily focus on weight loss and improving blood lipid control.

1. Introduction

Cardiovascular diseases (CVDs), an umbrella term to include such illnesses as coronary heart disease (CHD), stroke and hypertension, are generally complications of atherosclerosis and are among the leading causes of morbidity and mortality worldwide. It is projected that by 2030, 23.3 million deaths will be due to CVDs [1]. In the EU CVDs account for 40% of all-cause mortality [2], whereas in Lithuania 54% of deaths are due to CVDs, the worst figure among all of the EU member states [2,3].

An individual's propensity to develop CVD is strongly influenced by a set of risk factors including male sex, smoking, obesity, diabetes, stress, sedentary lifestyle, blood pressure and body mass index (BMI), all of which are normally ascertained as part of the standard medical evaluation. The levels of plasma lipids together with blood pressure (BP), smoking, age and sex are used in the assessment of CVD risk [4,5].

However, there is a growing body of evidence, that the CVD risk is affected not only by the environmental, but also by the genetic factors. For example, people of Afro-Caribbean or South-Asian

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origin are more likely to develop CVDs than people of European origin [6]. Likewise, individuals, carrying ε2 and ε4 alleles of *APOE*, are known to succumb to CVDs more frequently [7,8]. Therefore, currently there is a strive to include genetic testing into the CVD risk assessment [9]. To support this progress, multiple studies have tried to identify and characterize new alleles that render the individual more susceptible to develop a certain type of CVD [10,11].

The prevalence of the conventional CVD risk factors in Lithuania has been described by several population studies [12,13]. Yet the relative influence of the genetics to the CVD burden in Lithuania remains unknown. In the near future population genetic risk scoring may be able to identify pre-symptomatic intermediate or high risk individuals and allow the timely initiation of preventative intervention. However, to maximize the effectiveness of this public health measure, countries may need to make an informed decision, which key alleles to screen for. It remains unknown, what genetic CVD risk variants are more relevant to the Lithuanian population and there is no data to supplement the future diagnostic strategies. To find out why CVDs are so prevalent in Lithuania and to provide some data for future genetic risk assessment strategies, in this cross-sectional study we investigated the prevalence of classical and behavioural CVD risk factors and the prevalence of 60 selected CVD or atherosclerosis-associated alleles in the traditional Lithuanian population.

2. Materials and methods

2.1. Participants

In total 435 volunteers (216 males and 219 females), aged between 40 and 60 years, took part in this study. The participants were recruited at 20 local primary healthcare centres at six different geographical regions of Lithuania: Western, Eastern and Southern Aukštaitija (Higher Lithuania) and Northern, Western and Southern Žemaitija (Lower Lithuania). Lithuania's population is 2,923,360 people (as of December, 2014) [14]. An invitation to participate in the study was sent to all the 40–60 year old patients in a given healthcare centre. Volunteers who responded to an invitation to participate in the study and were available on the day of the investigators' team's visit at their primary healthcare centre were included in the study. To be included in the study participants had to be free of acute illnesses. The ethical approval for the biomedical research was obtained from Vilnius Regional Biomedical Research Ethics Committee (No.158200-05-329-79) and written informed consent was received from every participant.

2.2. Questionnaire

All of the study participants were asked to complete a questionnaire, designed by the research group, which surveyed participants' lifestyle, family history and health condition. To determine the reliability of the questionnaire designed for survey, the test-retest method was used following 30 days after the first survey. Fifty individuals were re-tested and overall Cronbach alpha coefficient for the internal consistency of the questionnaire and one pertaining to each subscale was 0.86. The questionnaire was not externally validated. The respondents were also asked about their history of CVDs (myocardial infarction, stroke and hypertension). In the present study we only analyzed the data from the following 18 out of 70 questions: 1–10, 45–48, 58, 59, 60 and 68. The full questionnaire is provided as Supplementary Material.

Hypertension was defined as history of hypertension diagnosed and treated with medications, diet and/or exercise, current treatment by antihypertensive medications or documented BP reading >140 mmHg systolic and/or >90 mmHg diastolic on 2 different occasions. CVD was self-reported by the respondents. The accuracy of responses was assessed in a randomly selected sample of respondents by cross-referencing their questionnaire information with their medical documents at the local primary healthcare centres.

Smoking, alcohol consumption and physical activity status were self-reported. History of everyday or occasional current tobacco smoking was regarded as current smoking; patients with no current tobacco smoking, but with previous history of tobacco smoking were classified as former smokers; no current or former history of smoking classified participant as never smoker. Participants, who responded 'often' and 'sometimes' to alcohol consumption were classified as drinkers. Participants, who answered 'No' or 'No current, but history of alcohol consumption in the past' were classified as non-consumers. Desk job or sedentary assembly job was classified as 'not active'; work involving a lot of walking, but no lifting of heavy items was classified as 'walking'; jobs requiring a lot of walking up and down the stairs and/or lifting heavy items and/or intensely physical jobs were classified as 'High level of activity'. As pertains to leisure, largely watching TV or reading books without any other significant physical activity was classified as 'sedentary' leisure; participants getting involved in walking, cycling, gardening or other medium-intensity movement at least 4 times per week were classified as having 'walking' levels of leisure activity; engagement in jogging, swimming, heavy gardening or other heavy physical activity at least 3 times per week or regular participation in intense sports events was classified as 'Higher level of activity'.

2.3. Biochemistry

Fasting venous blood samples were obtained before 11 am and immediately transferred to the Centre of Laboratory Medicine, Vilnius University Hospital "Santariškių klinikos". Biochemical blood parameters, including total cholesterol, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides (TG), fasting glucose, C-reactive protein (CRP), lipoprotein (a), Apo A1, ApoB, and the ratio of ApoB/ApoA1 were quantified using the standardized procedures.

2.4. Genotyping

Genotyping was performed on 166 selected participants: to ensure the study volunteers represent traditional Lithuanian population, participants of Lithuanian ethnicity that have lived in the same region for at least three generations were selected; to ensure that the six different geographical regions of Lithuania were represented proportionally to the current number of inhabitants only a random selection of suitable participants was chosen.

DNA was extracted from venous blood samples using either MagneSil® Genomic, Large Volume System (Promega Corp., USA), automated for the TECAN Freedom EVO® platform (TECAN Group Ltd., Switzerland), according to the manufacturer's instructions, or phenol-chloroform extraction method. We used Illumina HiScanSQ™ platform and Illumina HumanOmniExpress-12 v1.1 array, which is comprised of 719,666 SNP markers, adhering to the Infinium® HD Assay Ultra Protocol Guide (Illumina Inc., USA). The genotyping data visualization, primary quality control analysis, filtering and output file generation were accomplished using the Illumina GenomeStudio v2011.1 Genotyping Module software.

An extensive literature search was performed to identify 60 SNP loci that are associated with atherosclerosis or increased CVD risk. Only SNPs, whose association with CVDs has been reproduced, were selected for frequency evaluation in the traditional Lithuanian population. Hardy-Weinberg equilibrium and allele frequencies were determined using the PLINK v1.07 software (Purcell S et al., 2007; http://pngu.mgh.harvard.edu/purcell/plink/