VILNIUS UNIVERSITY

NERINGA BUROKIENĖ

INVESTIGATION OF RELATION BETWEEN THE EPIDEMIOLOGICAL FACTORS OF CARDIOVASCULAR DISEASES AND GENOME IN THE LITHUANIAN POPULATION

SUMMARY OF DOCTORAL DISSERTATION

Biomedical Sciences, Medicine (06 B)

Vilnius, 2016

The dissertation has been prepared at Vilnius University in the period 2011–2015.

Scientific supervisor:

Prof. Habil. Dr. Zita Aušrelė Kučinskienė (Vilnius University, Biomedical Sciences, Medicine – 06 B).

Consultant:

Prof. Habil. Dr. Vaidutis Kučinskas (Vilnius University, Biomedical Sciences, Medicine – 06 B).

The defence of the dissertation will take place at the Vilnius University Academic Board of Medicine:

Chairperson: Prof. Dr. Janina Tutkuvienė (Vilnius University, Biomedical Sciences, Medicine – 06 B).

Members:

Prof. Dr. Janina Didžiapetrienė (Vilnius University, Biomedical Sciences, Medicine –06 B);

Prof. Dr. Rimantas Stukas (Vilnius University, Biomedical Sciences, Public Health – 09 B);

Assoc. Prof. Dr. Gintarė Šakalytė (Lithuanian University of Health, Biomedical Sciences, Medicine – 06 B);

Dr. Osvaldas Pranevičius (W. Cornell University College of Medicine, Biomedical Sciences, Medicine – 06 B).

The public defence of the dissertation will take place at an open session of the Academic Council of Medicine at 14.00 on March 17, 2016 in the Red Audience of the Vilnius University Hospital Santariškių Clinics. Address: Santariškių St. 2, Vilnius, Lithuania.

The summary of the doctoral dissertation was distributed on the 18th of February, 2016.

The doctoral dissertation is available at the Vilnius University Library and Vilnius University website: **http://www.vu.lt/lt/naujienos/ivykiu-kalendorius**.

VILNIAUS UNIVERSITETAS

NERINGA BUROKIENĖ

ŠIRDIES IR KRAUJAGYSLIŲ LIGŲ EPIDEMIOLOGINIŲ VEIKSNIŲ IR GENOMO SĄVEIKOS TYRIMAS

DAKTARO DISERTACIJOS SANTRAUKA

Biomedicinos mokslai, medicina (06 B)

Vilnius, 2016

Disertacija rengta 2011–2015 metais Vilniaus universitete.

Mokslinis vadovas:

prof. habil. dr. akad. Zita Aušrelė Kučinskienė (Vilniaus universitetas, biomedicinos mokslai, medicina – 06 B).

Mokslinis konsultantas:

prof. habil. dr. akad. Vaidutis Kučinskas (Vilniaus universitetas, biomedicinos mokslai, medicina – 06 B).

Disertacija ginama Vilniaus universiteto Medicinos mokslo krypties taryboje.

Pirmininkė – prof. dr. Janina Tutkuvienė (Vilniaus universitetas, biomedicinos mokslai, medicina – 06 B).

Tarybos nariai:

prof. dr. Janina Didžiapetrienė (Vilniaus universitetas, biomedicinos mokslai, medicina - 06 B);

prof. dr. Rimantas Stukas (Vilniaus universitetas, biomedicinos mokslai, visuomenės sveikata – 09 B);

doc. Gintarė Šakalytė (Lietuvos sveikatos mokslų universitetas, biomedicinos mokslai, medicina – 06 B);

dr. Osvaldas Pranevičius (W. Cornell universiteto medicinos koledžas, biomedicinos mokslai, medicina – 06 B).

Disertacija bus ginama viešame Medicinos mokslo krypties tarybos posėdyje 2016 m. kovo mėn. 17 d. 14 val. Vilniaus universiteto ligoninės Santariškių klinikų Raudonojoje auditorijoje. Adresas: Santariškių g. 2, Vilnius, Lietuva.

Disertacijos santrauka išsiuntinėta 2016 m. vasario mėn. 18 d.

Disertaciją galima peržiūrėti Vilniaus universiteto bibliotekoje ir VU interneto svetainėje adresu: www.vu.lt/lt/naujienos/ivykiu-kalendorius.

LIST OF ABBREVIATIONS

AH – arterial hypertension

ABP – arterial blood pressure

Apo-A 1– apolipoprotein A1

Apo-B – apolipoprotein B

BMI – body mass index

Ch – cholesterol

CHD – coronary heart disease

CI – confidence interval

CVD – cardiovascular diseases

DM – diabetes mellitus

DHMG – Department of Human and Medical Genetics

DNA – deoxyribonucleic acid

HAD – *Hospital Anxiety and Depression* scale

HDL-Ch – high density lipoprotein cholesterol

hs CRP – high sensitivity C-reactive protein

Lp(a) – lipoprotein (a)

LDL-Ch – low density lipoprotein cholesterol

LITGEN – VU MF research project "Genetic diversity of the population of Lithuania and changes of its genetic structure associated with evolution and common diseases", 2011–2015

MCA – multiple correspondence analysis

MI – myocardial infarction

MS – metabolic syndrome

OR – odds ratio

PHC – primary healthcare centers

RA – risk allele

RAF – risk allele frequency

RF – risk factors

SD – standard deviation

SNP – single nucleotide polymorphism

TG – triglycerides

USA – Unites States of America

VU – Vilnius University

VUH SK CMG – Vilnius University Hospital Santariškių Clinics Centre for Medical Genetics

WHO – World Health Organisation

χ^2 – chi square statistics for the test of independence (in statistics)

1. INTRODUCTION

1.1. Topic and novelty

The total number of deaths from cardiovascular disseases in the European Union reaches 40 percent; cardiovascular morbidity and mortality in Lithuania is even higher to compare with other Western European countries and Scandinavia, and reaches 56 percent of all death cases. It is possible to overcome or change the recent situation partially by improving the control of risk factors, which are important in the etiopathogenesis of CVD.

The underlying pathology of CVD is atherosclerosis, starting to develop in childhood. Atherosclerosis is a chronic inflammatory disease, when accumulations of lipids and calcium on the vascular walls form ulcerating and scarring plaques, which results in the narrowing of vascular lumen and development of cardiovascular complications – myocardial ischemia, myocardial infarction, and stroke.

CVD have been studied for many years, and many risk factors of them have been described as far back as in the last century. However, findings of advanced scientific research disclose some new ones. Risk factors (RF) can be classified into groups based on the possibility to control them: modifiable (high blood pressure, smoking, dyslipidemia, stress, physical inactivity, use of alcohol, obesity, diabetes mellitus, metabolic syndrome) and unmodifiable (age, gender, personal and family history). The diversity of the above-mentioned modifiable factors and of unmodifiable factors is predisposed genetically.

Considering the novelty of the risk factors they can be traditional (smoking, dyslipidemia, hypertension, diabetes, abdominal obesity, psychosocial factors, insufficient consumption of fruits and vegetables, abstinence from alcohol and the lack of regular physical activity) and non-traditional (oxidative stress, coagulation factors, homocysteine, collagen vascular diseases, chronic kidney disease, hyperuricemia, high heart rate, left ventricular hypertrophy, increased vascular rigidity, endothelium

dysfunction, increased arterial intima-media thickness, reduced ankle-brachial index, and calcifications in coronary arteries, aortic arch and abdominal aorta). The effect of non-traditional risk factors on CVD is still under research.

Multifactorial diseases, such as CVD, are the result of interaction among genetic, environmental and life-style factors, where genetic and genomic factors predispose a tendency to the development of a disease but do not directly cause it. The development of a disease depends on the surroundings of living and the life-style. However, tendency- and environment-related genetic variations cannot act separately. The effects of genetic factors on cardiovascular diseases have been studied insufficiently in Lithuania. Assessment of genetic risk in the nearest future could help identifying asymptomic persons with a moderate or high risk for the development of cardiovascular diseases and to initiate the timely prevention treatment for such individuals. In the meantime, these are the initial attempts to identify the missing or damaging gene variations characteristic of Lithuanian population, candidates for the further diagnostic strategies.

Risk factors affect both the progress and the extent of the disease. Control of these risk factors reduces morbidity and mortality rates for both diagnosed and undiagnosed CVD cases. CVD increasingly affects younger working population. The programme for screening and prevention measures for the high risk CVD population has been implemented in Lithuania since 2006, involving 40–55-year-old males and 50–65 year-old-females. The age group people aged from 40 to 65 years is the part of population in which the control of risk factors is most effective and beneficial from the economical point of view.

Thus, it is very important to be aware of the prevalence of prevailing conditions and their correlation with the disease and genetic predisposition. The epidemiological analysis covering the whole Lithuania allows better understanding the issues of the control of CVD risk factors and accordingly adjusting the future treatment and prevention strategies.

Improved care of patients with CVD, early diagnosis in the primary healthcare establishments would help to recommend a timely and adequate treatment of patients with the high risk of CVD.

1.2. Relevance and importance of the study

Currently, the interactions between environmental and genetic factors predetermining the body features and risks of developing the disease have been extensively discussed. It is difficult to explain the very high CV morbidity and mortality rates in Lithuania based only on the prevalence of traditional risk factors such as obesity, arterial hypertension, metabolic syndrome and smoking, or the synergistic action of traditional RF. Assessment of all RF and effective control are important in clinical practice aiming to reduce the risk of acute CV and CVD, relapses and complications.

The infrastructure, the implemented new generation genome and exome, large-scale genotyping and bioinformatics analysis methods have been available in Lithuania for a few years, providing the opportunity for the further development of diagnostics and disease prognosis involving genetic risk factors.

Aiming to disclose the causes of the high prevalence of cardiovascular diseases in Lithuania and to provide data for the further development of genetic characteristic risk assessment strategies, life style correlation with the risk of CVD are analysed in this study, also including the genetic markers of atherosclerosis in the population of Lithuania.

This study, which aims to establish correlations among the risk factors of CVD, nutrition, anxiety/depression and genetic risk, is the first attempt to study the group of 40–65-year-old Lithuanian population.

1.3. Aim, goals, statements to defend

The main purpose of this study is to assess correlations among nutrition, anxiety/depression, biochemical and genetic markers of atherosclerosis and CVD risk among 40–65-year-old Lithuanian citizens.

The main **goals** to achieve the aim are:

1. To compare the frequency of risk factors among 40–65-year-old Lithuanian citizens with and without CVD.

2. To establish correlations between nutrition habits of the population and CVD.

3. To assess the biochemical markers of atherosclerosis and its correlation with CVD.

4. To analyse the level of depression and anxiety and their correlation with CVD.

5. To analyse single nucleotide polymorphisms (SNP), to define the genotype and to link the allele variations with predisposition to CVD.

Following the study results, the **statements to defend** are**:**

1. The high prevalence of dyslipidemia and obesity, predetermined by nutrition and life-style habits, is characteristic of the working age part of the Lithuanian population.

2. Anxiety and depression, along with traditional risk factors, increase the cardiovascular risk.

3. Based on the studied markers of the genetic risk of CVD (SNP), several genomic markers of CVD, significant for the Lithuanian population, can be detected.

2. METHODS

2.1. Study population

This doctoral dissertation has been prepared while participating in the project implemented by the Medical Faculty of the Vilnius University during the period 2011–2015 "Genetic Diversity and Structural Changes in Lithuanian Population Related to Evolution and Most Prevalent Diseases" (LITGEN), financed as a Public Grant measure from the European Social Fund and general financial instruments of the Republic of Lithuania. The project leader is Prof. Hab. Dr. V. Kučinskas.

The permission No. 158200-05-329-79 to conduct this study was issued by the Vilnius Regional Biomedical Research Ethics Committee. Signed Subject Information – Informed Consent Forms for the participation in the study were received from all study subjects.

The study population was selected during the implementation of the LITGEN project in 2011–2013. Based on the territorial division of Lithuania into six ethnolinguistic regions (Girdenis A., Zinkevičius Z., 1966) the primary healthcare centres were selected (**Figure 1**).

Figure 1. Primary healthcare centres, about 50 study subjects were enrolled in each centre. Lithuania was divided on the map into six ethnolinguistic regions according to the classification of dialects, proposed by A. Girdenis and

Z. Zinkevičius. The map template authors are from the Department of Human and Medical Genetics, Medicine Faculty of Vilnius University (based on "Lietuvių kalbos atlasas I" map No. 1 (1977)).

Individuals whose ancestry of at least two generations resided in the same region were enrolled in the study population. A randomised study group, stratified according to the age and gender, was defined in each of the below listed primary healthcare centers (PHC) based on the lists of notified citizens: both parents aged 40–65 years and one or several their children from all six ethnolinguistic regions;

- ✓ Viečiūnai, Alytus, Druskininkai, Lazdijai, Elektrėnai, Lazdijai PHC Southern Aukštaičiai;
- ✓ Širvintos, Panevėžys, Rokiškis, Molėtai, Ignalina PHC in the Eastern Aukštaičiai;
- ✓ Birštonas, Šakiai, Marijampolė, Kėdainiai and Šiauliai PHC in the Western Aukštaičiai;
- ✓ Šilalė, Varniai, Kelmė, Raseiniai, Tauragėė, Raseiniai PHC in the Southern Žemaičiai;
- ✓ Priekulė, Plikiai, Šilutė PHC in the Western Žemaičiai;
- ✓ Mažeikiai, Naujoji Akmenė, Palanga, Skuodas, Gargždai, Telšiai PHC in the Northern Žemaičiai.

The cross-sectional study design was was applied, and standard methods for epidemiological studies were used: interview, blood sample collection for further biochemical and genetic tests.

2.2. The research process

A questionnaire including demographic data, nutrition and lifestyle characteristics, health status of the study subjects and elements of family history was used for interviewing the individuals.

Food-frequency nutrition study method. Individuals were asked bout their eating habits: the frequency of consumption of different foods: fresh, boiled or stewed vegetables, grain products (bread, cereal porridge), fish and fish products, meat and meat

products, fast-made soups and bouillon cubes, eggs, milk and dairy products, confectionery. Options for answers: “Never eat”, “Less than once per week”, “1–2 times per week”, “3–5 times per week”, “Every day”, “Several times per day”.

The respondents were asked to specify their preferences for meat (poultry, beef or pork) and processing methods (boiled, fried or smoked).

Respondents were also asked about their preferences for fat they use for food preparation. Options for the answer were: butter, margarine, vegetable oil. The following question was presented aiming to find out the additional use of salt: “Do you add salt to already cooked food?” Options for answers: “never”, “a little bit if the food is not salted to my taste”, “almost always, even before tasting”. The following questions were asked to assess an appropriate nutrition: “How many times do you eat per day?” Options for answers: “One”, “Two”, “Three”, “Four and more”. “Do you eat the same time every day?” – “yes” or “no”. “Do you think that your nutrition is appropriate (good) and you receive a sufficient amount of nutrients?” Options for answers: “Yes. I think so”, “No, I don't think so”, “I do not know”.

Assessment of life-style factors. To clarify the habits of alcohol use, the individuals were asked: “Do you drink alcohol?“ Options for answers: “Yes, often“, “Yes, sometimes”, “No”, “I used to have them before, but currently I do not drink alcohol”. If the answer was “Yes”, a responder was asked to specify his (her) preferences based on taste: which would you like more – beer, wine or liqueurs?

For the analysis of smoking habits, the individuals were asked: “Are you a smoker?“ Options for answers: “Yes, I smoke every day”, “Yes, I smoke sometimes”, “No”, “I used to smoke before, but currently I do not smoke”.

Life descriptions of four groups were presented aiming to get information about physical and movement activity at work; respondents were asked to select one option: “Your work is mainly sedentary and you do not walk much at work. For instance, you work at a desk, or you are engaged in assembling separate elements, etc.” (Group I); “You need to walk a lot at your workplace, but you do not need to carry on or to lift heavy items. For example, providing services for your clients, light industrial work,

working as a salesman, teaching, involving much walking" (Group II); "You are walking a lot, also you are lifting items or you need to climb stairs up and down many times. For example, working as a postman, in heavier industrial works, construction activities, heavier duties in a restaurant" (Group III); "You are engaged in physically hard jobs, lifting heavy items and physically intensive activities. For example, provision of nursing services, activities requiring lifting heavy objects and carring out heavy clearing activities" (Group IV).

Life descriptions of the four groups were presented aiming to get information about their physical activity and movement at leisure time; respondents were asked to select one option: "You spend your leisure time mainly reading, watching TV, visiting cinema or for other physically not demanding activities" (Group I); "At least 4 times per week you go for a walk, bicycling or are involved in other movement-related activities. This includes walking or bicycling to your work and back home, weekend walking, unhurried work in your garden, fishing, playing table-tennis or playing at skittles" (Group II); "You spend your leisure time by jogging, swimming, playing tennis, badminton, aerobics and being involved in other similar brisk sport activities". This group also covers heavier activities in a garden. "You must be engaged in such activities for at least 3 times per week" (Group III); "You spend you leisure time for heavy exercises and your participate regularly in races, orientation, skiing, swimming, football, handball competitions twice a week" (Group IV).

The individuals completed a questionnaire form and presented information about their education and occupation, nationality, date of birth, entered data indicating their height and weight. Individuals had to mark a chronic disease they had in the past or have had currently. Options for answers: "cardiovascular diseases", "elevated blood pressure (hypertension)", "acute cerebrovascular circulation disorder (stroke)", "diabetes mellitus", "obesity", "myopia", "cancer", "pulmonary diseases (asthma, chronic obstructive pulmonary disease)", "depression", "alcoholism", "skin diseases (psoriasis, dermatitis)", "other – describe if your diseases are not mentioned above", "I do not have any disease".

We also asked to specify whether their first-degree (siblings, parents) and second-degree relatives (half-sibs and their parents) had any of the above-mentioned chronic diseases and to indicate the date of disease manifestation.

We interviewed about the health problems (diseases) in the families, i.e. relatives of the study individuals third generation), but because of incomplete and insufficient data (individuals were not aware about these issues) this information was not used. We also failed in collecting enough data about the age when a disease manifested in a family (study subjects were not able to answer / they were not aware); therefore, we could not use this information for the statistical analysis of data.

The HAD (Hospital Anxiety and Depression) scale was used to identify anxiety and depression disorders. The HAD scale was translated into Lithuanian in 1991 and has been extensively used for the screening of depression and anxiety disorders.

The HAD scale consists of 14 questions; there are four options to answer each question. They are rated accordingly from 0 to 3 points. Seven questions have been designed to assess the depression symptoms and seven other questions the anxiety symptoms. The total sum of points in the group of depression and anxiety symptoms can vary from 0 to 21. The rating values demonstrate the degree of severity of depression or anxiety symptoms: 0 to 7 points – normal state of anxiety or mood, 8 to 10 points – mild, 11 to 14 – moderate, and 15 to 21 points – severe symptoms of anxiety and depression.

Before starting to mark a scale, patients had been asked to mark the answer which most accurately reflects his (her) condition during the last week.

Blood samples for biochemical lipid tests were taken from the ulnar vein directly into a tube (BD Vacutainer®). All study persons had to abstain from smoking, eating, drinking beverage and coffee for at least 12 hours. The tubes with collected samples were stored at room temperature for 10–15 minutes. Then blood samples underwent centrifuging for 10 minutes with a speed of 3000 rpm. The serum was transferred into tubes and kept at –80^{o} C in a freezing box until the biochemical assay was done. At the same day, blood samples of the study participants were sent to the Centre of Laboratory Medicine of the public institution Santariškių Clinics of the Vilnius University Hospital.

Blood samples were prepared for testing using the automatised sample preparation system “Genesis Fe500” (Tecan, Switzerland). The following tests were carried out at the Biochemistry Laboratory: total cholesterol (Ch), high density lipoprotein cholesterol (HDL-Ch), low density lipoprotein cholesterol (LDL-Ch), fasting glucose, high sensitivity C-reactive protein (hs-CRP), apolipoprotein A1 (Apo-A1), apolipoprotein B (Apo-B), lipoprotein (a) and Apo-B/Apo-A1 ratio. Unused remaining serum samples were dispensed into a storage tubes and frozen at the temperature of – 80^{o} C.

Glucose and hs-CRP levels in the blood serum were tested applying the immunoturbidimetry method (Architect ci8200, Abbott, USA). Total cholesterol triglycerides and HDL-Ch were measured applying the enzyme colorimetric assay, LDL-Ch was calculated according to Friedewald's equation, if TG > 4.5 as measured by a direct enzymatic clearance assay (Architect ci 8200, Abbott, USA). Blood serum concentrations of Lp (a), Apo-A1 and Apo-B were measured applying an immunonephelometric assay (BN II, Siemens, Germany).

Assessment of nutrition appropriateness was based on the WHO recommendations on healthy nutrition according to the following nine nutrition habits:

1. Consumption of fresh vegetables 6–7 times per week.
2. Consumption of boiled and stewed vegetables 3 times per week or more (3–5 times per week).
3. Consumption of cereals every day or several times per day.
4. Eating fish at least once per week.
5. Eating meat at least 3–5 times per week.
6. Drinking milk every day, several times per day.
7. Uuse of vegetable oil for cooking.
8. Compliance with the eating regimen (eating at the same time every day).
9. Eating three and more times per day.

Coding – 1, other – 0. Nutrition is maximally appropriate if the total score is 9.

The values of biochemical tests were used to assess dyslipidemia: the levels of total cholesterol were > 5 mmol/l or LDL-Ch > 3 mmol/l, or HDL-Ch < 1.0 mmol/ for males, < 1.2 mmol/l for females or TG > 1.7 mmol/l.

Body mass was assessed using the body mass index:

$$BMI = \text{weight (kg)} / \text{height (m)}^2.$$

The normal body mass index is 18.5 – 24.9 kg/m^2. The values 25 to 29.9 kg/m^2 indicate overweight and 30 kg/m^2 and above – obesity.

DNA used for the study was extracted from the leukocytes of the venous blood at the Department of Human and Medical Genetic of Medicine Faculty, Vilnius University.

Good laboratory practice principles were followed at the laboratory during testing, as well as requirements for external quality control, laboratory surveillance, preparation for laboratory testing and protocol.

Aiming to define the profile of the genetic markers of CVD of general Lithuanian population and to assess its genetic diversity, large-scale genotyping was carried out for 295 unrelated individuals. The genotyping was performed on the *llumina HiScan*^{TM}*SQ* instrument using the *Illumina Infinium* (manufacturer Illumina Inc., USA) *Ilumina HumanOmniExpress*-12 v1.1 SNP assays at the Vilnius University Medical Faculty Department of Human and Medical Genetic (VU MF DHMG). Individuals whose data on diseases in the past were confirmed with medical records in the randomised group were selected for genotyping. The further bioinformatics-statistical analysis of primary data was also carried out. The genotyping was carried out by the personnel of the VU MF DHMG.

The list of general alleles and genes posing risk of SNPs correlated with cardiovascular diseases – coronary heart disease, stroke, myocardial infarction – was created based on published data and the analysis of databases. The frequencies of alleles and genotypes predetermining the risk were established in the study population during the study.

All data collected about the individuals were transferred into the electronic storing device on the BC | GENE data management platform.

2.3. The methods of statistical analysis

Data obtained during the study were processed using the statistical software package SPSS (*Statistical package for Social sciences*, "SPSS Inc." USA, version 20.0 for Windows). Descriptive and analytical statistical methods were applied.

The data were described indicating the frequencies and relative frequencies of quantitative variables in percent as well as arithmetic mean values, mode, median, standard deviations (SD) and 95% confidence intervals (CI) for qualitative variables. The chi square (χ^2) criterion was applied to check the independence of qualitative variables, and the Fisher exact criterion was applied in case when the frequency of observations was low (less than three). To assess the normality of the distribution of interval variables, the Shapiro–Wilk test was applied. Differences of mean values of interval variables were compared between two independent randomised groups applying the t-Student analysis; a comparison of three randomised groups was performed applying a single factor dispersion analysis (ANOVA), and multiple comparisons were performed applying the post-hoc Fisher least significant difference criterion. A covariant analysis (ANCOVA) for the comparison of mean values among the groups was applied when additional variables potentially affecting interval variables had to be considered. The variables not meeting assumptions for the application of parametric methods were compared using *the* Mann–Whitney–Wilcoxon criterion. The Spearman correlation coefficient (r) was calculated to establish a correlation among ranked variables. Multiple logistic regression was applied to assess the effect of independent factors on dependable variables.

The significance level of 0.05 was chosen for the verification of statistical hypotheses. Two-sided *p* values were presented in all cases. The *Microsoft Office Excel 2007* software was used for the graphical presentation of the results.

<u>Other bioinformatics-statistical methods</u>

The study population was divided into two groups for the analysis of genetic associations: 33 individuals with cardiovascular diseases and 265 individuals without diseases.

The primary analysis of genetic data was carried out using a genome analysis software, genotyping module *Illumina GenomeStudio™v2011.1 Genotyping Module v1.9*. The input files were generated using this software for the entire genome association analysis software package *PLINK v1.90b2o 64-bit (25 Nov 2014)* (Purcell S et al., 2007; internet access (https://www.cog-genomics.org/plink2).

Fisher's exact test was used for the analysis of genetic associations using the PLINK software package. Command row: --assoc fisher --file NERINGAI --out 1 --pheno pheno.txt --snps rs1042031, rs10757274, rs11185660, rs11203042, rs11206510, rs11556924, rs11591147, rs1260326, rs1412444, rs150468, rs15563, rs17145738, rs17228212, rs174547, rs17464857, rs17465637, rs17514846, rs1801133, rs2023938, rs2048327, rs2075650, rs2197089, rs2252641, rs2259816, rs2271293, rs2306374, rs2383206, rs2383207, rs261332, rs2774279, rs3217992, rs3764261, rs4252120, rs4420638, rs445925, rs4773144, rs4775041, rs4775065, rs4845625, rs4977574, rs501120, rs562338, rs579459, rs5882, rs5918, rs599839, rs646776, rs662, rs688, rs6903956, rs693, rs7173743, rs732314, rs780094, rs7893395, rs9369640, rs9515203, rs974819, rs9818870, rs879324, rs2107595
An association analysis was used to perform the data quality analysis; all cases of SNP and control group cases enrolled in the study met quality requirements for the association analysis.

The association analysis was carried out the applying conventional allele test to compare the frequency of alleles in the case and control groups as the well as Cochran–Armitage trend, genotype, gene dominant action and gene recessive action tests, using the function of the *PLINK model*. The values of χ^2 and the appropriate p values were calculated. If less than 5 cases of any genotype were found, NA values were obtained. The Cochran–Armitage test is designed to calculate the association among the genotypes regardless of the Hardy–Weinberg equilibrium, i.e. preference in this analysis is given to an individual rather than to the allele (NA values are not obtained in this case). The

genotype test is designed to calculate associations according to genotype frequencies and considering the Hardy–Weinberg equilibrium. The gene dominant action and gene recessive action tests were carried out for rare allele, i.e. if the D allele was the risk allele (and *d* is an alternative to the risk allele). Then,

dominating (DD, Dd) *versus* dd

recessive DD *versus* (Dd, dd)

In this analysis, allele frequencies, p-values for Fisher's test and odds ratio were calculated both in the group of individuals suffering from CVD and healthy ones. The frequencies of tested SNP genotypes were calculated based on the number of direct cases using the formula: a/x, b/x, c/x, where a is the number of homozygous genotypes, b is the number of heterozygous genotypes, c is the number of alternative homozygous genotypes, x is the number of the study subjects for the studies genotype (in the groups of individuals with and without CVD).

All SNPs in both case and control groups were consistent with the Hardy–Weinberg equilibrium. The Hardy–Weinberg equilibrium is calculated applying the exact test (Wigginton J. E. et al., 2005), using the function of the PLINK software – *hardy*. The significance level was 0.001. If SNPs genotypes and allele frequencies are consistent with the Hardy–Weinberg equilibrium, the distribution follows the rule: $p^2 + 2pq + q^2 = 1$ and $p + q = 1$, when p is the frequency of a single allele, q is the frequency of the alternative allele, p^2 is the frequency of single allele homozygotes, q^2 is the frequency of alternative allele homozygotes, and 2pq is the frequency of heterozygotes.

To dertermine the reliability of the questionnaire designed for the survey, the test–retest method was used following 30 days after the first survey. Fifty individuals were re-tested. The intraclass correlation coefficient (ICC) was calculated for the comparison of interval values; CI and p values were assessed. The Kappa coefficient was calculated for qualitative variables, standard errors, and the p-value was calculated for these rates.

The assumption was used for the calculation of the study sample that the prevalence of CVD in the adult population is 0.12 (12 percent).

Applying the alpha-error level of 0.05, the confidence level (1-alpha), the value precision equal to 0.05, the minimal study sample in 40–65 year old population should be at least 163 subjects, (calculated on-line: http://www.raosoft.com/samplesize.html).

3. RESULTS

3.1. Characteristics of the study population

In total, 469 individuals aged 40–65 years were interviewed and their personal data were analysed. 32.7 percent of all individuals had higher education, 0.7 percent had primary education, 15.3 percent had secondary and 51.4 special secondary education. Individuals living in the urbanised area made 69.2 percents of the respondents (30.8 percent of the respondents were from rural areas). Individuals of Lithuanian origin made up 97.6 percent of all study population.

The mean age of the study individuals was 48.59 ± 6.14 years. The clinical characteristics of the individuals are presented in **Table 3.1.1**.

Table 3.1.1. Clinical characteristics of the individuals

Indicator	**N**	**Mean ± SD**	**Minimal value**	**Maximal value**
Age, years	469	48.59 ± 6.14	40	65
Height (cm)	469	172.05 ± 8.63	148	201
Body mass, kg	469	83.54 ± 17.21	47	170
BMI, kg/m^2	469	28.13 ± 4.88	18.08	51.32
Glucose (mmol/l)	469	5.68 ± 1.51	3.71	23.97
Cholesterol (mmol/l)	469	6.24 ± 1.09	2.98	10.49
HDL-Ch (mmol/l)	469	1.41 ± 0.37	0.70	2.94
LDL-Ch (mmol/l)	469	4.12 ± 0.97	1.39	7.58
Triglycerides (mmol/l)	469	1.57 ± 1.35	0.37	17.37
Apo-A1, g/l	469	1.61 ± 0.27	0.96	2.46
Apo-B, g/l	469	1.06 ± 0.26	0.45	2.03
Apo-A / Apo-B	469	1.63 ± 0.56	0.65	4.55
LP(a), g/l	469	0.19 ± 0.26	0.02	1.37
hs CRP, mg/l	469	2.42 ± 3.94	0.20	37.60

SD – standard deviation; BMI – body mass index; HDL-Ch – high density lipoprotein cholesterol; LDL-Ch – low density lipoprotein cholesterol; hs-CRP – high sensitivity C-reactive protein; Apo-A1 – apolipoprotein A1; Apo-B – apolipoprotein B; LP(a) – lipoprotein (a).

Of the study individuals, 94% (231) were males and 50.7% (238) were females. A comparison of clinical data of males and females is presented in **Table 3.1.2**.

Table 3.1.2. Comparison of clinical date of males and females, mean value ± SD

Indicator	**Males (n = 231)**	**Females (n = 238)**	**p**
Age, years	49.02 ± 6.35	48.18 ± 5.91	0.136
Height (cm)	177.98 ± 6.29	166.30 ± 6.42	**< 0.0001**
Body mass, kg	90.12 ± 17.02	77.16 ± 14.86	**< 0.0001**
BMI, kg/m^2	28.36 ± 4.53	27.90 ± 5.20	0.315

SD – standard deviation; BMI – body mass index.

The age of males and females was not statistically significantly different. The heigh and weight of males was higher than those indicators of females, but no statistically significant difference in BMI between males and females was detected.

3.2. Analysis of risk factors in study individuals suffering from cardiovascular diseases and in individuals without CVD

In our study groups, 12.2% (57) were diagnosed with cardiovascular diseases, 49.1% (28) of them were males and 50.9% (29) females. The incidence of CVD was similar both in males and females ($p = 0.983$); 13.0% (42) of individuals with CVD were from urbanised areas and 10.4% (15) were from villages. No statistically significant difference in the incidence of CVD among people living in cities and rural areas was discovered ($p = 0.437$); 14.0% (42) of individuals having primary, secondary or special secondary education had CVD, and among individuals with higher education CVD was diagnosed in 9.0% (13) of individuals. The incidence of CDV among individuals with primary, secondary or special secondary education was similar ($p = 0.127$).

The mean age of individuals with CVD was 53.16 ± 5.98 years and of those without CVD 47.96 ± 5.89 years. The study individuals with CVD were statistically significantly older by 5.20 years on average ($p < 0.0001$). The mean age of males diagnosed with CVD was 52.54 ± 6.25 years. Males with CVD were statistically significantly older by 4.00 years on average than males without CVD ($p = 0.004$). The

mean age of females diagnosed with CVD was 53.76 ± 5.57 years. Females with CVD were statistically significantly older by 6.36 years on average than males without CVD ($p < 0.0001$). No statistically significant difference in the age of males and females diagnosed with CVD was discovered ($p = 0.446$).

The incidence of arterial hypertension (AH) was 31.1%. AH was diagnosed in 45.6% of individuals with CVD and in 29.1% of individuals free of CVD. Arterial hypertension was diagnosed in individuals with CVD and was reported more frequently than in individuals without CVD, and this difference was statistically significant ($\chi^2(1) = 6.35$, $p = 0.012$).

The incidence of diabetes mellitus (DM) in the study population was 4.7%. Among the individuals with CVD, diabetes mellitus was diagnosed in 8.8% of them; diabetes mellitus was diagnosed in 4.1% of individuals without CVD ($\chi^2(1) = 2.42$, $p = 0.120$).

During the interview, 15.4% (72) of individuals indicated that they were obese. Normal BMI was found in 26.4 of individuals, 46.9% of individuals were overweighted, and 26.7% were obese. The incidence of normal body mass, overweight and obesity among individuals with and without CVD did not reach the statistically significant difference ($\chi^2(2) = 2.11$, $p = 0.349$) (**Fig. 3.2.1**).

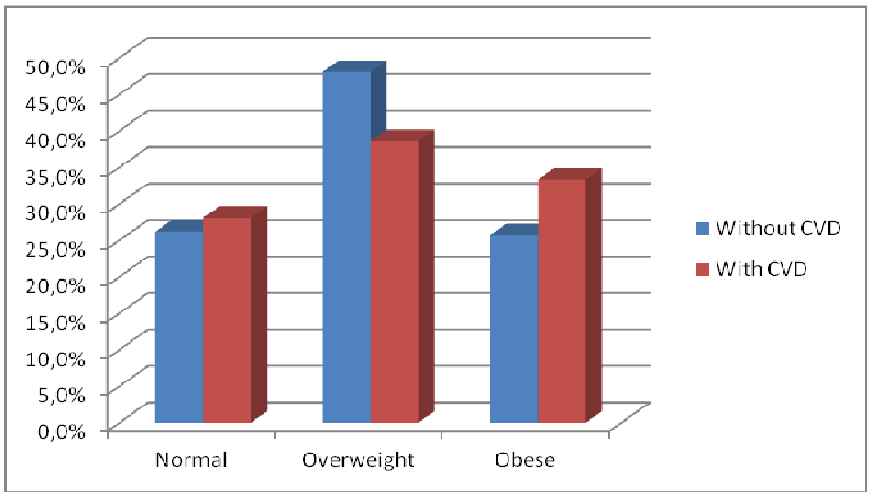

Fig. 3.2.1. BMI distribution in the groups of individuals with and without CVD ($p = 0.349$, χ^2 was used for a comparison among the groups).

The incidence of dyslipidemia reported during the study reached 94.2%, it was diagnosed in 442 individuals. In 93.9% of all cases a mixed type dyslipidemia was diagnosed. Blood lipid levels were normal in 27 (5.8%) individuals. Dyslipidemia was found in 94.8% (219) of males and in 93.7% (223) of females (p = 0.607). Dyslipidemia was diagnosed in 94.7% (54) of individuals with CVD and in 94.2% (388) of individuals without CVD. No statistically significant difference of dyslipidemia between individuals with and without CVD was detected (p = 0.864).

Life-style risk factors

As indicated by interviews on harmful behavioural habits, 79.3% (365) of respondents sometimes used alcohol, 6.1% (28) were using alcohol frequently, and 9.3% (43) did not use alcohol at all. More than half of the study individuals – 59.9% (260) – were non-smokers, 17.5 (80) had a smoking history but gave up smoking. Smokers made up 25.6% (117) of all study individuals.

Table 3.2.1. Comparison of life-style risk factors in individuals with and without CVD

Indicator	**N (%)**	**With CVD, N (%)**	**Without CVD, N (%)**	**p**
Consumption of alcohol, N = 460				0.001
Yes, often	28 (6.1)	2 (3.5)	26 (6.5)	
Yes, sometimes	365 (79.3)	37 (64.9)	328 (81.4)	
Do not consume	43 (9.3)	12 (21.1)	31 (7.7)	
Do not consume, but used before	24 (5.2)	6 (20.5)	18 (4.5)	
Smoking, N = 457				0.716
Yes, every day	80 (17.5)	10 (17.5)	70 (17.5)	
Yes, sometimes	37 (8.1)	6 (10.5)	31 (7.8)	
Not smoking	260 (56.9)	29 (50.9)	231 (57.8)	
Former smoker	80 (17.5)	12 (21.1)	68 (17.0)	
Activity at work, N = 449				0.077
Not active	140 (31.2)	12 (22.2)	128 (32.4)	
Walking	161 (35.9)	21 (38.9)	140 (35.4)	
Active	92 (20.5)	9 (16.7)	83 (21.0)	
Physically hard work	56 (12.5)	12 (22.2)	44 (11.1)	
Activity at leisure time, N4 = 51				0.335
Not active	174 (38.6)	25 (44.6)	149 (37.7)	
Walking	220 (48.8)	28 (50.0)	192 (48.6)	
Active	54 (12.0)	3 (5.4)	51 (12.9)	
Heavy exercises	3 (0.7)	0 (0)	3 (0.8)	

On the basis of the results obtained by interviewing the study individuals about their physical activity, 38.6% (174) of respondents were physically passive during their leisure time. Talking about forms of physical activities, the majority of individuals – 48.8% (220) – preferred walking, 12.0% and 0.7% of individuals accordingly were involved in active sport and in intensive workout. Every third study individual – 31.3% (140) – was engaged in a sedentary job. 35.9% (161) of individuals specified that they needed to walk a lot at their work. Among the individuals, 20.5% (92) were engaged in more intensive physical activities at work, and 12.5% (56) of respondents had duties associated with hard physical work (**Table 3.2.1**).

A statistically significant difference among the groups of individuals with and without CVD was discovered in this study in alcohol use only ($\chi^2(3) = 15.47$, $p = 0.001$). Statistically significant differences in the frequency of other life-style risk factors were not detected among the groups, either.

Family history of CVD

We strove to assess family CVD risk factors during this study. An important part in the questionnaire covered chronic diseases prevalent in a family. We aimed to find out whether the first-degree relatives (parents, siblings) and second-degree relatives (grandparents) of the individuals had CVD. The medical history of CVD, diagnosed in at least one relative, was found in 62.9% (295) of the study subjects, in 73.7% (42) of the study subjects with CVD, and in 61.4% (253) of individuals without CVD. The medical history of CVD was found statistically significantly more often among individuals with CVD ($\chi^2(1) = 3.23$, $p = 0.047$).

CVD in first-degree relatives of the individuals with CVD was diagnosed statistically significantly more frequently ($\chi^2(1) = 3.82$, $p = 0.034$): 66.7% of the study subjects with CVD have specified that their first-degree relatives have / had CVD; CVD in first-degree relatives was reported by 52.9% of individuals without CVD (**Fig. 3.2.2)**.

If at least one first-degree relative had a CVD, the odds ratio (OR) for a proband to get a CVD was 1.78 (95% CI 0.99 – 3.19).

Analysis of a correlation between the CVD morbidity of first-degree relatives and the CVD morbidity of a proband demonstrated that the incidence of cardiovascular diseases in mothers ($\chi^2(1) = 4.13$, $p = 0.042$) and sisters ($\chi^2(1) = 8.67$, $p = 0.003$) of probands with a CVD was statistically significantly higher than in mothers and sisters of probands without a CVD. If the mother had a CVD, the odds ratio (OR) for a proband to get a CVD was 1.79 (95% CI 1.02 – 3.16). If a sister had a CVD, the odds ratio (OR) for a proband to get a CVD was 4.86 (95% CI 1.53 – 15.40).

The incidence of CVD in fathers ($\chi^2(1) = 0.77$, $p = 0.380$) or brothers ($\chi^2(1) = 0.80$, $p = 0.559$) of probands with and without a CVD was similar (**Fig. 3.2.2**).

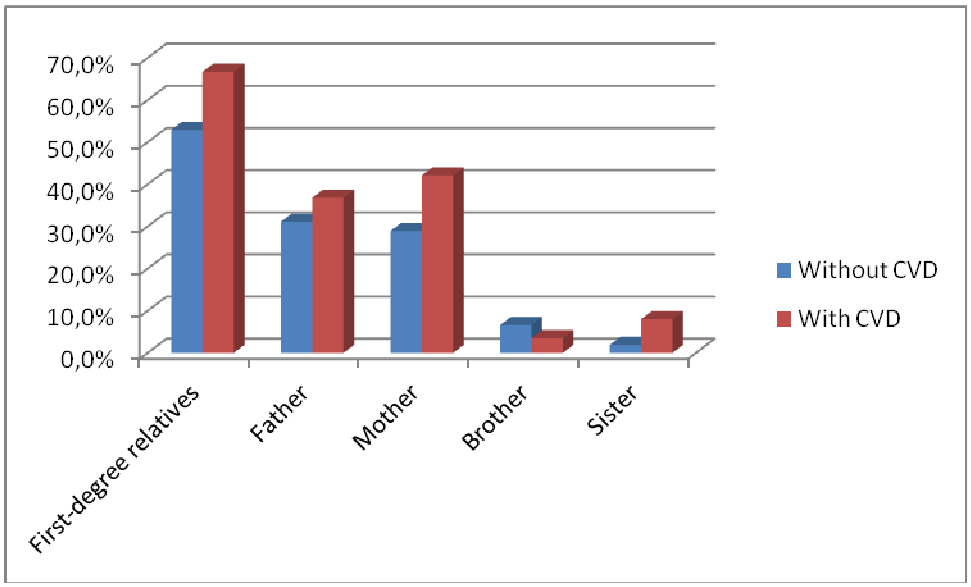

Fig. 3.2.2. CVD frequency among the first-degree relatives of individuals with and without CVD

We asked whether someone of grandparents (second-degree relatives) had a CVD. In case of the positive medical history of the mother's mother ($\chi^2(1) = 0.001$, $p = 0.64$) or mother's father ($\chi^2(1) = 0.76$, $p = 0.385$), or father's mother ($\chi^2(1) = 0.22$, $p = 0.643$) or father's father ($\chi^2(1) = 0.16$, $p = 0.599$), statistically no significant differences in the incidence of CVD in probands were discovered (**Fig. 3.2.3**).

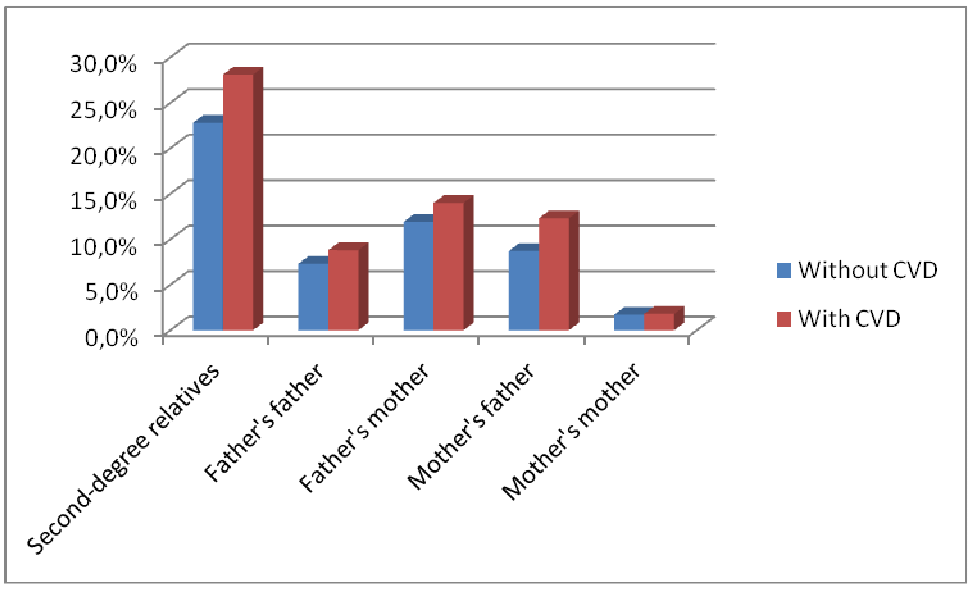

Fig. 3.2.3. CVD frequency among second-degree relatives of individuals with and without CVD

A correlation between CVD morbidity and the number of disease-affected relatives was also analysed. No statistically significant correlation between the incidence of CVD in probands and the number of relatives with CVD was detected ($\chi^2(5) = 7.72$, $p = 0.172$).

3.3. Comparison of nutrition habits of study individuals with and without cardiovascular disease

Nutrition habits were assessed applying the food frequency test method. We found that the main criteria for food product selection in the population of Lithuania (2011–2013) were the gustatory properties of food (54.2%) and the influence of family members (35.2%), and only every fourth person (23%) used to select food products for health improving purposes. Statistically significant differences in food selection criteria between individuals with and without CVD were not detected. Females more often than males used to choose food products for better health (disease prevention) ($\chi^2(1) = 8.38$, $p = 0.004$) and due to the need of a specific diet ($\chi^2(1) = 9.24$, $p = 0.002$), and males

more often than females choose food products on the basis of their price ($\chi^2(1) = 4.08$, $p = 0.043$); both these differences were statistically significant.

The frequency of the use of fresh, boiled and stewed vegetables is presented in **Table 3.3.1**.

Table 3.3.1. Habits of vegetable use

Frequency of use	**Fresh vegetables, N (%)**	**Boiled and stewed vegetables, N (%)**
Not once	6 (1.3)	24 (5.7)
1–2 times per week	184 (39.8)	258 (61.6)
3–5 times per week	203 (43.9)	119 (28.4)
6–7 times per week	69 (14.9)	18 (4.3)

The frequency of eating fresh vegetables in the groups of individuals with and without CVD was similar ($\chi^2(3) = 1.70$, $p = 0.637$). A statistically significant difference was discovered in the frequency of the consumption of fresh vegetables depending on sex ($\chi^2(3) = 12.66$, $p = 0.005$).

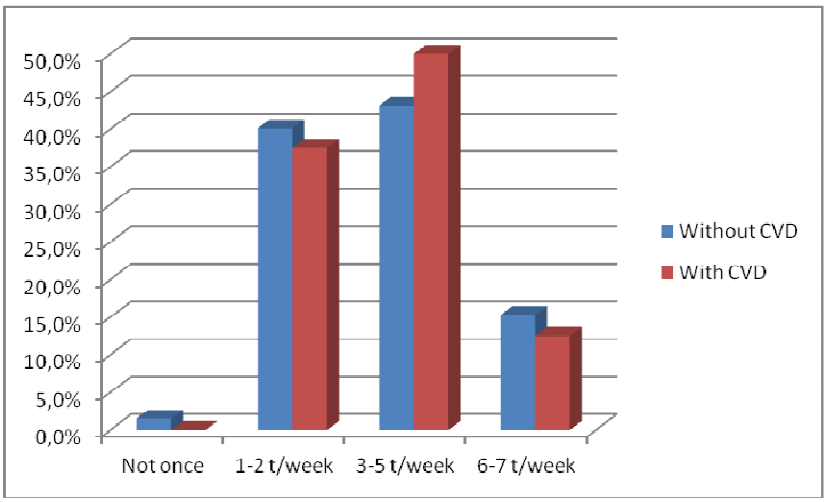

Fig. 3.3.1. Habits of eating fresh vegetables in the groups of study individuals with and without CVD ($p = 0.637$, χ^2 was used for the comparison between groups).

It was noticed that the use of fresh vegetables was insufficient among the adults living in Lithuania: only 14.9% of respondents have indicated that they eat fresh

vegetables every day or almost every day as recommended by the WHO. The consuption of fresh vegetables every day or almost every day indicated 12.3% of males and 17.4% of females. 12.5% (7) of individuals with CVD and 15.3% (62) of individuals without CVD used to eat fresh vegetables every day or almost every day ($\chi^2(1) = 0.30$, $p = 0.586$).

No statistically significant differences in the use of boiled and stewed vegetables between the groups of individuals with and without CVD ($\chi^2(3) = 3.58$, $p = 0.310$) and between males and females ($\chi^2(3) = 2.75$, $p < 0.432$) were discovered.

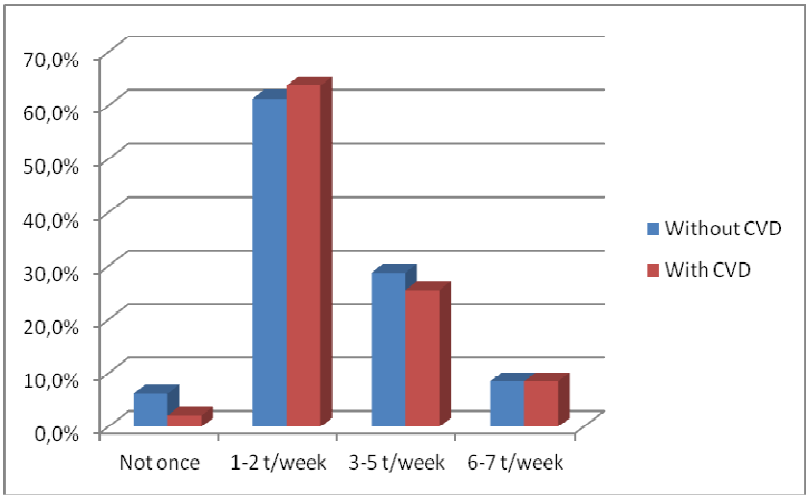

Fig. 3.3.2. Habits of eating boiled or stewed vegetables in the groups of individuals with and without CVD ($p = 0.310$, χ^2 was used for a comparison between the groups).

It was discovered that in total 32.7% of respondents, 34.0% with and 32.5% without CVD ate boiled and stewed vegetables three or more times per week (**Fig. 3.3.2**).

The frequency of consumption of cereals is presented in **Figure 3.3.3**.

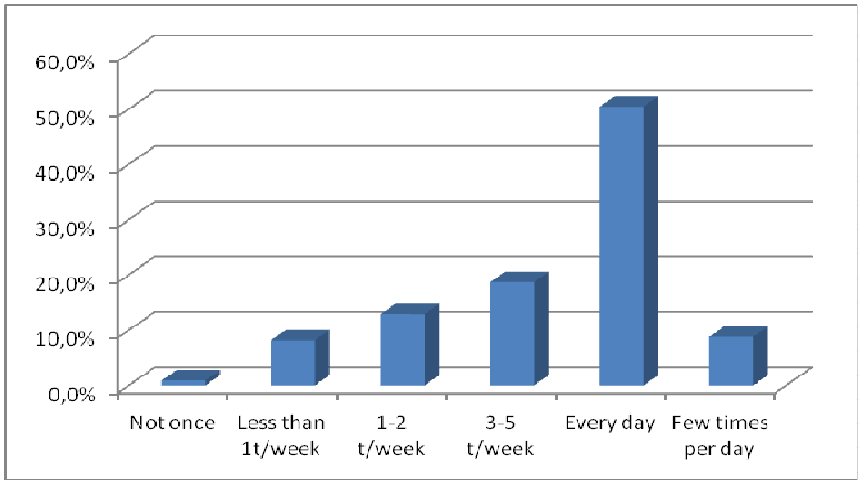

Fig. 3.3.3. Habits of cereal consumption

The frequency of cereal use was gender-dependent ($\chi^2(5) = 15.93$, $p = 0.007$), but in the groups of individuals with and without CVD it was similar ($\chi^2(5) = 2.61$, $p = 0.760$). (**Fig. 3.3.4.)**

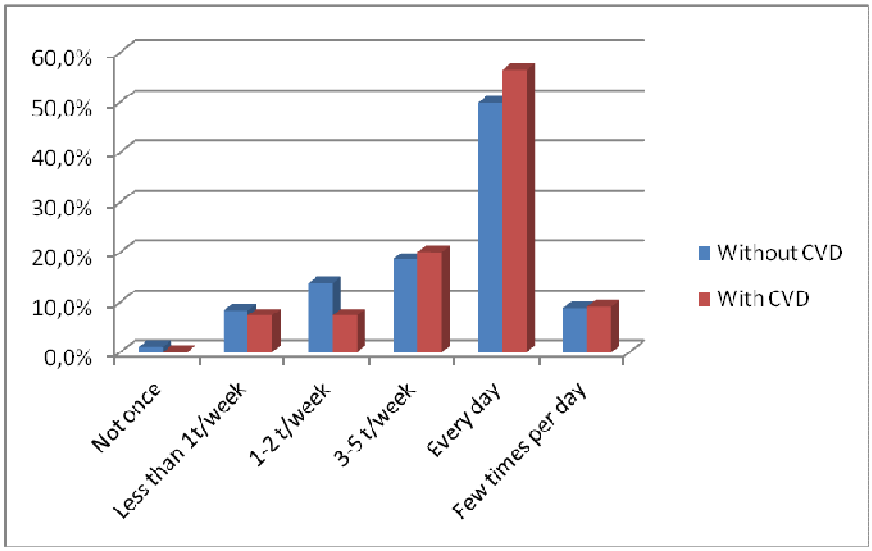

Fig. 3.3.4. Habits of cereal consumption in the groups of individuals with and without CVD ($p = 0.760$, χ^2 was used for a comparison between the groups).

We demonstrated that 59.4% of respondents ate cereals (including bread and cereal porridge) every day. Consumption of cereals every day was reported by 65.5% of respondents with CVD and by 58.8% of respondents without CVD ($\chi^2(1) = 0.96$, $p = 0.327$) as well as by 65.4 of males and 53.6% of females ($\chi^2(1) = 6.67$, $p = 0.010$).

Every third citizen of Lithuania (37.4%) ate fish and fish products 1–2 times a week. Only 5.3% of individuals ate fish 3–5 times a week and every second individual (55.1%) ate fish less than once a week (**Fig. 3.3.5.).**

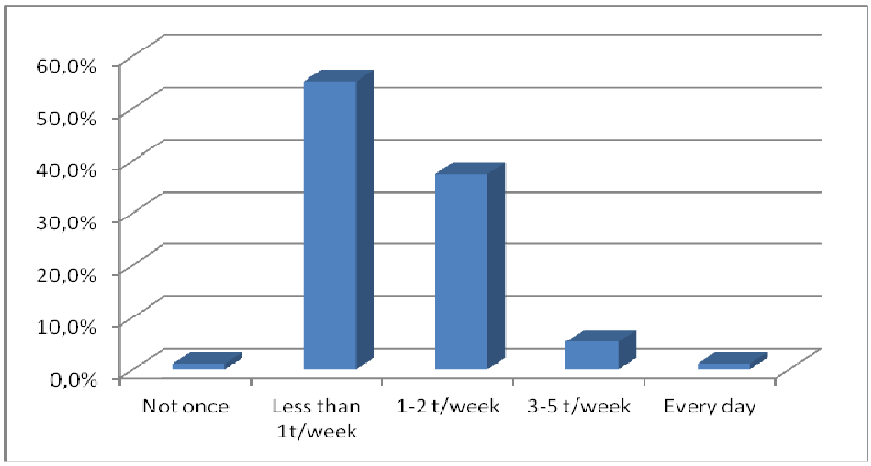

Fig. 3.3.5. Habits of using fish and fish products.

The frequency of fish and fish product use was different in the groups of individuals with and without CVD ($\chi 2(4) = 10.82$, $p = 0.029$) (**Figure 3.3.6)**, but was similar in groups of males and females ($\chi^2(4) = 2.98$, $p = 0.562$). The consumption of fish at least once a week was reported by 47.4% of respondents with CVD and by 43.3% of respondents without CVD ($\chi^2(1) = 0.34$, $p = 0.563$) as well as by 43.5% of males and 44.1% of females ($\chi^2(1) = 0.19$, $p = 0.889$).

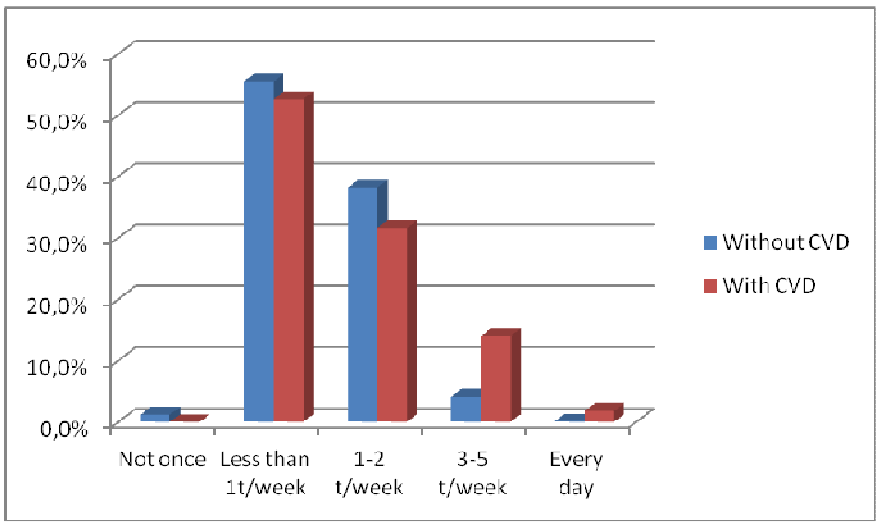

Fig. 3.3.6. Consumption habits of fish and fish products in the groups of individuals with and without CVD ($p = 0.029$, χ^2 was used for a comparison between the groups).

The consumption of meat and meat products every day was reported by 34.1% of respondents **(Figure 3.3.7**). The frequency of the consumption of meat and meat products was statistically significantly different in the groups of individuals with and without CVD ($\chi 2(5) = 12.88$, $p = 0.025$) and between males and females ($\chi 2(5) = 25.16$, $p < 0.0001$) (**Figure 3.3.8**).

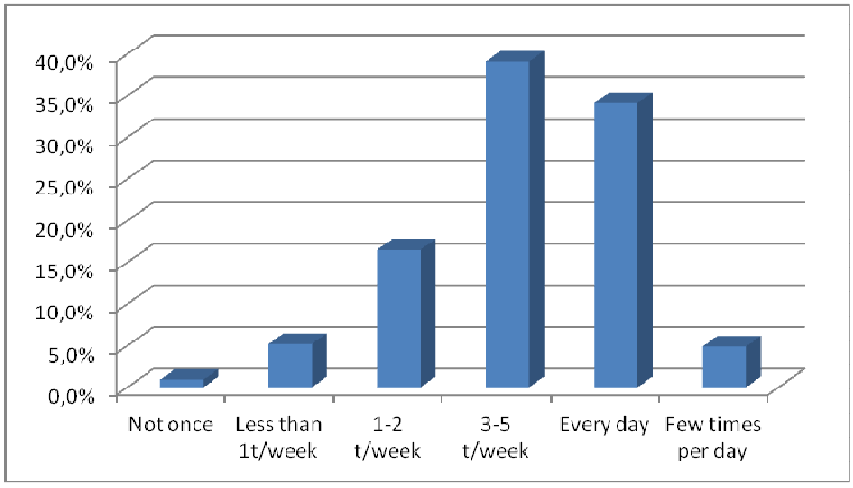

Fig. 3.3.7. Habits of using meat and meat products.

Following the recommendations of the WHO, consumption of meat and meat products 3–5 days per week was reported by 38.6% individuals with CVD and by 39.1% of individuals without CVD. Consumption of meat and meat products more than 3–5 times per week was reported by 28.1% of individuals with CVD and by 40.6% of those without CVD **(Figure 3.3.8**). 38.7% of males and 29.9% of females ate meat and meat products every day, and 8% of males and 2.1% of females ate meat even several times per day.

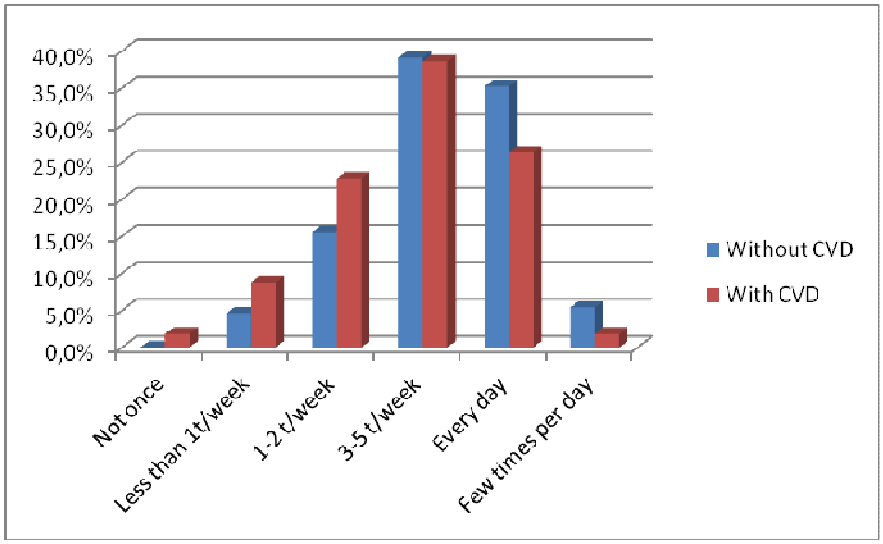

Fig. 3.3.8. Consumption habits of meat and meat products in the groups of individuals with and without CVD ($p = 0.025$, χ^2 was used for a comparison between the groups).

The majority of females (61.3%) have indicated that they prefer poultry, and the majority of males (67.7%) have stated that pork for them is more tasteful ($\chi^2(2) = 2.42$, $p < 0.0001$). Answering the question about the preferable method of meat processing, more than half of the respondents have stated that they like to eat fried meat (56.9% of males and 51.5% of females). Stewed meat was preferred by 44.5 % of females and only by every third male (32.2%) ($\chi^2(2) = 12.23$, $p = 0.002$). No statistically significant differences between the individuals with and without CVD in the use of different types of meat ($\chi^2(2) = 5.00$, $p = 0.082$) or the meat processing method ($\chi^2(2) = 1.72$, $p = 0.424$) were discovered.

The analysis of the frequency of using instant soups and bouillon cubes demonstrated that 30% of respondents used these products less than once per week, and 65.5% of females and 59.2% of males abstained from their use ($\chi^2(5) = 11.12$, $p = 0.049$). The frequency of using instant soups and bouillon cubes by the individuals with and without CVD was similar ($\chi^2(5) = 2.60$, $p = 0.762$).

Eggs as a separate serving 1–2 times per week were consumed by 44.1% of respondents, among them there were 46.7% males and 41.9% females. Consumption of eggs less than once per week was reported by 41.9% of respondents; 35.8% of them

were males, and 47.9% were females. Males more frequently (4.8%) ate eggs every day, as compared with females (0.8%) ($\chi^2(4) = 19.28$, $p < 0.0001$). The frequency of egg consumption by individuals with and without CVD was similar ($\chi^2(4) = 0.94$, $p = 0.919$).

Every fourth citizen in Lithuania (24%) is used to drinking milk and eating dairy products every day, and 3.9% of respondents did not use these products (**Fig. 3.3.9**). No statistically significant differences in the use of milk between the groups of individuals with and without CVD ($\chi^2(5) = 6.05$, $p = 0.302$) and between males and females ($\chi2(5) = 6.12$, $p < 0.294$) were discovered.

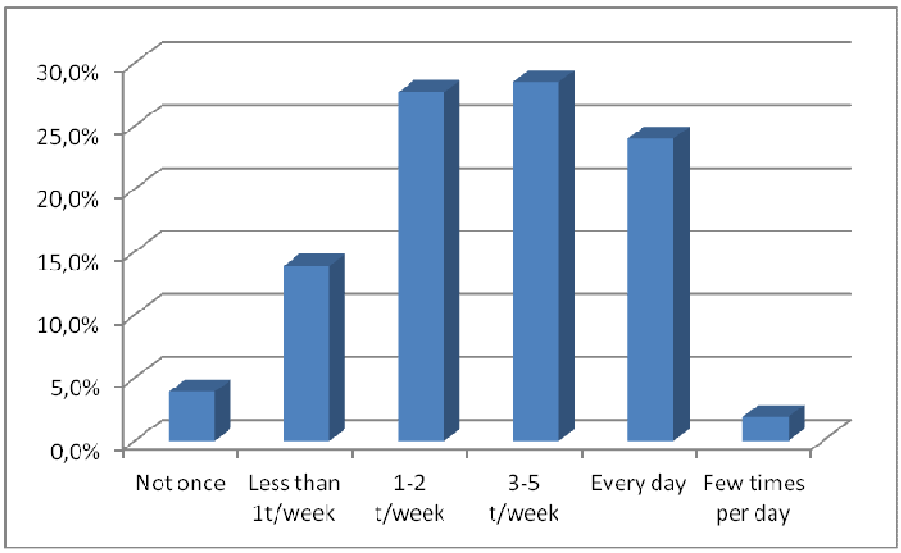

Fig. 3.3.9. Habits of using milk and dairy products

We have found that a smaller proportion of Lithuanian population (38%) do not salt food before eating (26.1% of males and 49.6% of females). Additionally, salt was almost always used by 4.9% of respondents. Females more frequently than males abstained from an additional use of salt ($\chi^2(2) = 31.25$, $p < 0.0001$), and this difference was statistically significant (**Fig. 3.3.10**). Statistically significant differences in salt use habits between individuals with and without CVD were not discovered ($\chi^2(2) = 1.06$, $p = 0.588$).

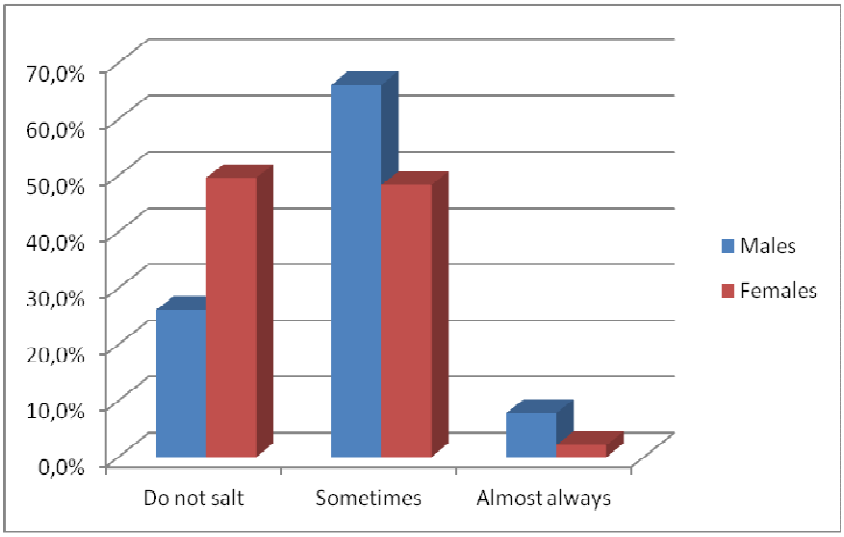

Fig. 3.3.10. Habits of salt use according to gender ($p < 0.001$, χ^2 was used for the comparison between groups).

This study has shown that the majority of individuals (88.3%) in the Lithuanian population used vegetable oil for cooking; 3.9% preferred using butter, 0.9% – margarine, and 6.9% – fat of animal origin. Statistically significant differences in the use of differed fat types between the individuals with and without CVD were not discovered ($\chi^2(3) = 0.94$, $p = 0.816$). However, the frequency of using various fat types was different between genders ($\chi^2(3) = 11.05$, $p = 0.011$): males, as compared with females, were more frequently using butter and animal fat and less frequently vegetable oil.

The consumption of confectionery products every day was reported by 15.4% of respondents. Every third individual (33.3%) used to eat confectionery products 1–2 times per week (35.2% of males and 31.5% of females). The consumption of confectionery products was similar between the individuals with and without CVD ($\chi^2(5) = 7.24$, $p = 0.203$) and between genders ($\chi^2(5) = 3.79$, $p = 0.579$).

The majority of individuals (73.5%) have stated that they do not eat at the same time every day. 76.3% of individuals sometimes take snacks between the main meals, and 15.7% of the individuals always have such snacks. The assessment of nutrition appropriateness has revealed that 50.2% of females and 30.4% of males consider their nutrition to be inappropriate. Among the individuals with and without CVD, 33.3% and 41% of them, accordingly, stated that their nutrition was inappropriate. The frequency of

nutrition appropriateness assessment was gender-dependent ($\chi^2(2) = 19.05$, $p < 0.0001$) (**Fig. 3.3.11**).

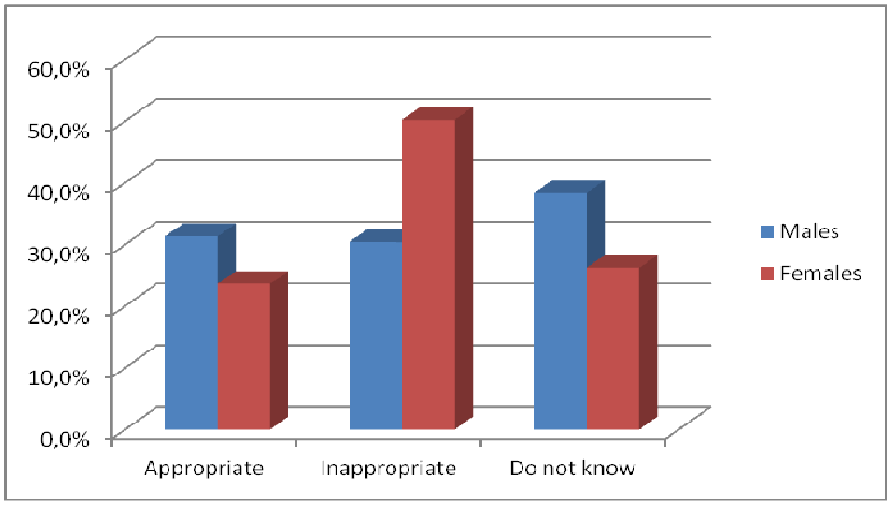

Fig. 3.3.11. Frequency of nutrition appropriateness assessment according to gender ($p < 0.0001$, $\chi 2$ was used for the comparison of the groups).

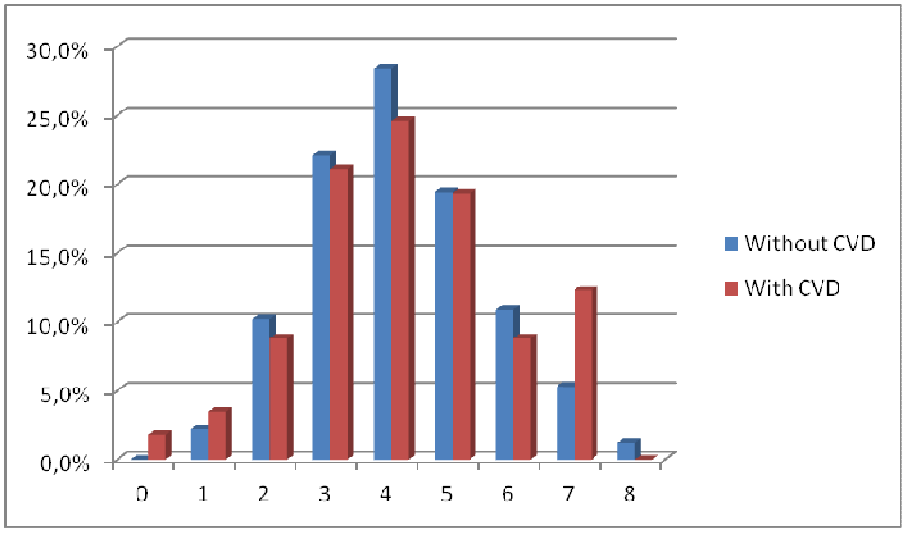

Fig. 3.3.12. Distribution of appropriate nutrition habits between the individuals with and without CVD ($p < 0.409$, $\chi 2$ was used for the comparison of the groups).

Groups of individuals with and without CVD were assessed according to the characteristics of appropriate nutrition (the maximal total score 9). No statistically significant difference in the frequency of appropriate nutrition between the groups was detected ($\chi^2(8) = 8.25$, $p = 0.409$) (**Fig. 3.3.12**).

3.4. The biochemical markers of atherosclerosis in the groups of individuals with and without cardiovascular diseases

A comparison of clinical data and laboratory test values between the individuals with and without CVD is presented in **Table 3.4.1**.

Table 3.4.1. Comparison of clinical data and laboratory test values between the individuals with and without CVD, mean value ± SD

Indicator	Suffering from CVD, N = 57	Not suffering from CVD, N = 412	p
Age, years	53.16 ± 5.98	47.96 ± 5.89	**<0.0001**
Height (cm)	172.53 ± 9.13	171.98 ± 8.57	0.689
Body mass, kg	84.76 ± 17.96	83.37 ± 17.12	0.393
BMI, kg/m^2	28.34 ± 4.72	28.1 ± 4.91	0.374
Glucose(mmol/l)	6.07 ± 2.76	5.63 ± 1.23	0.078
Cholesterol (mmol/l)	6.08 ± 1.31	6.26 ± 1.06	0.216
HDL-Ch (mmol/l)	1.34 ± 0.36	1.42 ± 0.37	0.146
LDL-Ch (mmol/l)	4.01 ± 1.14	4.14 ± 0.94	0.297
Triglycerides (mmol/l)	1.56 ± 1.06	1.57 ± 1.39	0.874
Apo-A1, g/l	1.56 ± 0.27	1.62 ± 0.27	0.134
Apo-B, g/l	1.03 ± 0.29	1.06 ± 0.25	0.336
Apo-A1 / Apo-B	1.65 ± 0.64	1.63 ± 0.55	0.963
LP(a), g/l	0.24 ± 0.30	0.18 ± 0.25	0.238
hs CRP, mg/l	3.22 ± 5.71	2.31 ± 3.63	0.060

CVD – cardiovascular diseases; SD – standard deviation; BMI – body mass index; HDL-Ch – high density lipoprotein cholesterol; LDL-Ch – low density lipoprotein cholesterol; hs-CRP – high sensitivity C-reactive protein; Apo-A1 – apolipoprotein A1; Apo-B – apolipoprotein B; LP(a) – lipoprotein (a).

Individuals with CVD were statistically significantly older (by 5.2 years on average) to compare with those without CVD ($p < 0.0001$). No statistically significant differences in biochemical blood parameters between the groups of individuals with and without CVD were detected.

A covariant analysis (ANCOVA) was applied to eliminate the impact of age in the comparison of mean values between the groups when an additional variable – age – was used, potentially affecting other variables.

Table 3.4.2. Comparison of clinical data and laboratory test values between the individuals with and without CVD considering age

Indicator	Suffering from CVD, N = 57		Not suffering from CVD, N = 412		p
	Mean	95 proc. CI	Mean	95 proc. CI	
Height (cm)	172.99	170.66–175.32	171.92	171.08–172.76	0.400
Body mass, kg	83.81	79.17–88.45	83.50	81.83–85.17	0.903
BMI, kg/m^2	27.82	26.51–29.12	28.17	27.7–28.64	0.619
Glucose (mmol/l)	5.91	5.51–6.32	5.65	5.51–5.80	0.232
Ch (mmol/l)	6.02	5.72–6.31	6.27	6.17–6.38	0.113
HDL-Ch (mmol/l)	1.35	1.25–1.45	1.42	1.39–1.46	0.161
LDL-Ch (mmol/l)	3.93	3.68–4.20	4.15	4.05–4.24	0.142
TG (mmol/l)	1.57	1.21–1.94	1.57	1.44–1.71	0.997
Apo-A1, g/l	1.56	1.48–1.63	1.62	1.59–1.64	0.126
Apo-B, g/l	1.02	0.95–1.09	1.06	1.04–1.09	0.232
Apo-A1 / Apo-B	1.68	1.52–1.83	1.62	1.57–1.68	0.537
LP(a), g/l	0.22	0.15–0.29	0.19	0.16–0.21	0.396
hs CRP, mg/l	3.08	2.02–4.14	2.33	1.94–2.71	0.194

CVD – cardiovascular diseases; CI – confidence interval; BMI – body mass index; Ch – cholesterol; HDL-Ch – high density lipoprotein cholesterol; LDL-Ch – low density lipoprotein cholesterol; hs-CRP – high sensitivity C-reactive protein; Apo-A1 – apolipoprotein A1; Apo-B – apolipoprotein B; LP(a) – lipoprotein (a)

The mean values were adjusted considering the mean age – 48.59 years.

After elimination of age effects, no statistically significant differences in the biochemical blood parameters between the groups of individuals with and without CVD were detected (**Table 3.4.2)**.

Groups of males and females with and without CVD were compared. Females with CVD were older by 6.36 years and males by 4 years on average as compared with individuals of the same gender without CVD ($p < 0.0001$ and $p = 0.003$, respectively); these differences were statistically significant. Blood glucose concentration in males with CVD was statistically significantly higher than in males without CVD ($p < 0.04$) (**Tables 3.4.3 and 3.4.4**).

Table 3.4.3. Comparison of clinical data and laboratory test values between females with and without CVD, mean value ± SD

Indicator	**Suffering from CVD, N = 29**	**Not suffering from CVD, N = 209**	**p**
Age, years	53.76 ± 5.57	47.40 ± 5.53	**<0.0001**
Height (cm)	165.97 ± 6.15	166.34 ± 6.47	0.667
Body mass, kg	77.47 ± 13.64	77.11 ± 15.05	0.490
BMI, kg/m^2	28.14 ± 4.88	27.87 ± 5.25	0.503
Glucose(mmol/l)	5.7 ± 1.78	5.55 ± 1.25	0.834
Cholesterol (mmol/l)	6.21 ± 1.05	6.20 ± 1.00	0.952
HDL-Ch (mmol/l)	1.43 ± 0.29	1.51 ± 0.35	0.289
LDL-Ch (mmol/l)	4.11 ± 0.97	4.10 ± 0.92	0.913
Triglycerides (mmol/l)	1.39 ± 1.14	1.28 ± 0.57	0.651
Apo-A1, g/l	1.62 ± 0.22	1.67 ± 0.27	0.543
Apo-B, g/l	1.05 ± 0.27	1.02 ± 0.24	0.688
Apo-A1 / Apo-B	1.69 ± 0.70	1.75 ± 0.59	0.397
LP(a), g/l	0.24 ± 0.31	0.18 ± 0.24	0.529
hs CRP, mg/l	2.22 ± 2.35	2.42 ± 3.77	0.335

CVD – cardiovascular diseases; BMI – body mass index; HDL-Ch – high density lipoprotein cholesterol; LDL-Ch – low density lipoprotein cholesterol; hs-CRP – high sensitivity C-reactive protein; Apo-A1 – apolipoprotein A1; Apo-B – apolipoprotein B; LP(a) – lipoprotein (a)

Table 3.4.4. Comparison of clinical data and laboratory test values between males with and without CVD, mean value ± SD

Indicator	**Suffering from CVD, N = 28**	**Not suffering from CVD, N = 203**	**p**
Age, years	52.54 ± 6.43	48.54 ± 6.20	**0.003**
Height (cm)	179.33 ± 6.27	177.79 ± 6.29	0.183
Body mass, kg	92.31 ± 18.98	89.81 ± 16.76	0.367
BMI, kg/m^2	28.54 ± 4.64	28.33 ± 4.52	0.590
Glucose(mmol/l)	6.46 ± 3.50	5.71 ± 1.21	**0.040**
Cholesterol (mmol/l)	5.94 ± 1.54	6.33 ± 1.11	0.092
HDL-Ch (mmol/l)	1.24 ± 0.39	1.33 ± 0.37	0.173
LDL-Ch (mmol/l)	3.9 ± 1.30	4.17 ± 0.96	0.117
Triglycerides (mmol/l)	1.74 ± 0.95	1.87 ± 1.85	0.605
Apo-A1, g/l	1.49 ± 0.31	1.57 ± 0.27	0.068
Apo-B, g/l	1.01 ± 0.32	1.10 ± 0.26	0.084
Apo-A1 / Apo-B	1.60 ± 0.59	1.51 ± 0.49	0.521

LP(a), g/l	0.23 ± 0.29	0.18 ± 0.26	0.325
hs CRP, mg/l	4.24 ± 7.73	2.18 ± 3.48	0.069

CVD – cardiovascular diseases; BMI – body mass index; HDL-Ch – high density lipoprotein cholesterol; LDL-Ch – low density lipoprotein cholesterol; hs-CRP – high sensitivity C-reactive protein; Apo-A1 – apolipoprotein A1; Apo-B – apolipoprotein B; LP(a) – lipoprotein (a).

After elimination of age effects, no statistically significant differences in the biochemical blood parameters between the groups of individuals with and without CVD were detected.

Table 3.4.5.. Comparison of clinical data and laboratory test values stratified by BMI groups, mean value ± SN

Indicator	**Normal, N = 124**	**Overweight, N = 220**	**Obese, N = 125**	**p**
Age, years	46.62 ± 5.37	49.01 ± 6.21	49.82 ± 6.31	**<0.0001**
Height (cm)	171.00 ± 8.53	172.07 ± 8.28	173.06 ± 9.28	0.171
Body mass, kg	67.50 ± 8.27	81.32 ± 8.67	103.35 ± 15.99	**<0.0001**
BMI, kg/m^2	23.02 ± 1.47	27.41 ± 1.37	34.46 ± 4.12	**<0.0001**
Glucose(mmol/l)	5.34 ± 0.66	5.56 ± 1.10	6.24 ± 2.35	**<0.0001**
Cholesterol (mmol/l)	6.09 ± 1.04	6.34 ± 1.03	6.22 ± 1.22	0.125
HDL-Ch (mmol/l)	1.55 ± 0.35	1.45 ± 0.38	1.21 ± 0.29	**<0.0001**
LDL-Ch (mmol/l)	4.01 ± 0.94	4.19 ± 0.90	4.11 ± 1.09	0.221
Triglycerides (mmol/l)	1.17 ± 0.55	1.54 ± 1.32	2.04 ± 1.78	**<0.0001**
Apo-A1, g/l	1.69 ± 0.28	1.63 ± 0.27	1.49 ± 0.23	**<0.0001**
Apo-B, g/l	0.98 ± 0.25	1.07 ± 0.25	1.11 ± 0.25	**<0.0001**
Apo-A1 / Apo-B	1.86 ± 0.68	1.63 ± 0.53	1.41 ± 0.39	**<0.0001**
LP(a), g/l	0.17 ± 0.23	0.21 ± 0.28	0.17 ± 0.24	0.353
hs CRP, mg/l	2.09 ± 4.82	2.04 ± 3.44	3.41 ± 3.66	**0.004**

CVD – cardiovascular diseases; BMI – body mass index; HDL-Ch – high density lipoprotein cholesterol; LDL-Ch – low density lipoprotein cholesterol; hs-CRP – high sensitivity C-reactive protein; Apo-A1 – apolipoprotein A1; Apo-B – apolipoprotein B; LP(a) – lipoprotein (a).

A post-hoc Fisher's least significant difference (LSF) criterion was applied for multiple comparisons. Obese individuals were statistically significantly older by 3.92 years on average to compare with those having a normal body weight ($p < 0.0001$). Individuals with overweight were statistically significantly older by 2.39 years on average to compare with those having a normal body weight ($p < 0.0001$).

Blood glucose levels in obese and overweighted individuals were statistically significantly higher than in individuals with the normal body weight.

Cholesterol concentration in individuals with overweight was statistically significantly higher by 0.25 mmol/l that in individuals with the normal body weight. The lowest levels of HDL cholesterol were reported in obese individuals; they were statistically significantly lower by 0.24 mmol/l than in individuals with overweight ($p < 0.0001$) and by 0.34 mmol/l lower than in individuals with a normal body weight. The HDL cholesterol concentration in individuals with overweight was statistically significantly lower by 0.1 mmol/l than in individuals with a normal body weight. Statistically significant difference in LDL cholesterol levels was not discovered between the groups. The concentration of triglycerides was the highest in obese individuals. It was statistically significantly higher by (0.51 mmol/l on average) than in individuals with overweight ($p = 0.001$) and by 0.87 mmol/l higher that in individuals with a normal body weight; TG levels in individuals with overweight were statistically significantly higher by 0.37 mmol/l than in individuals with normal weight ($p = 0.013$).

The lowest levels of Apo-A1 were reported in obese individuals; they were statistically significantly lower than in individuals with overweight ($p < 0.0001$) and lower than in individuals with a normal body weight ($p < 0.0001$). The levels of Apo-B were higher in obese individuals than in individuals having a normal body weight ($p < 0.0001$); these levels were also higher in individuals with overweight than in individuals with a normal body weight ($p = 0.002$).

The highest levels of CRP were reported in obese individuals; they were statistically significantly higher by 1.37 mg/l on average than in individuals with overweight ($p = 0.002$) and by 1.32 mg/l higher than in individuals with a normal body weight ($p = 0.008$). A statistically significant difference in CRP levels between the individuals with overweight and with a normal body weigh was not detected.

Statistically significant differences in glucose, HDL cholesterol, TG, Apo-A1 and Apo-B as well as CRP were reported also after the elimination of age affects.

3.5. Correlation of anxiety and depression with cardiovascular diseases

The Hospital Anxiety and Depression (HAD) scale was used to identify anxiety and depression disorders. The distribution of depression and anxiety symptoms reported by the individuals according to severity is presented in **Fig. 3.5.1.**

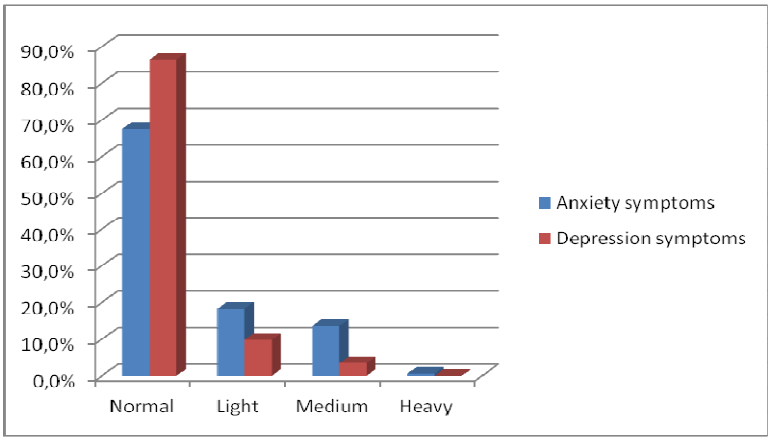

Fig. 3.5.1. Distribution of individuals according to the severity of anxiety and depression symptoms, percentage

Clinical symptoms of anxiety were reported by 177 (37.7%) of individuals (anxiety symptoms assessed by 8 or more points on the HAD scale). Clinical symptoms of depression were reported by 82 (17.5%) of individuals (depression symptoms assessed by 8 or more points on the HAD scale).

A statistically significant correlation of moderate strength was established with anxiety and depression symptoms ($r = 0.562$, $p < 0.0001$). Statistically significant correlations with the values of laboratory test results were not detected.

Anxiety and depression symptoms according to gender were assessed. The values of anxiety assessment among females were statistically significantly higher than in males ($p < 0.0001$), and the depression assessment values were not statistically significantly different ($p = 0.221$).

The clinical symptoms of anxiety were reported statistically significantly more often in females ($\chi^2(1) = 21.23$, $p < 0.0001$). 47.9% (114) of females experienced clinical symptoms of anxiety. Clinical symptoms of anxiety were also reported by 27.3% (63) of

males (anxiety symptoms assessed by 8 or more points on the HAD scale). 18.2% (42) of males experienced clinical symptoms of depression (depression symptoms assessed by 8 or more points on the HAD scale). Clinical symptoms of depression were reported also by 16.8% (40) of females ($\chi^2(1) = 7.65$, $p < 0.695$).

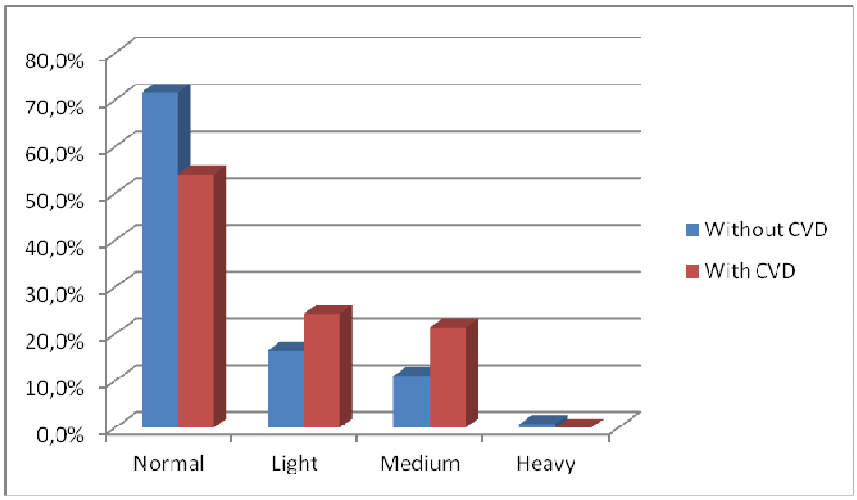

Fig. 3.5.2. Distribution of anxiety symptoms between individuals with and without CVD, percentage ($p = 0.038$, χ^2 was used for comparison between the groups)

Anxiety symptoms were reported statistically significantly more often in the group of individuals with CVD than among individuals without CVD ($\chi^2(3) = 8.42$, $p = 0.038$) (**Figure 3.5.2).** Depression symptoms were reported somewhat more often in the group of individuals with CVD than among individuals without CVD ($\chi^2(3) = 7.49$, $p = 0.058$), however, the difference was not statistically significant (**Figure 3.5.3).**

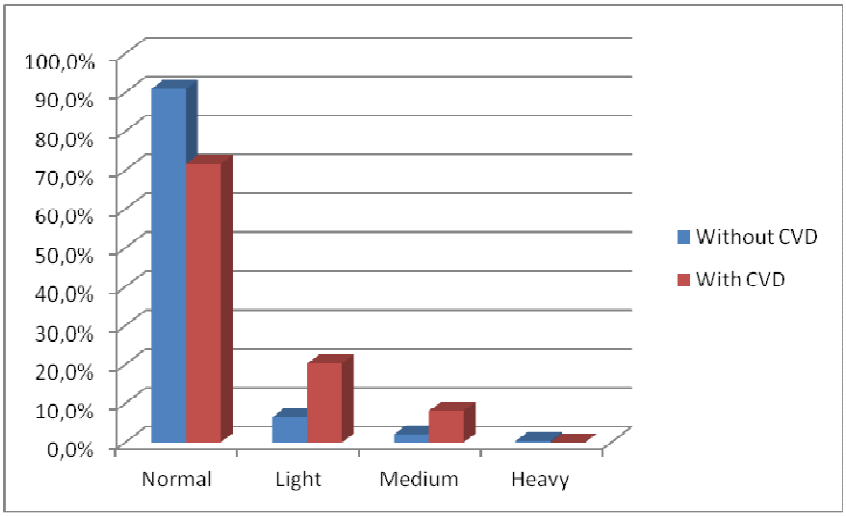

Fig. 3.5.3. Distribution of depression symptoms between the groups of individuals with and without CVD, percentage ($p = 0.058$, χ^2 was used for comparison between the groups)

The values of anxiety assessment ($p < 0.0001$) and the values of depression assessment ($p = 0.006$) were statistically significantly higher in individuals with CVD than in those without CVD.

Clinical symptoms of anxiety were reported statistically significantly more often in individuals with CVD than in those without CVD ($\chi^2(1) = 7.65$, $p = 0.006$). Clinical symptoms of anxiety were reported by 54.4% of individuals with CVD and by 35.4% of individuals without CVD (anxiety symptoms assessed by 8 or more points on the HAD scale) (**Table 3.5.1)**.

Table 3.5.1. Assessment of anxiety and depression symptoms in the study groups of individuals with and without CVD

Indicator	**Suffering from CVD, N = 57**	**Not suffering from CVD, N = 412**	***p***
Anxiety symptoms	31 (54.4%)	146 (35.4%)	0.006
Depression symptoms	17 (29.8%)	65 (15.8%)	0.009

The clinical symptoms of depression were reported statistically significantly more often in individuals with CVD than in individuals without CVD ($\chi^2(1) = 6.85$, $p = 0.009$). Clinical symptoms of depression were reported by 29.8% of individuals with CVD and by 15.8% of individuals without CVD (depression symptoms assessed by 8 or more points on the HAD scale) (**Table 3.5.1)**.

A logistic regression analysis was carried out to examine the correlation between cardiovascular diseases and the symptoms of depression and anxiety. A dependent variable presence of CVD was included in the primary regression model along with the following independent variables: assessment of anxiety and depression symptoms, age. The general proper classification of the model was 68.2%, the Cox–Snell coefficient of determination was $r_{CS}^2 = 0.086$, the Nagelkerke coefficient of determination was $r_N^2 = 0.117$.

A statistically significant correlation between the presence of CVD and anxiety symptoms was established: an increase of anxiety symptoms' value by one point was associated with an increase of the CVD risk 1.10 times ($\beta = 0.10$, OR = 1.10 (1.03 – 1.17), $p = 0.003$). A statistically significant correlation between CVD and depression symptoms was not detected ($p = 0.792$).

3.6. Risk-related allele frequences in the groups of individuals with and without cardiovascular diseases

The etiopathogenesis of atherosclerosis involves many factors, including several biochemical pathways. One of the most important is the disorder of blood lipid metabolism. This has provided the base for gene and SNP screening.

Genes and SNPs for this study were selected on the basis of analysis covering the recent scientific publications and studies which were not primary but repeatedly demonstrating and proving already established or new statistically significant associations in large study samples. Data from scientific publications were supplemented with information from databases: Ensembl, NCBI Gene, dbSNP, 1000 Genomes. SNPs

significantly related to cardiovascular diseases of atherosclerotic origin such as the coronary heart disease, stroke and dyslipidemia were selected for the further study .

Aiming to assess the genetic risk of CVD in the Lithuanian population, a study of the genetic structure and diversity according to selected 61 SNPs was carried out, involving 295 individuals. After genotyping (Illumina BeadChip Array 12-sample HD DNA Analysis BC HumanOmniExpress-12v1-1), the quality control testing was carried out using the *GenomeStudio*™ *v2011.1* software according to recommendations of the manufacturer.

The frequencies of the tested SNPs genotypes are presented in **Table 3.6.1.**

Table 3.6.1. Frequencies of SNPs genotypes in the groups of study subjects with and without CVD

		Frequencies				Frequencies	
SNP	**Genotype**	**Suffering from CVD**	**Not suffering from CVD**	**SNP**	**Genotype**	**Suffering from CVD**	**Not suffering from CVD**
rs1801133	*AA*	*0.0909091*	*0.0725191*	***rs2383206***	*GG*	*0.1818182*	*0.2366412*
	AG	0.5151515	0.4389313		GA	0.4545455	0.5
	GG	0.3939394	0.4885496		AA	0.3636364	0.2633588
rs11206510	GG	0	0.0229008	**rs2383207**	GG	0.1818182	0.2366412
	GA	0.2121212	0.259542		GA	0.4545455	0.5038168
	AA	0.7878788	0.7175573		AA	0.3636364	0.259542
rs11591147	AA	0	0	**rs579459**	GG	0.1212121	0.0572519
	AC	0	0.0152672		GA	0.3333333	0.3816794
	CC	1	0.9847328		AA	0.5454545	0.5610687
rs646776	GG	0.1212121	0.0536398	**rs11185660**	GG	0.0909091	0.03861
	GA	0.4242424	0.3639847		GA	0.4242424	0.3552124
	AA	0.4545455	0.5823755		AA	0.4848485	0.6061776
rs599839	GG	0.0909091	0.0538462	**rs7893395**	AA	0	0.0152672
	GA	0.4545455	0.3653846		AG	0.1212121	0.2748092
	AA	0.4545455	0.5807692		GG	0.8787879	0.7099237
rs4845625	GG	0.3030303	0.240458	**rs501120**	GG	0	0.0076923
	GA	0.4545455	0.4923664		GA	0.09375	0.2346154
	AA	0.2424242	0.2671756		AA	0.90625	0.7576923
rs2774279	AA	0.1212121	0.0763359	**rs11203042**	AA	0.0606061	0.129771
	AG	0.3939394	0.4198473		AG	0.4848485	0.4770992
	GG	0.4848485	0.5038168		GG	0.4545455	0.3931298
rs732314	GG	0.2424242	0.1832061	**rs1412444**	AA	0.0606061	0.0916031
	GA	0.5151515	0.4503817		AG	0.3939394	0.4770992
	AA	0.2424242	0.3664122		GG	0.5454545	0.4312977

rs17464857	CC	0	0.0343511	**rs174547**	GG	0.030303	0.0839695
	CA	0.2121212	0.3091603		GA	0.4242424	0.3854962
	AA	0.7878788	0.6564885		AA	0.5454545	0.5305344
rs17465637	AA	0.1818182	0.1111111	**rs974819**	AA	0.0606061	0.0881226
	AC	0.5151515	0.4252874		AG	0.3030303	0.3831418
	CC	0.3030303	0.4636015		GG	0.6363636	0.5287356
rs1042031	AA	0.030303	0.0267176	**rs2259816**	AA	0.1515152	0.1412214
	AG	0.3939394	0.3358779		AC	0.3636364	0.4274809
	GG	0.5757576	0.6374046		CC	0.4848485	0.4312977
rs693	AA	0.1515152	0.2099237	**rs4773144**	GG	0.2727273	0.1423077
	AG	0.5454545	0.5763359		GA	0.5151515	0.4961538
	GG	0.3030303	0.2137405		AA	0.2121212	0.3615385
rs562338	AA	0.030303	0.0343511	**rs9515203**	GG	0.0909091	0.0381679
	AG	0.2727273	0.3053435		GA	0.3636364	0.3778626
	GG	0.6969697	0.6603053		AA	0.5454545	0.5839695
rs1260326	AA	0.1212121	0.1183206	**rs4775041**	GG	0.0909091	0.0763359
	AG	0.5757576	0.5419847		GC	0.3939394	0.4122137
	GG	0.3030303	0.3396947		CC	0.5151515	0.5114504
rs780094	AA	0.1212121	0.1183206	**rs261332**	AA	0.030303	0.0648855
	AG	0.5757576	0.5381679		AG	0.5151515	0.3664122
	GG	0.3030303	0.3435115		GG	0.4545455	0.5687023
rs2252641	GG	0	0.1564885	**rs4775065**	AA	0	0.0383142
	GA	0.4242424	0.3969466		AG	0.3636364	0.3180077
	AA	0.5757576	0.4465649		GG	0.6363636	0.6436782
rs2306374	GG	0	0.0152672	**rs17228212**	GG	0.030303	0.0687023
	GA	0.3333333	0.2519084		GA	0.4545455	0.3740458
	AA	0.6666667	0.7328244		AA	0.5151515	0.5572519
rs9818870	AA	0	0.0152672	**rs7173743**	GG	0.2424242	0.1930502
	AG	0.3333333	0.2519084		GA	0.5454545	0.5328185
	GG	0.6666667	0.7328244		AA	0.2121212	0.2741313
rs6903956	AA	0.2121212	0.091954	**rs17514846**	CC	0.1212121	0.2615385
	AG	0.4848485	0.4176245		CA	0.4545455	0.4923077
	GG	0.3030303	0.4904215		AA	0.4242424	0.2461538
rs9369640	CC	0.1515152	0.1068702	**rs150468**	CC	0.030303	0.015444
	CA	0.4242424	0.519084		CA	0.2424242	0.2277992
	AA	0.4242424	0.3740458		AA	0.7272727	0.7567568
rs2048327	GG	0.1818182	0.1030534	**rs3764261**	AA	0.0606061	0.0839695
	GA	0.3636364	0.480916		AC	0.3939394	0.4160305
	AA	0.4545455	0.4160305		CC	0.5454545	0.5
rs4252120	GG	0.0606061	0.0648855	**rs5882**	GG	0.0909091	0.0877863
	GA	0.4545455	0.3206107		GA	0.4242424	0.4160305
	AA	0.4848485	0.6145038		AA	0.4848485	0.4961832
rs2023938	GG	0.030303	0.0038314	**rs2271293**	AA	0.030303	0.0152672
	GA	0.2424242	0.1149425		AG	0.2424242	0.2175573
	AA	0.7272727	0.8812261		GG	0.7272727	0.7671756
rs2107595	AA	0.09375	0.03861	**rs879324**	AA	0.030303	0.019084
	AG	0.28125	0.2741313		AG	0.2424242	0.2519084

	GG	0.625	0.6872587		GG	0.7272727	0.7290076
rs17145738	AA	0	0.0114504	**rs5918**	GG	0.030303	0.0153257
	AG	0.1515152	0.2290076		GA	0.3939394	0.2605364
	GG	0.8484848	0.759542		AA	0.5757576	0.7241379
rs662	GG	0.0606061	0.0725191	**rs15563**	GG	0.2727273	0.2337165
	GA	0.3333333	0.3549618		GA	0.4242424	0.48659
	AA	0.6060606	0.5725191		AA	0.3030303	0.2796935
rs11556924	AA	0.0606061	0.1946565	**rs688**	AA	0.1515152	0.1679389
	AG	0.5757576	0.4961832		AG	0.4848485	0.5038168
	GG	0.3636364	0.3091603		GG	0.3636364	0.3282443
rs2197089	GG	0.3333333	0.2298851	**rs2075650**	GG	0.0606061	0.0305344
	GA	0.3636364	0.4904215		GA	0.3030303	0.2977099
	AA	0.3030303	0.2796935		AA	0.6363636	0.6717557
rs3217992	AA	0.1818182	0.1609195	**rs445925**	AA	0	0.0114504
	AG	0.4848485	0.51341		AG	0.2727273	0.1679389
	GG	0.3333333	0.3256705		GG	0.7272727	0.8206107
rs10757274	GG	0.1515152	0.221374	**rs4420638**	GG	0.0606061	0.019084
	GA	0.4545455	0.4770992		GA	0.3030303	0.240458
	AA	0.3939394	0.3015267		AA	0.6363636	0.740458
rs4977574	GG	0.1515152	0.2251908				
	GA	0.4545455	0.4732824				
	AA	0.3939394	0.3015267				

Statistically significant differences are highlighted; SNP – single nucleotide polymorphism, GT – genotype, CVD – cardiovascular diseases.

Statistically significant associations between the six SNPs genotypes and CVD were found.

A comparison was carried out for the risk allele. In the group of individuals with CVD, the SNP rs4773144 (risk allele G) genotype GG ratio was significantly higher than in the group of individuals without CVD ($\chi2(1) = 4.9$, $p < 0.026$); the SNP rs2023938 (risk allele G) genotype GG frequency was significantly higher in the group of individuals with CVD than in the group of individuals without CVD ($\chi2(1) = 7$, $p < 0.008$).

Both homozygous genotypes (GG) of the risk allele (G) are dominating in the group of individuals with CVD as compared with individuals without CVD. A risk allele is characterised by the recessive action, and heterozygotes are not a sufficient condition for the disease predisposition.

The frequency of the CC genotype of SNP rs6903956 (C) in the group of individuals with CVD was significantly higher ($\chi2(1) = 6.2$, $p < 0.01$) than in individuals

without CVD. Thus, in conclusion, it could be stated that three SNPs are associated with the CVD in the study population.

SNPs' rs7893395 (A) AA and AG, rs2252641 (G) GG as well as rs17514846 (C) CC genotypes were found statistically significantly more often in the group of individuals without CVD ($\chi 2$ respectively $\chi 2(1) = 4.3$, $p < 0.03$; $\chi 2(1) = 4.8$, $p < 0.02$; $\chi 2(1) = 5.8$, $p < 0.01$); thus, they can be considered as protective ones.

The frequencies of 61 SNPs alleles located in 47 loci were analysed in the groups of individuals with and without CVD. All frequencies of SNPs and genotypes listed below were consistent with the Hardy–Weinberg equilibrium, $p \geq 0.001$. The results are presented in **Table 3.6.2**.

Table 3.6.2. Differences in the frequency of SNPs alleles in the Lithuanian population of individuals with and without CVD

CHR	SNP	A1	RAF suffering from CVD	RAF not suffering from CVD	A2	*P*	OR
1	rs1801133	A	0.3485	0.292	G	0.3916	1.297
1	rs11206510	G	0.1061	0.1527	A	0.3625	0.658
1	rs11591147	A	0	0.007634	C	1	0
1	rs646776	G	0.3333	0.2356	A	0.09526	1.622
1	rs599839	G	0.3182	0.2365	A	0.1713	1.506
1	rs4845625	G	0.5303	0.4866	A	0.5166	1.191
1	rs2774279	A	0.3182	0.2863	G	0.5685	1.164
1	rs732314	G	0.5	0.4084	A	0.1854	1.449
1	rs17464857	C	0.1061	0.1889	A	0.1246	0.509
1	rs17465637	A	0.4394	0.3238	C	0.0721	1.637
2	rs1042031	A	0.2273	0.1947	G	0.5152	1.217
2	rs693	A	0.4242	0.4981	G	0.2964	0.742
2	rs562338	A	0.1667	0.187	G	0.8663	0.869
2	rs1260326	A	0.4091	0.3893	G	0.7897	1.086
2	rs780094	A	0.4091	0.3874	G	0.7892	1.095
2	**rs2252641**	G	0.2121	0.355	A	0.02654	0.489
3	rs2306374	G	0.1667	0.1412	A	0.5777	1.216
3	rs9818870	A	0.1667	0.1412	G	0.5777	1.216
6	**rs6903956**	A	0.4545	0.3008	G	0.01648	1.937
6	rs9369640	C	0.3636	0.3664	A	1	0.988
6	rs2048327	G	0.3636	0.3435	A	0.7841	1.092
6	rs4252120	G	0.2879	0.2252	A	0.279	1.391
7	**rs2023938**	G	0.1515	0.0613	A	0.01806	2.734

7	rs2107595	A	0.2344	0.1757	G	0.3017	1.436
7	rs17145738	A	0.07576	0.126	G	0.3156	0.568
7	rs662	G	0.2273	0.25	A	0.7635	0.882
7	rs11556924	A	0.3485	0.4427	G	0.1499	0.673
8	rs2197089	G	0.5152	0.4751	A	0.6015	1.174
9	rs3217992	A	0.4242	0.4176	G	1	1.028
9	rs10757274	G	0.3788	0.4599	A	0.2384	0.716
9	rs4977574	G	0.3788	0.4618	A	0.2378	0.710
9	rs2383206	G	0.4091	0.4866	A	0.2426	0.730
9	rs2383207	G	0.4091	0.4885	A	0.2412	0.724
9	rs579459	G	0.2879	0.2481	A	0.5475	1.225
9	rs11185660	G	0.303	0.2162	A	0.1193	1.576
10	**rs7893395**	A	0.06061	0.1527	G	0.04089	0.358
10	rs501120	G	0.04688	0.125	A	0.09496	0.344
10	rs11203042	A	0.303	0.3683	G	0.3422	0.745
10	rs1412444	A	0.2576	0.3302	G	0.2651	0.704
11	rs174547	G	0.2424	0.2767	A	0.6604	0.836
11	rs974819	A	0.2121	0.2797	G	0.3042	0.693
12	rs2259816	A	0.3333	0.355	C	0.7856	0.908
13	**rs4773144**	G	0.5303	0.3904	A	0.03335	1.763
13	rs9515203	G	0.2727	0.2271	A	0.4394	1.276
15	rs4775041	G	0.2879	0.2824	C	1	1.027
15	rs261332	A	0.2879	0.2481	G	0.5475	1.225
15	rs4775065	A	0.1818	0.1973	G	0.8699	0.904
15	rs17228212	G	0.2576	0.2557	A	1	4.200
15	rs7173743	G	0.5152	0.4595	A	0.4328	4.203
15	**rs17514846**	C	0.3485	0.5077	A	0.01822	0.518
16	rs150468	C	0.1515	0.1293	A	0.5668	1.202
16	rs3764261	A	0.2576	0.292	C	0.6656	0.841
16	rs5882	G	0.303	0.2958	A	0.8872	1.035
16	rs2271293	A	0.1515	0.124	G	0.5556	1.261
16	rs879324	A	0.1515	0.145	G	0.8541	1.053
17	rs5918	G	0.2273	0.1456	A	0.1023	1.726
17	rs15563	G	0.4848	0.477	A	1	1.032
19	rs688	A	0.3939	0.4198	G	0.7913	0.898
19	rs2075650	G	0.2121	0.1794	A	0.502	1.232
19	rs445925	A	0.1364	0.09542	G	0.2803	1.497
19	rs4420638	G	0.2121	0.1393	A	0.1386	1.663

Statistically significant differences are highlighted. CHR – chromosome; A1 – risk allele; SNP – identification number of single nucleotide polymorphism; RAF – frequency of risk allele, A2 – alternative to risk allele; p – *p*-value; OR – odds ratio.

Six loci were associated with CVD in the Lithuanian study population. Statistically significant differences of allele and genotype frequencies between the groups of individuals with and without CVD were detected: rs2252641 located on the 2nd chromosome and associated with the *ZEB2* gene region ($p < 0.026$); rs6903956

located on the 6th chromosome and associated with the *C6orf105* gene ($p < 0.01648$); rs2023938 located on the 7th chromosome and associated with the *HDAC9* gene ($p < 0.01806$); rs789335 located on the 10th chromosome and associated with the *CUBN* gene ($p < 0.04089$); rs4773144 located on the13th chromosome and associated with the region of *COL4A1/COL4A2* genes ($p < 0.033$), and rs17514846 located on the 15th chromosome and associated with the region of *FURIN/FES* genes ($p < 0.018$).

CONCLUSIONS

1. Risk factors of CVD are highly prevalent in the middle-aged Lithuanian population: normal body weight has been reported in every fourth Lithuanian citizen only, half of interviewed individuals had overweight, and 26.7% were obese. We have also stated a low physical activity during leisure time. The family history of the disease is a very important CVD risk factor and can be used as an instrument for CVD risk identification among young symptoms-free individuals, considering the medical history of CVD of his (her) mother and sister(s).

2. Assessment of nutrition habits in the adult Lithuanian population has revealed that the nutrition of the majority of Lithuanian citizens does not meet recommendations for a healthy diet: the low frequency of the daily consumption of fresh vegetables and fruits, grains and cereals as well as fish and fish products, the high frequency of consumption of foods of animal origin, i.e. meat.

3. The prevalence of dyslipidemia reported in our study was 94.2%, We have demonstrated that the concentration of total Ch, LDL-Ch and TG in the individuals of the Lithuanian population is higher than recommended. No correlations between the biochemical markers tested and CVD were discovered.

4. We have found that every third (37.7%) interviewed person in the Lithuanian population experienced symptoms of anxiety and depression reported by 17.5% of the study subjects. We have established a significant correlation between the presence of CVD and anxiety: the increase of anxiety symptoms by one point was associated with

the increase of the CVD risk 1.10 times. No statistically significant correlation between CVD and depression symptoms was detected.

5. We have found statistically significant difference among individuals with and without CVD in the Lithuanian population regarding the frequency of six alleles *(genes ZEB2, CUBN, FURIN/FES, C6orf105, HDAC9 and COL4A1/COL4A2*).

rs2252641 (G) GG located in the 2nd chromosome associated with the region of the *ZEB* gene, rs789335 (A) located in the 10th chromosome and associated with the *CUBN* gene, and rs17514846 (C) CC located in the 15th chromosome and associated with the region of *FURIN/FES* genes were found statistically significantly more often in the group of individuals without CVD, thus these genes can be considered as protective.

We also found three regions strongly associated with CVD: rs6903956 (A) located in the 6th chromosome and associated with the gene *C6orf105*; rs2023938 (G) located in the 7th chromosome and associated with the *HDAC9* gene, and rs4773144 (G) located in the 13th chromosome and associated with the region of the *COL4A1/COL4A2* genes.

SANTRAUKA

Širdies ir kraujagyslių ligos (ŠKL) yra pagrindinė mirtingumo Lietuvoje priežastis. Mirtingumas nuo ŠKL yra beveik du kartus didesnis nei kitose senosiose ES valstybėse. Tai lemia keletas priežasčių: gyvensenos įpročiai ir / arba genetiniai veiksniai. Šio tyrimo tikslas buvo įvertinti 40–65 m. amžiaus Lietuvos gyventojų mitybos, nerimo ir depresijos, biocheminių ir genetinių aterosklerozės žymenų sąsajas su ŠKL rizika.

Tiriamųjų kontingentą sudarė asmenys, kurių mažiausiai dvi protėvių kartos gyveno tame pačiame Lietuvos regione. Tyrime dalyvavo 469 asmenys. Anketinės apklausos būdu buvo surinti duomenys apie tiriamųjų mitybos bei gyvensenos ypatumus, šeimos anamnezės komponentus, tiriamųjų asmenų sveikatos būklę, atlikti bendrojo cholesterolio, trigliceridų (TG), gliukozės, C reaktyviojo baltymo (CRB), Apo A1, ApoB, ApoB/ApoA1 santykio, lipoproteino (a) tyrimai bei genotipavimas.

Pagal tirtus ŠKL rizikos veiksnius nustatėme, kad normalų kūno svorį turi vos kas ketvirtas Lietuvos gyventojas, pusė apklaustųjų turi antsvorio, o 26,7 proc. yra nutukę. Taip pat nustatėme mažą fizinį aktyvumą laisvalaikiu. Vertindami Lietuvos suaugusių gyventojų mitybos įpročius nustatėme, kad daugumos Lietuvos gyventojų mityba neatitinka sveikos mitybos rekomendacijų: mažas šviežių daržovių ir vaisių, grūdų ir grūdinių produktų, žuvies ir jos produktų kasdienio vartojimo dažnis, didelis gyvulinės kilmės produktų, t. y. mėsos, vartojimo dažnis. Taip pat nustatėme dislipidemijos paplitimą – 94,2 proc. Mūsų tyrimo duomenimis, Lietuvos gyventojai turi didesnę, nei rekomenduojama, vidutinę bendrojo Ch, MTL-Ch ir TG koncentraciją. Nerimo simptomus patiria kas trečias (37,7 proc.) apklaustas asmuo Lietuvos populiacijoje, depresijos simptomus nustatėme 17,5 proc. tiriamųjų. Gavome reikšmingas sąsajas tarp sirgimo ŠKL ir nerimo: nerimo simptomų įvertinimo padidėjimas vienu balu ŠKL šansų santykį didino 1,10 karto. Tiriamoje Lietuvos populiacijoje nustatėme šešių alelių dažnių statistiškai reikšmingus skirtumus (*ZEB2, CUBN, FURIN/FES, C6orf105, HDAC9* ir *COL4A1/COL4A2* genuose) ŠKL nesergančių ir sergančių asmenų grupėje. Mes įvertinome sąsajas tradicinių ir netradicinių rizikos veiksnių su ŠKL, tačiau norint

įvertinti netradicinių ir genetinių rizikos veiksnių reikšmingumą prognozuojant ŠKL, būtų reikalingas perspektyvinis tyrimas.

APPROBATION

Publications (3)

1. N. **Burokienė**, V. Kasiulevičius, Z. A. Kučinskienė. Koronarinės širdies ligos išsivystymo rizika, epidemiologinių ir genomo žymenų sąveikos tyrimas (literatūros apžvalga). Medicinos teorija ir praktika 2013; 19 (2): 200–205.

2. **N. Burokienė**, D. Karčiauskaitė, V. Kasiulevičius, V. Kučinskas, Z. A. Kučinskienė. Manifestation of anxiety and depression and their association with cardiovascular disease in Lithuanian population. Acta medica Lituanica 2014; 21(3): 123–130.

3. **N. Burokienė**, D. Karčiauskaitė, V. Kasiulevičius, V. Kučinskas, Z.A. Kučinskienė Širdies ir kraujagyslių ligų sąsajos su aterosklerozės rizikos veiksniais ir biochemiais žymenimis Lietuvos populiacijoje. Laboratorinė medicina 2015; 2(66): 47–52.

Oral presentations (2)

1. **N. Burokienė**. Širdies ir kraujagyslių ligų rizikos vertinimas Lietuvos populiacijoje: biocheminių ir genetinių žymenų atranka ir vertinimas. Mokslinė-praktinė konferencija LITGEN: Lietuvos populiacijos genetinė įvairovė ir sandaros kitimai, susiję su evoliucija ir dažniausiai paplitusiomis ligomis, 2015-03-06.

2. **N. Burokienė**. Why cardiovascular diseases are so prevalent in Lithuania. 9-oji Baltijos šalių šeimos medicinos konferencija, 2015-03-27.

Poster presentations (7)

1. Neringa **Burokienė**, Asta Mažeikienė, Zita Aušrelė Kučinskienė. Nutritional habits and prevalence of the multiple cardiovascular risk factors among children and young

adults. Tarptautinėje konferencijoje „Evoliucinė medicina: nauji senų problemų sprendimai“, 2012 m. birželio 12–15 d., Vilnius, Lietuva**.**

2. Asta Mažeikienė, **Neringa Burokienė**, Dovilė Karčiauskaitė, Zita Aušrelė Kučinskienė. Relation between family history of common chronic diseases, nutritional habits and blood biochemical parameters. 20-asis Tarptautinės ir Europos Klinikinės Chemijos ir Laboratorinės Medicinos federacijų (IFCC-EFLM) Europos klinikinės chemijos ir laboratorinės medicinos kongresas (EUROMEDLAB), 2013 m. gegužės 18–24 d., Milanas, Italija.

3. Karčiauskaitė D., Mažeikienė A., **Burokienė N**., Kučinskienė Z.A. Impact of anxiety and depression on biochemical blood parameters in general Lithuanian population. 81-asis Europos aterosklerozės draugijos kongresas (EAS), 2013 m. birželio 1–6 d., Lionas, Prancūzija.

4. **Burokienė N.**, Karčiauskaitė D., Kasiulevičius V., Kučinskas V., Kučinskienė Z. A. Manifestation of anxiety an depression and their association with cardiovascular disease in Lithuanian population. Tarptautinėje konferencijoje „Evoliucinė medicina: sveikatos sampratos ir ligų suvokimo perspektyvos“. 2014 m. gegužės 27–30 d., Vilnius, Lietuva.

5. **Burokienė N**., Karčiauskaitė D., Mažeikienė A., Kučinskienė Z.A. Impact of risk factors on cardiovascular disease in general Lithuanian populiation; 19-a pasaulinė šeimos gydytojų konferencija WONCA. 2014 m. liepos 2–5 d., Lisabona, Portugalija.

6. Domarkienė I., Uktverytė I., Ambrozaitytė L., Meškienė R., **Burokienė N.**, Kasiulevičius V., Kučinskienė Z. A., Kučinskas V. „Evaluation of CVD genetic risk in the Lithuanian population; Europos žmogaus genetikos konferencija, 2015 m. birželio 6–9 d., Glasgow, Škotija, UK.

7. **Burokienė N.,** Karčiauskaitė D., Mažeikienė A., Daunoravičius Ž., Miklošiūtė V., Kučinskienė Z. Serum malondialdehyde concentration and its relation to CVD risk markers and morbidity in Lithuanian population. 17-as tarptautinis aterosklerozės simpoziumas ISA. 2015 m. gegužės 23–25 d., Amsterdamas, Olandija.

ABOUT THE AUTHOR|

Personal data: Neringa Burokienė, born 12 February 1971.

Contact information: Clinic of Internal Diseases, Family Medicine and Oncology of Medicine Faculty, Vilnius University, Santariškių Str. 2, Vilnius

E-mail: neringa.burokiene@santa.lt

Education:	
1989–1995	Faculty of Medicine, Vilnius University
1995–1999	Residency of family physician.
Working experience:	
2000–2006	Employed by UAB "Schering", manager of gynaecology unit
2007–2010	Employed by UAB "Bayer", regional manager
1 February – 18 June 2011	Apprenticeship at the Family Medicine Centre of Santariškių Clinics, Vilnius University Hospital
October 2011 – October 2015	Doctorate at the Clinic of Internal Diseases, Family Medicine and Oncology of Medicine Faculty, Vilnius University
16 January 2012 – currently	Northway Clinic, family physician (Licence of professional qualification of family physician No. MPL–16946)
1 February 2014 – currently	Family physician at the Family Medicine Centre of Santariškių Clinics, Vilnius University Hospital
September 2011 – currently	Lecturer at the Clinic of Internal Diseases, Family Medicine and Oncology of Medical Faculty, Vilnius University
September 2011 – currently	Assistant at the Clinic of Internal Diseases, Family Medicine and Oncology of Medicine Faculty, Vilnius University

August 2011 March 2015	The author participated in the project implemented according to general grant measure – the priority 3 of Human Resource Development Action Program Strengthening researchers' abilities. Project title: "Genetic diversity of the population of Lithuania and changes of its genetic structure associated with evolution and common diseases" *"LITGEN"*.
April 2015 – currently	The author participates in the implementation of the Research Project of the Research Council of Lithuania "Particularities of atherosclerosis process predisposed by chronic stress: search and study of new biomarkers" "*ATEROSTRES"*.