Modern Molecular Genomic Biomarkers with Emphasis on Atherosclerosis

Professor Daniel Petrovič MD PhD, FESC

Institute of Histology and Embryology
Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

International Center for Cardiovascular Diseases Medicor, Izola, Slovenia
FLOW CHART OF THE LECTURE

• Concept of genomic biomarkers (DNA and RNA biomarkers)

• Genomic biomarkers and complex disorders

• Atherosclerosis and risk factors

• Markers of atherosclerosis (i.e. increased serum cholesterol and dyslipidemias...)
  – Screening for monogenic dyslipidemias → familial hypercholesterolemia and other monogenic disorders
  – Genetic testing → next generation sequencing

• Genetic markers of subclinical atherosclerosis and CAD: i.e. CIMT, carotid plaques, coronary calcium score...
  – candidate gene approach
  – GWAS

• Atherosclerosis and development of new biomarkers

• Predictions for genetic testing in future → genetic smart card

• Conclusions
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Biomarkers have been used in clinical practice and drug development for many years.

The concept of “Genomic Biomarker” (GB) was introduced by regulatory agencies several years ago (CHMP/ICH/437986/2006).

GB is defined as a DNA or RNA characteristic that is an indicator of normal/pathogenic biologic processes, and/or response to therapeutic or other intervention.

Genetic tests and genomic biomarkers: regulation, qualification and validation
Giuseppe Novelli, Cinzia Ciccacci, Paola Borgiani, Marisa Papaluca Amati, Eric Abadie
GENOMIC BIOMARKERS (DNA, RNA)

**DNA characteristics** include, but are not limited to:
1. Single nucleotide polymorphisms (SNPs) – i.e. gene polymorphism
2. Variability of short sequence repeats
3. DNA modification, e.g. methylation
4. Insertions
5. Deletions
6. Copy number variation
7. Cytogenetic rearrangements, e.g. translocations, duplications, deletions or inversions

**RNA characteristics** include, but are not limited to:
1. RNA sequence
2. RNA expression levels
3. RNA processing, e.g. splicing and editing
4. MicroRNA levels
GENOMIC versus GENETIC BIOMARKERS

• Genomic biomarkers versus genetic biomarkers versus gene polymorphisms (popular expression) → affects number of hits I Pub Med
BIOMARKERS AND ATEROSCLEROSIS

GENETIC BIOMARKERS versus GENE POLYMORPHISM

- Biomarkers versus genetic biomarkers versus gene polymorphisms (popular expression) → affects number of hits I Pub Med
BIOMARKERS AND REGULATION OF VALIDITY

- Biomarkers are emerging as key indices for individualized patient management, however regulation of their safety and validity should be available.

- Formal approval of biomarkers as diagnostic tests → from regulatory agencies is needed.

- An increased focus on more regulated process of validation is appreciated.

Genetic tests and genomic biomarkers: regulation, qualification and validation
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• **Complex disorders** such as heart disease, diabetes, and cancer → **caused by** multiple genetic and environmental factors

• **complicating factor in biomarker development** for complex traits is → the difficulty to understand the role of the genetic factors in the pathophysiology of the disease

• The **identification of “key” genes** influencing **complex traits** is **crucial**, and:
  – may help in **predicting those individuals who are predisposed** to disease
  – may lead to **new targets, and better classification of diseases** → may have **significant impact** on the pharmaceutical industry
A major challenge facing the development of GBs for complex diseases:
- is in the **etiologic or phenotypic heterogeneity** of the clinical conditions.
- **Heterogeneity** influences the ability:
  - to **discover a biomarker** and
  - to **prove the clinical utility** of the biomarker once identified.

A specific polymorphism **may have utility in one but not in another population** → **studies in a variety of populations** are needed
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• **Coronary artery disease and cerebrovascular diseases** are the leading causes of morbidity and mortality in the developed world.

• CVDs are an appropriate model of **complex disorders**

• **Atherosclerosis** is the **underlying cause** of the majority of CV events.

Atherosclerosis and Goals of Personalized Medicine

- Atherosclerosis develops over many years starting from childhood.
  - Thus, the clinical manifestations of disease occur after a prolonged “silent” period.

- Identification of individuals with high risk for developing atherosclerosis → is based on the understanding the pathogenesis and risk factors.
  - Risk factors are:
    1. modifiable → related to lifestyle
    2. non-modifiable → genetic factors

Therefore, goals of personalized medicine are to:

1. identify individuals at high risk of developing a disease (stroke, MI)
   - Use of markers (genetic…)
2. offer preventive measures tailored to those identified risks
Several traditional markers of atherosclerosis are available for risk assessment in clinical practise and for research purposes:

- Increased lipid levels (total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides) → dyslipidemias
- hsCRP
- common carotid intima media thickness
- coronary Calcium score
- Positive family history ...

DYSLIPIDEMIAS AND SCREENING FOR MONOGENIC DYSLIPIDEMIAS

- A significant proportion of individuals with dyslipidaemia remains undiagnosed

- It was estimated that 75% of persons with familial hypercholesterolemia (FH) are not diagnosed → therefore not treated, or not treated appropriately.

- Therefore, health systems face the important challenge of how to identify individuals and their families at risk.

LIPID-LOWERING TREATMENT IN CHILDHOOD AND CORONARY HEART DISEASE RISK

• Atherosclerotic process **starts** in FH-predisposed patients already in childhood.

• Lipid-lowering treatment in children can reduce lipid concentrations in the childhood, while there is currently no evidence:
  – on the long-term benefits or harms of beginning lipid-lowering treatment in childhood.

• There is some evidence that treatment started early in childhood could be associated with **lower coronary heart disease risk.**
GENOMIC SCREENING TOOLS

• A large number of genes leading to monogenic dyslipidaemias → associated with atherosclerosis

• New methods such as next generation sequencing (NGS) provide an opportunity for genomic screening.

• There are several Pros and Cons to consider regarding screening with NGS in general population

NEXT-GENERATION SEQUENCING (NGS)

- In contrast to the Sanger sequencing approach (i.e. testing one gene at a time), several human genes can be analysed in a single genetic test → exome sequencing

- Types of exome sequencing:
  - clinical exome sequencing → human monogenic disorders
  - whole exome sequencing – WES → all human genes
  - whole genome sequencing – WGS → all human genome
Clinical exome sequencing – one genetic test may identify several mutations


**Sandhoff disease:** HEXB deletion of exons 1-5 deletion

**Leigh disease:** chrM:8993T>G (mutation in ATPase 6, homoplasia)

**Progressive syndromic cardiomyopathy:** complex chromosomal rearrangement
• In addition to positive family history, genetic variation of several genes, combined in the polygenic risk score has shown potential to identify a subgroup of individuals at increased risk for subclinical atherosclerosis and CAD.


Screening programs targeted to identify individuals and families with monogenic genetic predisposition have so far been mainly focused on the most frequent genetic disorder associated with dyslipidaemia - FH.

MONOGENIC DISORDERS ASSOCIATED WITH DYSLIPIDAEMIA AND ATHEROSCLEROSIS

• Recently we have performed a review of genes associated with atherosclerosis.

• We found 17 genes responsible for 5 phenotypes
  – Hypercholesterolemia
  – Hypolipoproteinaemia
  – Hyperlipidaemia
  – Lipid storage disease
  – Lipodystrophy

MONOGENIC DYSLIPIDAEMIAS ASSOCIATED WITH ATHEROSCLEROSIS

- **Hypercholesterolemia** is characterized by **genetic heterogeneity**

- **5 genes** are known so far → to be associated with the disease

- So far, **1317** likely or clearly pathogenic gene **variants** are included in the UCL LDL-R gene variant **database**

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<tr>
<th>Phenotype</th>
<th>Disease</th>
<th>OMIM</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Onset</th>
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Systematic analysis of 4 genes (LDLR, APOE, PCSK9, STAP1) using exome sequencing has revealed hypercholesterolemia in 5% of patients with premature MI and positive family history for CAD.

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GENETIC MARKERS OF SUBCLINICAL ATHEROSCLEROSIS = CIMT AND CAROTID PLAQUES

- CIMT and risk assessment
  - CIMT → predictor of future CV events (MI, ischaemic stroke)
  - 0.1 mm change in CIMT corresponds to an increase of 10-15% in the MI risk and 13-18% in the stroke risk (Simon et al., 2010)
  - Genetic basis for CIMT variation/carotid plaques remains to be determined

2 MAIN TYPES OF GENETIC ASSOCIATION STUDIES

• **CANDIDATE GENE APPROACH**
  - we choose potential candidate genes and select potential genetic markers (SNPs) according to “a priori” hypothesis
    * Based on the knowledge of pathophysiology for some phenotype

• **GENOME WIDE ASSOCIATION STUDIES**
  - Scanning of the whole genome without “a priori” hypothesis

• **With both approaches we compare cases and controls with regard to genotype distribution** and try to find risk genotypes for different polymorphisms (rs)/genes
CIMT AND CANDIDATE GENE APPROACH

- **Historical reports** - Polymorphisms in 3 genes were associated with CIMT
  - methyltetrahydrofolate reductase
  - angiotensin I converting enzyme
  - apoprotein E

- When the analysis was restricted to larger studies (>1000 subjects), apo E polymorphism was the only polymorphism with a persistent, statistically significant association with CIMT.

CIMT AND GWAS - CHARGE consortium

- In **meta-analysis** of **GWAS data** (genome wide association studies) involved over 40,000 subjects of European ancestry (**CHARGE consortium**)
  - associations between **3 regions (polymorphisms)** and common CIMT
    - chromosome 8q23.1 (**rs6601530**) in the **Pin2-interacting protein 1 gene**
    - chromosome 8q24 (**rs11781551**) - the **ZHX2 gene** - nuclear homodimeric transcriptional repressors
    - chromosome 9q13 (**rs445925**), fell upstream of the **APOC1 gene**

Carotid plaques and GWAS - CHARGE consortium

In meta-analysis of GWAS data (genome wide association studies) involved over 40,000 subjects of European ancestry (CHARGE consortium)

- association between 2 regions (polymorphisms) and the presence of carotid plaques
  - Chromosome 7q22 (rs17398575), close to the **PIK3CG** gene
  - Chromosome 4q31 (rs1878406), located 8.5 kb from **EDNRA**

**Figure 3. Regional plots for plaque SNPs**

The Wellcome Trust Case Control Consortium

- Measurements of CCA-IMT were available on 31,210 participants from 9 studies
- Measurements of carotid artery plaques were available on 25,179 participants from 7 studies

- 2 SNPs (rs11984041 and rs2107595) of the gene HDAC9 (histone deacetylase 9) → associated with:
  - common CIMT (rs2107595 p=0.0018)
  - presence of carotid plaque (rs2107595 p=0.0022)
GWAS AND CAD

- GWAS studies (CARDIOGRAM + C4D Consortium) have identified 58 independent loci associated with CAD contributing 13.3% to CAD heritability.

- Most identified loci have low allele frequency (<5%) with minor contributions to CAD development.

- Their exact function is known only for some of them and is related to inflammatory response, oxidative stress regulation, lipid function, transportation, endothelial dysfunction and other pathogenic processes involved in atherosclerosis.

According to 2016 ESC Guidelines, the generalized use of DNA testing is not recommended in the CVD risk assessment (in clinical practice).
**EPIGENETICS AND BIOMARKERS OF ATHEROSCLEROSIS**

- A plethora of **environmental risk factors** may result in **epigenetic modifications** leading to **alterations of gene expression** relevant to the onset or progression of CVD.

- **Epigenetics** consists of → **three interrelated mechanisms**:
  - DNA-based modifications
  - the histone modifications
  - RNA-based mechanisms

- **Atherosclerosis** can arise at the **early stages of development and growth** during pregnancy.
  - **Fetal exposure** to high-fat diet or dietary imbalance, gestational diabetes, maternal obesity, and smoking are associated with **increased risk and progression of atherosclerosis**.

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**Figure 1** The relevance of epigenetics in the atherosclerosis.
Epigenetic biomarkers of atherosclerosis consist of three distinct processes: DNA methylation, histone protein methylation and acetylation, and RNA mechanisms including activity of mi-RNA.
Abbreviations: Ac, acetylation; DNMTs, methyltransferases; Me, methylation.
2016 European Guidelines on cardiovascular disease prevention in clinical practice

The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)

2.4.1.3 Epigenetics

Epigenetics studies the chemical changes in DNA that affect gene expression. Methylation of genes related to CV risk factors is associated with variation in CV risk factor levels, and lower DNA methylation levels are associated with an increased risk of CAD or stroke. No information exists, however, regarding the effect of epigenetic markers in improving CVD risk prediction beyond conventional risk factors. Thus, epigenetic screening of CVD is not recommended.
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NEW BIOMARKERS AND ATHEROSCLEROSIS

Some new promising **serologic and genetic biomarkers** that provide significant **diagnostic and prognostic information** about **cardiovascular risk prediction and atherosclerosis** have so far been reported.

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Abbreviations: ACS, acute coronary syndrome; AP, angina pectoris; CAD, coronary artery disease; CV, cardiovascular; CVD, cardiovascular disease; HD, heart disease; HF, heart failure; MI, myocardial infarction.

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• Genetic markers of CAD

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Goals of personalized medicine are to:
1. identify individuals at high risk of developing a disease (stroke, MI)
   • Environmental risk factors
   • Genetic markers
   • Epigenetic markers
2. offer preventive measures tailored to these identified risks
3. decrease the burden of CV diseases (stroke, MI)
In the future (? years), doctors will be able to:

1) determine genetic risk score by defining risk polymorphisms for atherosclerosis

2) select the best drug to treat the disease (hypercholesterolemia/atherosclerosis) and the appropriate dose based on knowledge of each specific genetic makeup!
Vision for the Transformation of Medicine in the 21st Century

= PRECISION or “4P MEDICINE”

Predictive  Personalized  Preemptive

+ PARTICIPATORY

Leading to Patient Empowerment !!

Comprehensive, genomics-based health care is expected to become the norm with individualized preventive medicine and early detection of illnesses....
SHIFT FROM “CLASSICAL” BIOMARKERS TO VARIOUS “-OMICS” TECHNOLOGIES

• Interest in application of biomarkers for diagnostics and drug discovery has increased remarkably since the discovery of GBs.

• GBs originate from various “-omics” technologies and combine genomics, proteomics and metabolomics.

• The role of GBs in various therapeutic areas particularly cancer, cardiovascular diseases and disorders of the central nervous system, is expected to change in near future the modern medicine.
STUDENTS FROM ABROAD via STUDENT ORGANISATION SLOMSIC or via CMEPIUS
http://www2.cmepius.si/en/index.html
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Approaches to the Choice of new Biomarkers in Research with Emphasis on Atherosclerosis

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GENE BIOLOGY AND BIOMARKERS

• Increased knowledge of genes biology is generating promising marker candidates for more accurate diagnosis, prognosis assessment, and therapeutic targeting.

• Well-designed clinical trials for assessing the utility of markers are needed.

• Ideally, the population studied should be one:
  – in which knowledge of the marker would have substantial clinical relevance and
  – where the feasibility of obtaining appropriate specimens is established.
COMPLEX TRAITS AND DEVELOPMENT OF NEW BIOMARKERS

- **Complex disorders** such as **atherosclerosis** (CAD, carotid disease), **diabetes** with micro- /macrovascular complications → **caused by multiple genetic and environmental factors**

- **Difficulties in developing biomarkers** for complex traits are:
  - **phenotypic heterogeneity** of disorder
  - understanding the role of the **genetic factors** in the pathophysiology of the disease
  - Differences in **environmental factors** (in adulthood and during early human development) → **epigenetic effect**
  - **Interaction among factors** involved in the development of disorder

- The **identification of candidate genes** influencing **complex traits**
  - may help **predict the individuals who are predisposed** to disease
  - may lead to **new drug targets**
COMPLEX DISORDERS AND DEVELOPMENT OF GENOMIC BIOMARKERS

• Phenotypic and etiologic heterogeneity of complex disorders influences the ability:
  • to discover a biomarker and
  • to prove the clinical utility of the biomarker once identified.

• Very importantly, a specific polymorphism may have utility in one but not in another population → studies in a variety of populations are needed
2 MAIN TYPES OF GENETIC STUDIES WITH REGARD TO MARKER IDENTIFICATION

• CANDIDATE GENE APPROACH
  – we choose potential candidate genes and select potential genetic markers (SNPs) according to “a priori” hypothesis → gene/protein may or may not be confirmed as biomarker for disorder → test whether some polymorphism is functional (increased expression in blood, tissue)

• GENOME WIDE ASSOCIATION STUDIES
  – Scanning of the whole genome without “a priori” hypothesis → test whether some polymorphism is functional (increased expression in blood, tissue)
GWAS AND CAD

- GWAS studies have identified **58 independent loci** associated with CAD contributing **13.3%** to CAD heritability.

- Most identified loci have **low allele frequency (<5%)** with minor contributions to CAD development.

- Their **exact function** is known only for some of them and is related to **inflammatory response, oxidative stress regulation, lipid function, transportation, endothelial dysfunction** and other pathogenic processes involved in atherosclerosis.

Some new promising **serologic and genetic biomarkers** that provide significant **diagnostic and prognostic information** about cardiovascular risk prediction and atherosclerosis have so far been reported.

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- **GENOME WIDE ASSOCIATION STUDIES**
  - Scanning of the whole genome without "*a priori*" hypothesis → test whether some polymorphism is functional (increased expression in blood, tissue)
PROSTATIC CANCER – BIOMARKERS versus GENETIC BIOMARKERS
In prostate cancer - the prostate-specific antigen (PSA) changes is an accepted pre-clinical biomarker → far from perfect in terms of specificity and sensitivity.

Prostate biopsies are invasive, must be performed repeatedly, and only sample a fraction of the prostate.

Several new potential GBs with potentially better specificity and sensitivity than PSA have been discovered and published.

- These include specific gene mutations (e.g., HPC1, RNase-L) and combination of SNPs
• An increasing number of clinical studies are employing gene expression profiling PBMCs for the identification of novel transcriptional biomarkers of disease and markers predictive of clinical outcomes.
• Oligoarray ("AndroChip 2") containing 190 genes → was selected on the basis of their proved or potential role in prostate cancerogenesis related to androgen signalling.

• Multiple genes were identified exhibiting differential expression
  – in androgen-dependent and androgen-independent cells.

• All genes on the "Androchip 2" show a detectable expression levels in peripheral blood cells (PBMC).
PROSTATIC CANCER AND POTENTIAL NEW BIOMARKERS

Classification of prostate cancer using a protease activity nanosensor library.

Proteases are an important class of enzymes that play a role in every hallmark of cancer; their activities could be leveraged as biomarkers.

- Panel of prostate cancer proteases through transcriptomic and proteomic analysis.
- This activity-based nanosensor library could be useful throughout clinical management of prostate cancer, with both diagnostic and prognostic utility.

2 MAIN TYPES OF GENETIC STUDIES WITH REGARD TO MARKERS IDENTIFICATION

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  - we choose potential candidate genes and select potential genetic markers (SNPs) according to "a priori" hypothesis → gene/protein may or may not be confirmed as biomarker for disorder → test whether some polymorphism is functional (increased expression in blood, tissue) **MODEL 2 DIABETIC RETINOPATHY**

- **GENOME WIDE ASSOCIATION STUDIES**
  - Scanning of the whole genome without "a priori" hypothesis → test whether some polymorphism is functional (increased expression in blood, tissue)
The aim of this study was to examine the role of the rs6060566 polymorphism of the reactive oxygen species modulator 1 (Romo-1) gene in the development of diabetic retinopathy (DR) in Caucasians with type 2 diabetes (T2DM).

METHODS:

A total of 806 subjects with T2DM were enrolled in cross-sectional case-control study: 278 patients with DR and 528 subjects without clinical signs of DR.

Moreover, immunohistochemical analysis of 40 fibrovascular membranes of patients with proliferative DR was performed. The number of positive (labelled) cells per area - numerical areal density of the Romo-1-positive cells (the number of positive cells/mm(2) ) - was calculated.
RESULTS:
A significantly higher frequency of the CC genotype of the rs6060566 polymorphism of the Romo-1 gene was found in subjects with T2DM with DR compared to those without DR (odds ratio=3.3, 95% confidence interval=1.1-8.8; p = 0.024).

Moreover, the Romo-1 C allele was found to effect Romo-1 expression in fibrovascular membranes of patients with proliferative DR.

CONCLUSIONS:
The rs6060566 polymorphism of the Romo-1 gene was found to be an independent risk factor for DR in Caucasians with T2DM. Moreover, the rs6060566 is most probably functional and its effect might be mediated through the increased expression of Romo-1 in the retina.
2 MAIN TYPES OF GENETIC STUDIES WITH REGARD TO MARKERS IDENTIFICATION

- **CANDIDATE GENE APPROACH**
  - we choose potential candidate genes and select potential genetic markers (SNPs) according to “a priori” hypothesis → gene/protein may or may not be confirmed as biomarker for disorder → test whether some polymorphism is functional (increased expression in blood, tissue) **MODEL 3 – CAROTID ATHERO рассода**

- **GENOME WIDE ASSOCIATION STUDIES**
  - Scanning of the whole genome without “a priori” hypothesis → test whether some polymorphism is functional (increased expression in blood, tissue)
SUBJECTS WITH T2DM AND CAROTID ATHEROSCLEROSIS – SLOVENIAN STUDY

- The **design of the study** was cross-sectional with follow-up for up to 4 years.
- **Consecutive patients** with **type 2 diabetes** from different General hospitals in Slovenia were enrolled.

**Inclusion criteria for cases:**
- Caucasians above >50 years
- &
- with DM 2

**Exclusion criteria:**
- Evident CAD (history of myocardial infarction)
- &
- CV stroke

DNA extraction
- Combine sample with TaqMan SNP Genotyping Assay
- Run on real-time PCR System
- Analyze data
Patients examination

- **Doppler examinations of carotid arteries**
  - Morphological data
  - Functional data

**CIMT**
- Cut-off > 75 percentile

**Plaque type**
- No plaque (0)
- Unstable plaque (1, 2, 3) + plaque thickness
- Stable plaque (4, 5) + plaque thickness

**Plaque score**
- Number of affected arteries (CCA, bifurcation, ICA)
  - (0, 1, 2)
  - (3, 4, 5, 6)

- **CT angio of coronary arteries**
  - Coronary calcium score
1000 subjects are enrolled

UB Slovenj Gradec
UB Murska Sobota
UKC Maribor
UKC Ljubljana
Medicor d.o.o.
SB Izola
MC Medicor d.d.

700 cases
Doppler exams are available

300 cases
Doppler exams - are ongoing (2018)
We evaluated gene polymorphisms of different pathogenetic systems (oxidative stress, inflammatory, growth factors etc.)

...as genetic markers of **carotid atherosclerosis** in type 2 DM

We wanted to learn if there are any differences in CIMT and presence of carotid plaques according to genotypes (risk genotypes vs. non-risk genotypes).


<table>
<thead>
<tr>
<th></th>
<th>Subjects with T2DM</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.39 ± 9.61</td>
<td>60.07 ± 9.18</td>
<td>0.008</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>338 (56.8)</td>
<td>92 (46.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>11.25 ± 7.88</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cigarette smoking (%)</td>
<td>53 (8.91)</td>
<td>34 (17.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>108.65 ± 12.88</td>
<td>93.31 ± 13.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.00 ± 4.74</td>
<td>27.90 ± 4.42</td>
<td>0.16</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>147.1 ± 19.80</td>
<td>143.3 ± 16.6</td>
<td>0.86</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>85.78 ± 11.60</td>
<td>84.7 ± 11.6</td>
<td>0.19</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>8.04 ± 2.57</td>
<td>5.27 ± 0.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.89 ± 3.56</td>
<td>4.79 ± 0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.70 ± 1.18</td>
<td>5.36 ± 1.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.20 ± 0.35</td>
<td>1.43 ± 0.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.63 ± 0.94</td>
<td>3.24 ± 0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.9 (1.2–2.7)</td>
<td>1.3 (0.9–1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>3.5 ± 1.18</td>
<td>2.2 ± 1.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CIMT (µm)</td>
<td>958 ± 194</td>
<td>890 ± 212</td>
<td>0.007</td>
</tr>
<tr>
<td>Statin therapy (%)</td>
<td>375 (63.0)</td>
<td>62 (31.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihypertensive agents (%)</td>
<td>499 (83.9)</td>
<td>58 (29%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Continuous variables were expressed as means ± standard deviations when normally distributed and as median (interquartile range) when asymmetrically distributed. Categorical variables were expressed as frequency (percentage). BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: glycated haemoglobin; hs-CRP: high sensitivity C-reactive protein.
We found an association between the rs2071559 (KDR) and either CIMT or sum of plaque thickness in subjects with T2DM.
### Vascular endothelial growth factor (VEGF) - rs2010963
Receptor for VEGF (KDR) - rs2071559

**Table 2: VEGF serum levels in subjects with and without T2DM with regard to the rs2010963 and rs2071559 genotypes.**

<table>
<thead>
<tr>
<th>rs2010963</th>
<th>Mean (95% CI)</th>
<th></th>
<th>Linear trend analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC (52)</td>
<td>63.5 ± 29.2</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CG + GG (543)</td>
<td>46.1 ± 22.3</td>
<td>F</td>
<td>3.22</td>
</tr>
<tr>
<td>rs2071559</td>
<td>Mean (95% CI)</td>
<td></td>
<td>Linear trend analysis</td>
</tr>
<tr>
<td>CC (131)</td>
<td>69.4 ± 25.1</td>
<td></td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CT + TT (464)</td>
<td>40.9 ± 28.3</td>
<td>F</td>
<td>3.70</td>
</tr>
</tbody>
</table>

**Higher serum levels of VEGF were found in subjects with the CC genotypes of both polymorphisms (rs2010963, rs2071559) in comparison with subjects with other genotypes.**

**Increased expression of VEGF receptor was found in atherosclerotic plaques (endarterectomy sequester).**
## Table 5: Association of the rs1801282 genotypes with the presence of plaques and presence of unstable plaques in patients with T2DM at the time of recruitment.

<table>
<thead>
<tr>
<th>rs1801282</th>
<th>Presence of plaque</th>
<th></th>
<th>Presence of unstable plaque</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p</td>
<td>OR (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td>Hypertension (0 = no; 1 = yes)</td>
<td>1.71 (0.93–2.58)</td>
<td>0.04</td>
<td>1.25 (0.88–2.64)</td>
<td>0.97</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>1.07 (0.92–1.007)</td>
<td>0.17</td>
<td>1.11 (0.86–1.44)</td>
<td>0.32</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>1.21 (0.78–1.89)</td>
<td>0.40</td>
<td>1.08 (0.75–1.56)</td>
<td>0.67</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.18 (0.05–0.63)</td>
<td><strong>0.008</strong></td>
<td>0.30 (0.08–1.13)</td>
<td>0.08</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.28 (0.63–1.03)</td>
<td>0.09</td>
<td>1.09 (0.66–1.37)</td>
<td>0.34</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>1.14 (0.64–1.54)</td>
<td>0.28</td>
<td>1.22 (0.74–1.92)</td>
<td>0.42</td>
</tr>
<tr>
<td>GC + GG*</td>
<td>0.79 (0.48–1.14)</td>
<td><strong>0.04</strong></td>
<td>0.83 (0.34–1.91)</td>
<td>0.65</td>
</tr>
</tbody>
</table>

*GC + GG represents the combined genotypes of rs1801282.

---

**Polymorphisms of the PPAR-γ (rs1801282) and Its Coactivator (rs8192673) Have a Minor Effect on Markers of Carotid Atherosclerosis in Patients with Type 2 Diabetes Mellitus.**

Plesković A, Šantl Letonja M, Cokan Vujkovac A, Starčević JN, Petrović D.


PMID: 26949382  Free PMC Article
In our study, we found an association between the rs12762303 and coronary calcium score in subjects with T2DM. Moreover, we found an association between the rs3802278 and CIMT in subjects with T2DM.
ECHO markers of subclinical carotid atherosclerosis in T2DM

ASSOCIATION

CIMT
- rs2071559 KDR
- rs4646994 ACE
- rs3802278 ALOX5AP
- rs275651, rs931490 AT1R
- MMP3 (rs3025058)

SUM OF PLAQUE THICKNESS
- rs2071559 KDR
- rs4646994 ACE
- rs699 AGT
- IL-1α (rs1800587)
- IL-1β (rs1143634)
- PPARγ (rs1801282)
- SPP1 (rs4754)
- OPG (rs2073618)

PLAQUE PROGRESSION
- IL-1β (rs1143634)
- PPARGC1A (rs8192678)
ECHO markers of subclinical carotid atherosclerosis – NO ASSOCIATION

**CIMT**
- MMP3 (rs3025058)
- IL-1α (rs1800587)
- IL-1β (rs1143634)
- PPARγ (rs1801282)
- SPP1 (rs4754)
- OPG (rs2073618)
- AGT (rs47629 AGT1R (rs275561, rs931490, rs5182)
- ALOX5 (ss12762303)

**SUM OF PLAQUE THICKNESS**
- MMP3 (rs3025058)
- IL-1α (rs1800587)
- IL-1β (rs1143634)
- PPARγ (rs1801282)
- SPP1 (rs4754)
- OPG (rs2073618)
- AGT (rs47629 AGT1R (rs275561, rs931490, rs5182)
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**PLAQUE PROGRESSION**
- MMP3 (rs3025058)
- IL-1α (rs1800587)
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- OPG (rs2073618)
- AGT (rs47629 AGT1R (rs275561, rs931490, rs5182)
- ALOX5 (ss12762303)
2016 European Guidelines on cardiovascular disease prevention in clinical practice

The Sixth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of 10 societies and by invited experts)

2.4.1.3 Epigenetics

Epigenetics studies the chemical changes in DNA that affect gene expression. Methylation of genes related to CV risk factors is associated with variation in CV risk factor levels, and lower DNA methylation levels are associated with an increased risk of CAD or stroke. No information exists, however, regarding the effect of epigenetic markers in improving CVD risk prediction beyond conventional risk factors. Thus, epigenetic screening of CVD is not recommended.
Glutathione S-transferases (M1, T1) null alleles and ischemic vascular disease

- In meta-analysis including almost 20,000 cases with IVD (ischemic heart disease or ischemic stroke) and almost 50,000 controls in general population, GST (M1, T1) deletion genotypes did not associate with risk of IVD or with markers of inflammation (Norskov et al., 2011).

- In our study in diabetics, **smokers with GSTM1 null genotype (deletion) had increased CIMT** in comparison with smokers **without null genotype** (1.18 vs. 1.11 mm) - **epigenetic effect** (interaction between genetic and environmental factors)

- **Smokers with GSTM1 null genotype** were shown to have a greater CIMT as well as a higher 2-year progression rate of CIMT in general population (De Waart. 2001).


RESEARCH AND PhD STUDENTS AND MD STUDENTS

Jana Makuc MD, PhD
Andreja Vujkovac MD

Matej Završnik MD, PhD

Marija Šantl Letonja MD, PhD
Dražen Popović MD, PhD
Maja Šeruga MD, PhD

UB Slovenj Gradec

UKC Maribor

UB Murska Sobota

UB Ptuj

Mitja Letonja MD, PhD

Assist. dr. Ines Cilenšek DVM, PhD
Assist. professor Sara Mankoč MD, PhD
Jovana Nikolajević Starčević MD, PhD

Medicor d.o.o.

Sebastjan Merlo, MD, PhD
Nataša Gorkič MD
Hrvoje Reschner MD, PhD
Aleš Pleskovič MD, PhD

UB Izola

Stojan Kariž MD, PhD
Recently several gene polymorphisms of oxidative stress, inflammatory, growth factors and RAS genes have been reported to be associated with macrovascular complications (MI, carotid atherosclerosis) in pts with type 2 diabetes.


STUDENTS FROM ABROAD via STUDENT ORGANISATION SLOMSIC or via CMEPIUS

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STUDENTS FROM ABROAD
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http://www2.cmepius.si/en/index.html
Approaches to the choice of new biomarkers in research with emphasize on atherosclerosis

Professor Daniel Petrovič MD PhD, FESC

Institute of Histology and Embryology
Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

International Center for Cardiovascular Diseases Medicor, Izola, Slovenia
Approaches to the Choice of new Biomarkers in Research with Emphasis on Atherosclerosis

Professor Daniel Petrovič MD PhD, FESC

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International Center for Cardiovascular Diseases Medicor, Izola, Slovenia
Increased knowledge of genes biology is generating promising marker candidates for more accurate diagnosis, prognosis assessment, and therapeutic targeting.

Well-designed clinical trials for assessing the utility of markers are needed.

Ideally, the population studied should be one:
- in which knowledge of the marker would have substantial clinical relevance and
- where the feasibility of obtaining appropriate specimens is established.
COMPLEX TRAITS AND DEVELOPMENT OF NEW BIOMARKERS

- **Complex disorders** such as **atherosclerosis** (CAD, carotid disease), **diabetes** with micro-/macrovascular complications → **caused by** multiple genetic and environmental factors

- **Difficulties in developing biomarkers** for complex traits are:
  - phenotypic heterogeneity of disorder
  - understanding the role of the **genetic factors** in the pathophysiology of the disease
  - Differences in **environmental factors** (in adulthood and during early human development) → epigenetic effect
  - Interaction among factors involved in the development of disorder

- The **identification of candidate genes** influencing **complex traits**
  - may help predict the individuals who are predisposed to disease
  - may lead to **new drug targets**
COMPLEX DISORDERS AND DEVELOPMENT OF GENOMIC BIOMARKERS

• Phenotypic and etiologic heterogeneity of complex disorders influences the ability:
  • to *discover a biomarker* and
  • to *prove the clinical utility* of the biomarker once identified.

• a specific polymorphism may have utility in one but not in another population → *studies in a
diversity of populations* are needed
2 MAIN TYPES OF GENETIC STUDIES WITH REGARD TO MARKER IDENTIFICATION

• CANDIDATE GENE APPROACH
  – we choose potential candidate genes and select potential genetic markers (SNPs) according to "a priori" hypothesis → gene/protein may or may not be confirmed as biomarker for disorder → test whether some polymorphism is functional (increased expression in blood, tissue)

• GENOME WIDE ASSOCIATION STUDIES
  – Scanning of the whole genome without "a priori" hypothesis → test whether some polymorphism is functional (increased expression in blood, tissue)
PROSTATIC CANCER – BIOMARKERS versus GENETIC BIOMARKERS

Search results
Items: 1 to 20 of 29310

1. Serum DHEA-S is a Predictive Parameter of Abiraterone Acetate in Patients with Castration-resistant Prostate Cancer.
   Similar articles

2. Distinct urinary glycoprotein signatures in prostate cancer patients.
   PMID: 30237853 Free PMC Article
   Similar articles

3. The impact of chronic pelvic ischemia on LUTS and urinary levels of neuro-inflammatory, inflammatory and oxidative stress markers in elderly men: a case-control study.
   PMID: 30219559 Similar articles

4. Cyclin-Dependent Kinase 12, Immunity, and Prostate Cancer.
   PMID: 30209714 Similar articles

5. Are localized prostate cancer biomarkers useful in the clinical practice?
   Similar articles

Search results
Items: 1 to 20 of 2568

1. miR-21-5p, miR-141-3p, and miR-205-5p levels in urine-promiseing biomarkers for the identification of prostate and bladder cancer.
   PMID: 30194779
   Similar articles

2. Genetics and biology of prostate cancer.
   PMID: 30181359
   Similar articles

3. Multi-region proteome analysis quantifies spatial heterogeneity of prostate tissue biomarkers.
   PMID: 30020875 Free PMC Article
   Similar articles

4. Sigmoid Colon Adenocarcinoma with Isolated Loss of PMS2 Presenting in a Patient with Synchronous Prostate Cancer with Inactive MMR: Diagnosis and Analysis of the Family Pedigree.
   PMID: 30061258
   Similar articles

5. ZNF184 is a promising diagnostic biomarker and predicts biochemical recurrence in prostate cancer.
   Similar articles

   PMID: 31273923
   Similar articles
In prostate cancer - the prostate-specific antigen (PSA) changes is an accepted pre-clinical biomarker → far from perfect in terms of specificity and sensitivity.

Several new potential GBs with potentially better specificity and sensitivity than PSA have been discovered and published.

- These include specific gene mutations (e.g., HPC1, RNase-L) and combined SNPs
PERSONALIZED MEDICINE AND BIOMARKERS
EMPLOYING GENE EXPRESSION PROFILING PBMCs

• An increasing number of clinical studies are employing gene expression profiling PBMCs for the identification of novel transcriptional biomarkers of disease and markers predictive of clinical outcomes.
Oligoarray ("AndroChip 2") containing 190 genes → selected on the basis of their proved or potential role in prostate cancerogenesis related to androgen signalling.

This array was successfully utilized to monitor the gene expression profiles in androgen-dependent and androgen-independent cells.

- Multiple genes were identified exhibiting differential expression during drug treatment.

All genes fixed on the “Androchip 2” show a detectable expression levels in peripheral blood cells (PBMC).
The current gold standards have poor predictive value.

Tests for circulating PSA levels are susceptible to various non-cancer comorbidities and do not provide prognostic information.

Prostate biopsies are invasive, must be performed repeatedly, and only sample a fraction of the prostate.

Proteases are an important class of enzymes that play a role in every hallmark of cancer; their activities could be leveraged as biomarkers.

Panel of prostate cancer proteases through transcriptomic and proteomic analysis.

This activity-based nanosensor library could be useful throughout clinical management of prostate cancer, with both diagnostic and prognostic utility.

2 MAIN TYPES OF GENETIC STUDIES WITH REGARD TO MARKER IDENTIFICATION

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**GENOME WIDE ASSOCIATION STUDIES**

- Scanning of the whole genome without "a priori" hypothesis → test whether some polymorphism is functional (increased expression in blood, tissue)
GWAS AND CAD

- GWAS studies have identified **58 independent loci** associated with CAD contributing **13.3%** to CAD heritability.

- Most identified loci have **low allele frequency (<5%)** with minor contributions to CAD development.

- Their **exact function** → is known only for some of them → and is related to **inflammatory response, oxidative stress regulation, lipid function, transportation, endothelial dysfunction** and other pathogenic processes involved in atherosclerosis.

NEW BIOMARKERS AND Atherosclerosis

Some new promising **serologic and genetic biomarkers** that provide significant **diagnostic and prognostic information** about **cardiovascular risk prediction and atherosclerosis** have so far been reported.

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Predictive ability</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-sensitivity C-reactive protein</td>
<td>↑risk for CV events and mortality</td>
<td>[40,41]</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>↑risk of premature atherosclerosis</td>
<td>[42,43]</td>
</tr>
<tr>
<td>Apolipoprotein-associated phospholipase A2</td>
<td>correlates with the coronary HD and its severity</td>
<td>[44–46]</td>
</tr>
<tr>
<td>Matrix metalloproteinases</td>
<td>markers of plaque vulnerability and subclinical atherosclerosis, predictors of CVD and mortality</td>
<td>[47–51]</td>
</tr>
<tr>
<td>Myeloperoxidase</td>
<td>early detection of subclinical CAD, its severity, diagnosis of MI,</td>
<td>[52,53]</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>correlates with increased CAD and ACS risk and severity</td>
<td>[54–56]</td>
</tr>
<tr>
<td>Natriuretic peptides</td>
<td>↑risk for CV events and mortality</td>
<td>[57,58]</td>
</tr>
<tr>
<td>High-sensitivity assays for cardiac troponin</td>
<td>predictor of HF, mortality, and incident coronary HD</td>
<td>[59–62]</td>
</tr>
<tr>
<td>Pregnancy-associated plasma protein-A</td>
<td>marker of plaque vulnerability and predictor of CVD and mortality</td>
<td>[63]</td>
</tr>
<tr>
<td>Growth differentiation factor 15</td>
<td>predictor of CV and all-cause mortality, unstable AP</td>
<td>[64,65]</td>
</tr>
<tr>
<td>Micro-RNAs</td>
<td>association with the acute MI, predictor of atherosclerosis</td>
<td>[25,37]</td>
</tr>
</tbody>
</table>

**Table 2** New promising biomarkers of cardiovascular risk prediction and atherosclerosis.

Abbreviations: ACS, acute coronary syndrome; AP, angina pectoris; CAD, coronary artery disease; CV, cardiovascular; CVD, cardiovascular disease; HD, heart disease; HF, heart failure; MI, myocardial infarction.

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• **GENOME WIDE ASSOCIATION STUDIES**
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The aim of this study was to examine the role of the rs6060566 polymorphism of the reactive oxygen species modulator 1 (Romo-1) gene in the development of diabetic retinopathy (DR) in Caucasians with type 2 diabetes (T2DM).

METHODS:
A total of 806 subjects with T2DM were enrolled in cross-sectional case-control study: 278 patients with DR and 528 subjects without clinical signs of DR. Moreover, immunohistochemical analysis of 40 fibrovascular membranes of patients with proliferative DR was performed. The number of positive (labelled) cells per area - numerical areal density of the Romo-1-positive cells (the number of positive cells/mm(2) ) - was calculated.
DIABETIC RETINOPATHY AND POTENTIAL BIOMARKER ROMO-1

RESULTS:
A significantly higher frequency of the **CC genotype** of the rs6060566 polymorphism of the Romo-1 gene was found in subjects with T2DM with DR compared to those without DR (odds ratio=3.3, 95% confidence interval=1.1-8.8; p = 0.024).

Moreover, the **Romo-1 C allele** was found to effect Romo-1 expression in fibrovascular membranes of patients with proliferative DR.

CONCLUSIONS:
The rs6060566 polymorphism of the **Romo-1 gene** was found to be an independent risk factor for DR in Caucasians with T2DM. Moreover, the rs6060566 is most probably functional and its effect might be mediated through the increased expression of Romo-1 in the retina.
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- **Doppler examinations of carotid arteries**
  - Morphological data
  - Functional data

- **CIMT**
  - Cut-off > 75 percentile

- **Plaque type**
  - No plaque (0)
  - Unstable plaque (1,2,3) + plaque thickness
  - Stable plaque (4,5) + plaque thickness

- **Plaque score**
  - Number of affected arteries (CCA, bifurcation, ICA)
    - (0,1,2)
    - (3,4,5,6)

- **CT angio of coronary arteries**
  - Coronary calcium score

ACI = 1
BIFF = 1
ACC = 1
1000 subjects are enrolled

UB Slovenj Gradec
UKC Ljubljana
Medicor d.o.o.

UKC Maribor

UB Murska Sobota

SB Izola
MC Medicor d.d.

700 cases
Doppler exams are available

300 cases
Doppler exams - are ongoing (2018)
WE EVALUATED GENE POLYMORPHISMS OF DIFFERENT PATHOGENETIC SYSTEMS (OXIDATIVE STRESS, INFLAMMATORY, GROWTH FACTORS etc.)

...as genetic markers of carotid atherosclerosis in type 2 DM

We wanted to learn if there are any differences in CIMT and presence of carotid plaques according to genotypes (risk genotypes vs. non-risk genotypes).


<table>
<thead>
<tr>
<th></th>
<th>Subjects with T2DM ( n = 595 )</th>
<th>Control group ( n = 200 )</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>62.39 ± 9.61</td>
<td>60.07 ± 9.18</td>
<td>0.008</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>338 (56.8)</td>
<td>92 (46.0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>11.25 ± 7.88</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cigarette smoking (%)</td>
<td>53 (8.91)</td>
<td>34 (17.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>108.65 ± 12.88</td>
<td>93.31 ± 13.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.00 ± 4.74</td>
<td>27.90 ± 4.42</td>
<td>0.16</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>147.1 ± 19.80</td>
<td>143.3 ± 16.6</td>
<td>0.86</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>85.78 ± 11.60</td>
<td>84.7 ± 11.6</td>
<td>0.19</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>8.04 ± 2.57</td>
<td>5.27 ± 0.87</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.89 ± 3.56</td>
<td>4.79 ± 0.29</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.70 ± 1.18</td>
<td>5.36 ± 1.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.20 ± 0.35</td>
<td>1.43 ± 0.37</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.63 ± 0.94</td>
<td>3.24 ± 0.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.9 (1.2–2.7)</td>
<td>1.3 (0.9–1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>hs-CRP (mg/L)</td>
<td>3.5 ± 1.18</td>
<td>2.2 ± 1.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CIMT (µm)</td>
<td>958 ± 194</td>
<td>890 ± 212</td>
<td>0.007</td>
</tr>
<tr>
<td>Statin therapy (%)</td>
<td>375 (63.0)</td>
<td>62 (31.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Antihypertensive agents (%)</td>
<td>499 (83.9)</td>
<td>58 (29%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Continuous variables were expressed as means ± standard deviations when normally distributed and as median (interquartile range) when asymmetrically distributed. Categorical variables were expressed as frequency (percentage). BMI: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HbA1c: glycated haemoglobin; hs-CRP: high sensitivity C-reactive protein.
We found an association between the rs2071559 (KDR) and either CIMT or sum of plaque thickness in subjects with T2DM.
Vascular endothelial growth factor (VEGF) - rs2010963
Receptor for VEGF (KDR) - rs2071559

<table>
<thead>
<tr>
<th></th>
<th>rs2010963</th>
<th>rs2071559</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td></td>
<td>CC (52)</td>
<td>CG + GG (543)</td>
</tr>
<tr>
<td>VEGF (ng/L)</td>
<td>63.5 ± 29.2</td>
<td>46.1 ± 22.3</td>
</tr>
<tr>
<td></td>
<td>CC (131)</td>
<td>CT + TT (464)</td>
</tr>
<tr>
<td>VEGF (ng/L)</td>
<td>69.4 ± 25.1</td>
<td>40.9 ± 28.3</td>
</tr>
</tbody>
</table>

Higher serum levels of VEGF were found in subjects with the CC genotypes of both polymorphisms (rs2010963, rs2071559) in comparison with subjects with other genotypes.

Increased **expression** of VEGF **receptor** was found in atherosclerotic plaques (endarterectomy sequester).
Polymorphisms of the **PPAR-γ** - rs1801282

**TABLE 5: Association of the rs1801282 genotypes with the presence of plaques and presence of unstable plaques in patients with T2DM at the time of recruitment.**

<table>
<thead>
<tr>
<th></th>
<th>Presence of plaque</th>
<th>Presence of unstable plaque</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>p</td>
</tr>
<tr>
<td><strong>rs1801282</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (0 = no; 1 = yes)</td>
<td>1.71 (0.93–2.58)</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>1.07 (0.92–1.007)</td>
<td>0.17</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>1.21 (0.78–1.89)</td>
<td>0.40</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.18 (0.05–0.63)</td>
<td><strong>0.008</strong></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.28 (0.63–1.03)</td>
<td>0.09</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>1.14 (0.64–1.54)</td>
<td>0.28</td>
</tr>
<tr>
<td>GC + GG*</td>
<td>0.79 (0.48–1.14)</td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>

---

**Polymorphisms of the PPAR-γ (rs1801282) and Its Coactivator (rs8192673) Have a Minor Effect on Markers of Carotid Atherosclerosis in Patients with Type 2 Diabetes Mellitus.**


PMID: 26949382  Free PMC Article
In our study, we found an association between the \textbf{rs12762303} and \textbf{coronary calcium score} in subjects with T2DM. Moreover, we found an association between the \textbf{rs3802278} and CIMT in subjects with T2DM.
ECHO markers of subclinical carotid atherosclerosis in T2DM

ASSOCIATION

CIMT
- rs2071559 KDR
- rs4646994 ACE
- rs3802278 ALOX5AP
- rs275651, rs931490 AT1R
- MMP3 (rs3025058)

SUM OF PLAQUE THICKNESS
- rs2071559 KDR
- rs4646994 ACE
- rs699 AGT
- IL-1α (rs1800587)
- IL-1β (rs1143634)
- PPARγ (rs1801282)
- SPP1 (rs4754)
- OPG (rs2073618)

PLAQUE PROGRESSION
- IL-1β (rs1143634)
- PPARGC1A (rs8192678)
ECHO markers of subclinical carotid atherosclerosis – NO ASSOCIATION

- **CIMT**
  - MMP3 (rs3025058)
  - IL-1α (rs1800587)
  - IL-1β (rs1143634)
  - PPARγ (rs1801282)
  - SPP1 (rs4754)
  - OPG (rs2073618)
  - AGT (rs47629 AGT1R (rs275561, rs931490 rs5182))
  - ALOX5 (ss12762303)

---

- **SUM OF PLAQUE THICKNESS**
  - MMP3 (rs3025058)
  - IL-1α (rs1800587)
  - IL-1β (rs1143634)
  - PPARγ (rs1801282)
  - SPP1 (rs4754)
  - OPG (rs2073618)
  - AGT (rs47629 AGT1R (rs275561, rs931490 rs5182))
  - ALOX5 (ss12762303)

---

- **PLAQUE PROGRESSION**
  - MMP3 (rs3025058)
  - IL-1α (rs1800587)
  - IL-1β (rs1143634)
  - PPARγ (rs1801282)
  - SPP1 (rs4754)
  - OPG (rs2073618)
  - AGT (rs47629 AGT1R (rs275561, rs931490 rs5182))
  - ALOX5 (ss12762303)
RESEARCH AND PhD STUDENTS AND MD STUDENTS

UB Slovenj Gradec
- Jana Makuc MD, PhD
- Andreja Vujkovac MD

UB Murska Sobota
- Matej Završnik MD, PhD

UKC Maribor

UB Ptuj
- Mitja Letonja MD, PhD

Medicor d.o.o.
- Assist. dr. Ines Cilenšek DVM, PhD
- Assist. professor Sara Mankoč MD, PhD
- Jovana Nikolajević Starčević MD, PhD
- Sebastjan Merlo, MD, PhD
- Nataša Gorkič MD
- Hrvoje Reschner MD, PhD
- Aleš Pleskovič MD, PhD

UB Izola
- Stojan Kariž MD, PhD
Recently several gene polymorphisms of oxidative stress, inflammatory, growth factors and RAS genes have been reported to be associated with macrovascular complications (MI, carotid atherosclerosis) in pts with type 2 diabetes.


Approaches to the choice of new biomarkers in research with emphasize on atherosclerosis

Professor Daniel Petrovič MD PhD, FESC

Institute of Histology and Embryology
Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

International Center for Cardiovascular Diseases Medicor, Izola, Slovenia
SUBJECTS WITH T2DM AND CAROTID ATHEROSCLEROSIS – SLOVENIAN STUDY

• The design of the study was cross-sectional with follow-up for up to 4 years
• 595 consecutive patients with type 2 diabetes from different General hospitals in Slovenia were enrolled
• 200 healthy controls → represented reference for CIMT measurement and genotype distribution.

Inclusion criteria for cases:
- Caucasians above >50 years
- with DM 2

Exclusion criteria:
- Evident CAD (myocardial infarction)
- CV stroke

DNA extraction
Combine sample with TaqMan SNP Genotyping Assay
Run on real-time PCR System
Analyze data
Epigenome-wide association study of body mass index, and the adverse outcomes of adiposity

- BMI (a key measure of adiposity) is associated with widespread changes in DNA methylation
- alterations in DNA methylation are predominantly the consequence of adiposity, rather than the cause
- methylation loci identify genes involved in lipid and lipoprotein metabolism, substrate transport and inflammatory pathways
- the disturbances in DNA methylation predict future development of type 2 diabetes (relative risk per 1 SD increase in methylation risk score: 2.3 (2.07–2.56); $P = 1.1 \times 10^{-54}$).
Integration of genome-scale metabolic models and other biological networks – scaffold for integration of omics data (incl. transcriptomics, proteomics and metabolomics)

Biomarkers predicted for CVD can be used together with other risk estimating algorithms for personalized risk prediction of CVD.

Discoveries from basic research
- Reception of specimens
- DNA, RNA sequencing
- Clinical Exome sequencing
- Omics data, cloud computing
- Disease signature
- GWAS, HapMap, Bioinformatics
- Molecular causes of disease
- Integration of functional pathways
- Genome-/phenome-wide analysis

- predictive,
- diagnostic,
- pronostic biomarkers

Clinical practice
- Moving evidence-based guidelines into health practice
- Delivery of recommended care to the right patient

Traditional medicine (one-size-fit all)
Stratified medicine (Precision medicine)

Presonalized medicine (One patient one clinical protocol)

Translational pathway
- Epidemiologic studies, Familial history
- Molecular Imaging
- Drug response monitoring
- Biobanks
- Special cohorts for PM
- Electronic medical records
- Algorithms guided dosing
- Genetic testing
- Implementation of new technologies

Policy and practice
- Inform genetic specialized physicians
- Educate patients
- Counselling methods
- Assessment of benefit/harm
- Regulation issues
In the future (? years), doctors will be able to select the best drug to treat your disease and the appropriate dose based on knowledge of your specific genetic makeup!
“We need to learn to measure what we value, not value what we can easily measure...”

Roman Emperor & Philosopher
Marcus Aurelius AD 120
Towards conclusions.....

Prevention

Interventions
**PPARγ:** rs1801282 polymorphism in exon 1 (Pro12Ala)

**PPARGC1A:** rs8192678 polymorphism in exon 8 (Gly482Ser)
Glutathione S- transferases (M1, T1, P1), manganese superoxide dismutase and carotid atherosclerosis

<table>
<thead>
<tr>
<th></th>
<th>CIMT&gt;1 mm</th>
<th>Plaque type 1,2,3</th>
<th>Plaque score 3,4,5,6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>p</td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>GSTM1-0</td>
<td>0.97</td>
<td>1.01</td>
<td>0.56-1.80</td>
</tr>
<tr>
<td>GSTT1-0</td>
<td>0.20</td>
<td>1.60</td>
<td>0.78-3.28</td>
</tr>
<tr>
<td>GSTP1</td>
<td>0.84</td>
<td>0.94</td>
<td>0.51-1.73</td>
</tr>
<tr>
<td>Ile/Val</td>
<td>0.29</td>
<td>0.56</td>
<td>0.19-1.63</td>
</tr>
<tr>
<td>Val/Val</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MnSOD</td>
<td>0.67</td>
<td>0.85</td>
<td>0.39-1.81</td>
</tr>
<tr>
<td>VA</td>
<td>0.89</td>
<td>1.06</td>
<td>0.46-2.45</td>
</tr>
<tr>
<td>VV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GSTM1-0/GSTT1-0</td>
<td>0.19</td>
<td>1.70</td>
<td>0.76-3.98</td>
</tr>
</tbody>
</table>

After adjustment for age, sex, smoking, BMI, lipid parameters, duration of hypertension and diabetes, carriers of GSTT1-0 (deletion) genotype showed an increased risk for higher plaque score (higher number of affected carotid segments) (OR=2.29; p=0.012), but no association with CIMT and plaque stability was observed.