Modern Molecular Genomic Biomarkers with Emphasis on Atherosclerosis

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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
GENOMIC BIOMARKERS (DNA, RNA)

DNA markers are:
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 markers are:
1. RNA sequence
2. RNA expression levels
3. RNA processing, e.g. splicing and editing
4. MicroRNA levels

PROTEIN BIOMARKERS
COMPLEX DISORDERS AND BIOMARKERS

• **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**

Genetic tests and genomic biomarkers: regulation, qualification and validation
Giuseppe Novelli, Cinzia Ciccacci, Paola Borgiani, Marisa Papaluca Amati, Eric Abadie
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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 practice 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.
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**.


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

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.

Table 1. Monogenic dyslipidaemias associated with atherosclerosis.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Disease</th>
<th>OMIM</th>
<th>Inheritance</th>
<th>Gene</th>
<th>Onset</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypercholesterolemia</td>
<td>Familial hypercholesterolaemia</td>
<td>143890</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AD/AR</td>
<td>LDLR</td>
<td>all ages</td>
<td>1: 200-250</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AD/AR</td>
<td>PCSK9</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AD</td>
<td>STAP1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AD</td>
<td>APOE</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>AR</td>
<td>LDLRAP1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
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
CHARGE 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**

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

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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)

The Wellcome Trust Case Control Consortium 2 Ischaemic Stroke Study; Stroke 2013; 44:1220-5. Markus HS et al.
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.

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,\(^\text{87,88}\) and lower DNA methylation levels are associated with an increased risk of CAD or stroke.\(^\text{89}\) 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.

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

<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>

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.
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4. MicroRNA levels

PROTEIN BIOMARKERS
CAROTID ATHEROSCLEROSIS
Endarteriectomy sequester

Normal CAROTID ARTERY
2 MAIN TYPES OF GENETIC STUDIES WITH REGARD TO MARKER IDENTIFICATION (DNA)

• **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)
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
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
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).

**CAROTID ATHEROSCLEROSIS AND POTENTIAL DNA BIOMARKERS**


### Table 1: Baseline characteristics of subjects with T2DM and subjects without T2DM (control group).

<table>
<thead>
<tr>
<th>Characteristic</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

**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>
<thead>
<tr>
<th>rs2010963</th>
<th>Mean (95% CI)</th>
<th>p</th>
<th>Linear trend analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>63.5 ± 29.2</td>
<td>CC (52)</td>
<td></td>
<td>0.01</td>
</tr>
<tr>
<td>46.1 ± 22.3</td>
<td>CG + GG (543)</td>
<td></td>
<td>3.22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>0.03</td>
</tr>
<tr>
<td>rs2071559</td>
<td>Mean (95% CI)</td>
<td>p</td>
<td>Linear trend analysis</td>
</tr>
<tr>
<td>69.4 ± 25.1</td>
<td>CC (131)</td>
<td></td>
<td>0.01</td>
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<tr>
<td>40.9 ± 28.3</td>
<td>CT + TT (464)</td>
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<td>3.70</td>
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<td></td>
<td>0.02</td>
</tr>
</tbody>
</table>

CAROTID ATHEROSCLEROSIS AND POTENTIAL DNA BIOMARKERS
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)

CAROTID ATHEROSENOSCLEROSIS AND POTENTIAL DNA BIOMARKERS
ECHO markers of subclinical carotid atherosclerosis – NO ASSOCIATION

CAROTID Atherosclerosis AND POTENTIAL DNA BIOMARKERS

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)
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PLAQUE PROGRESSION
- MMP3 (rs3025058)
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2 MAIN TYPES OF GENETIC STUDIES WITH REGARD TO MARKER IDENTIFICATION (DNA)

• 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.

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 2  New promising biomarkers of cardiovascular risk prediction and atherosclerosis.

<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, correlates with increased CAD and ACS risk and severity</td>
<td>[52,53]</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td></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>
</tbody>
</table>
| Growth differentiation factor 15                    | predictor of CV and all-cause mortality, unstable AP association with the acute MI, predictor of atherosclerosis | |}

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

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**GENOMIC BIOMARKERS (DNA, RNA)**

**DNA markers are:**
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 markers are:**
1. RNA sequence
2. RNA expression levels
3. RNA processing, e.g. splicing and editing
4. MicroRNA levels

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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, \(^{87,88}\) and lower DNA methylation levels are associated with an increased risk of CAD or stroke. \(^{89}\) 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.
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.


# Foreign Students

## Tuji Študenti

### 2019
- August 2019
  - Chen Dong (China)
  - Yaman Akhtar (Bangladesh)
  - Agnieszka Akidela (Poland)
- July 2019
  - Praskova Polna (Bulgaria)
  - Lee Jiwan (Korea)
  - Sekhawati Rishu (Indonesia)
  - Celija Rebecca (Malta)

### 2018
- September 2018
  - Hazar Samad Hasan Al Safar (Iraq)
- August 2018
  - Mariana Ortega (Mexico)
  - Maciejek Robert (Poland)
  - Marta Petrusev (Bulgaria)
  - Lynna Marcy (Lithuania)
- July 2018
  - Magdalena Barteczko (Poland)
  - Akhadi Krest Antoli (Ukraine)
  - Sarah-Leen Latifi (Ghana)

### 2017
- Luca Ronen (Belgium)
- Teyrome de Vale (Brazil)
- Mihailo Angeloska (Greece)
- Csata Bara (Portugal)
- Klaas Trougou (Greece)

### 2016
- Bernarda Fudal Paramita Rahayu (Indonesia)
- Samir Gharai
- Nasr Tutun Voro (Ukraine)

### 2015
- Jorge Alberto Marzanares Espinoza (Mexico)
- Paul Scavino (Mali)

### 2014
- Jen Novak (Czech)
- Nikola K. Cvetkovic

### 2013
- Marcela Kelkoe Spagolla Uehara (Brazil)

### 2012
- Aki Ricart Hernandez (Panama)
- Cheong Chun Yang (Singapore)
- Maciejek Robert (Poland)
- Andrei Calboca (Romania)
- Petko Kusman (Bulgaria)
- Damasco Cristina Bento Balsis (Portuguese)
STUDENTS FROM ABROAD
via STUDENT ORGANISATION SLOMSIC or via CMEPIUS
http://www2.cmepius.si/en/index.html
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Modern Molecular Genomic Biomarkers with Emphasis on Atherosclerosis

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1000 subjects are enrolled

Doppler exams are available

700 cases

Doppler exams are ongoing (2018)

300 cases
PREVENTATIVE MEDICINE KILLS
RETURN BUSINESS

FIRST DAY OF MED SCHOOL
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); $P = 1.1 \times 10^{-54}$).
Biomarkers predicted for CVD can be used together with other risk estimating algorithms for personalized risk prediction of CVD.

Integration of genome-scale metabolic models and other biological networks – scaffold for integration of omics data (incl. transcriptomics, proteomics and metabolomics)

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

Transition to Personalized Medicine
- Predictive, diagnostic, pronostic biomarkers

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

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

Presonalized medicine
- One patient one clinical protocol

Policy and practice
- Inform genetic specialized physicians
- Educate patients
- Counselling methods
- Assessment of benefit/harm
- Regulation issues

Discoveries from clinical research and practice
- 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
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!
Towards conclusions.....