Genetic factors and genetic variants associated with atherosclerosis: opportunity for personalized medicine

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• Atherosclerosis and risk factors

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

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

• Predictions for genetic testing in future → genetic smart card

• Conclusions
CARDOVASCULAR DISEASES

• Coronary artery disease and cerebrovascular diseases are the leading causes of morbidity and mortality in the developed world.

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

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 factors → 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 these identified risks

![Diagram showing the relationship between environment, genotypes, and subclinical disease.](image-url)
MARKERS OF ATHEROSCLEROSIS

- Several 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 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.

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 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 cardiovascular diseases.

SCREENING FOR DYSLIPIDAEMIAS IN GENERAL POPULATION

- 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.

There is a need for a translation of genetic studies from the research field into every day clinical practice.

17 genes responsible for 5 phenotypes:
- Hypercholesterolemia
- Hypolipoproteinaemia
- Hyperlipidaemia
- Lipid storage disease
- Lipodystrophy

→ to be associated with atherosclerosis

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

<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>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>
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<tr>
<td></td>
<td></td>
<td></td>
<td>AD</td>
<td>STAP1</td>
<td></td>
<td></td>
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<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

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

• 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
• **Early 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)

The Wellcome Trust Case Control Consortium 2 Ischaemic Stroke Study; Stroke 2013; 44:1220-5. Markus HS et al.
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 (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 Gene Polymorphism (rs2010963) and Its Receptor, Kinase Insert Domain-Containing Receptor Gene Polymorphism (rs2071559), and Markers of Carotid Atherosclerosis in Patients with Type 2 Diabetes Mellitus.

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>0.04</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>

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**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.**

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


PMID: 26949382  Free PMC Article
We found an association between the rs4646994 (ACE) and progression of atherosclerosis, i.e., change in the sum of plaque thickness in subjects with T2DM.

### Table 3. Changes of markers of carotid atherosclerosis in subjects with type 2 diabetes mellitus between first the examination and the examination at the end of the study with regard to the rs4646994 (angiotensin-converting-enzyme insertion/deletion) and rs4341 polymorphisms.

<table>
<thead>
<tr>
<th>rs4646994 ACE I/D</th>
<th>II</th>
<th>ID</th>
<th>DD</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual increase in CIMT (μm/year)</td>
<td>14.28 (5.35-26.83)</td>
<td>22.43 (16.73-32.19)</td>
<td>20.34 (10.53-33.65)</td>
<td>0.34</td>
</tr>
<tr>
<td>Δ Number of segments with plaques</td>
<td>3.0 (1.0-3.0)</td>
<td>1.0 (0.5-2.5)</td>
<td>2.0 (1.0-3.0)</td>
<td>0.42</td>
</tr>
<tr>
<td>Δ Sum of carotid plaques thickness (mm)</td>
<td>4.00 (2.30-5.30)</td>
<td>4.68 (3.30-7.60)</td>
<td>6.22 (3.90-8.10)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>rs4341</th>
<th>GG</th>
<th>GC</th>
<th>CC</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Annual increase in CIMT (μm/year)</td>
<td>21.05 (14.28-33.65)</td>
<td>20.69 (16.55-24.26)</td>
<td>14.28 (10.71-20.08)</td>
<td>0.26</td>
</tr>
<tr>
<td>Δ Number of segments with plaques</td>
<td>2.0 (1.0-3.5)</td>
<td>2.0 (1.0-2.5)</td>
<td>3.0 (2.0-3.0)</td>
<td>0.49</td>
</tr>
<tr>
<td>Δ Sum of carotid plaques thickness (mm)</td>
<td>4.60 (3.40-7.90)</td>
<td>5.60 (4.35-8.55)</td>
<td>5.6 (2.60-6.90)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

ACE I/D: angiotensin-converting-enzyme insertion/deletion. Annual increase in CIMT (carotid intima media thickness) was calculated as CIMT(beginning)-CIMT(endpoint)/follow-up in years. Change in number of plaques is expressed as number of segments with plaque at the endpoint minus the number at the beginning. Sum of plaque thickness is calculated as the end sum minus the beginning sum. Data are expressed as median and range.
With linear regression analysis, we demonstrated that subjects with T2DM with the DD genotype of the rs4646994 had faster progression of atherosclerosis in comparison with other genotypes.
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.
An association was demonstrated between the **rs16933090** and coronary calcium score 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)
- According to **2016 ESC Guidelines** the generalized use of DNA testing is not recommended in the CVD risk assessment (in clinical practice).

### 2.4.1 Family history/(epi)genetics

**Key messages**
- Family history of premature CVD in first-degree relatives, before 55 years of age in men and 65 years of age in women, increases the risk of CVD.
- Several genetic markers are associated with an increased risk of CVD, but their use in clinical practice is not recommended.

### Recommendations for assessment of family history/(epi)genetics

<table>
<thead>
<tr>
<th>Assessment of family history of premature CVD (defined as a fatal or non-fatal CVD event or/and established diagnosis of CVD in first degree male relatives before 55 years or female relatives before 65 years) is recommended as part of cardiovascular risk assessment.</th>
<th>Class</th>
<th>Level</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>C</td>
<td>71</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>The generalized use of DNA-based tests for CVD risk assessment is not recommended.</th>
<th>Class</th>
<th>Level</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>B</td>
<td>72, 73</td>
<td></td>
</tr>
</tbody>
</table>
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 CIMT and smoking (epigenetics)

- **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).

- 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).

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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”

P predictive  P personalized  P 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....
RESEARCH AND PhD STUDENTS AND MD STUDENTS

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

Sebastjan Merlo, MD, PhD
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Hrvoje Reschner MD, PhD
Stojan Kariž MD, PhD

UB Slovenj Gradec
UKC Maribor
UB Ptuj
UB Murska Sobota
UB Izola
Medicor d.o.o.
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.


Table 4. Multiple linear regression analysis for association of rs4646994 (angiotensin-converting-enzyme insertion/deletion) with carotid atherosclerosis progression in patients with type 2 diabetes mellitus.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Δ CIMT/Year</th>
<th>p Value</th>
<th>Δ Number of Segments</th>
<th>p Value</th>
<th>Δ Sum of Plaque Thickness</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td></td>
<td>β</td>
<td></td>
<td>β</td>
<td></td>
</tr>
<tr>
<td>A) rs4646994</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension (yes/no)</td>
<td>0.144</td>
<td>0.59</td>
<td>0.206</td>
<td>0.88</td>
<td>0.272</td>
<td>0.53</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>0.031</td>
<td>0.42</td>
<td>0.027</td>
<td>0.15</td>
<td>0.014</td>
<td>0.69</td>
</tr>
<tr>
<td>ID</td>
<td>0.141</td>
<td>0.49</td>
<td>0.116</td>
<td>0.51</td>
<td>0.845</td>
<td>0.26</td>
</tr>
<tr>
<td>DD</td>
<td>0.102</td>
<td>0.63</td>
<td>0.146</td>
<td>0.41</td>
<td>0.952</td>
<td>0.04</td>
</tr>
</tbody>
</table>

All models were adjusted to age, sex, smoking habits, serum levels of HbA1c, statin treatment and initial values of the dependent variables. Reference groups are homozygotes for the I allele.

Association of the C242T polymorphism in the NADPH oxidase p22 phox gene with carotid atherosclerosis in Slovenian patients with type 2 diabetes.

Leotonja MS, Nikolajević-Starčević J, Batista DC, Osredkar J, Petrović D.
PMID: 22932942
Similar articles
Genetic factors and genetic variants associated with atherosclerosis: opportunity for personalized medicine

Professor Daniel Petrovič MD PhD, FESC

Institute of Histology and Embryology
Faculty of Medicine
University of Ljubljana, 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
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); \( P = 1.1 \times 10^{-54} \)).
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.

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.....
*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><strong>GSTM1-0</strong></td>
<td>0.97</td>
<td>1.01</td>
<td>0.56-1.80</td>
</tr>
<tr>
<td><strong>GSTT1-0</strong></td>
<td>0.20</td>
<td>1.60</td>
<td>0.78-3.28</td>
</tr>
<tr>
<td><strong>GSTP1</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Ile/Val</td>
<td>0.84</td>
<td>0.94</td>
<td>0.51-1.73</td>
</tr>
<tr>
<td>Val/Val</td>
<td>0.29</td>
<td>0.56</td>
<td>0.19-1.63</td>
</tr>
<tr>
<td>MnSOD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VA</td>
<td>0.67</td>
<td>0.85</td>
<td>0.39-1.81</td>
</tr>
<tr>
<td>VV</td>
<td>0.89</td>
<td>1.06</td>
<td>0.46-2.45</td>
</tr>
<tr>
<td><strong>GSTM1-0/GSTT1-0</strong></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.