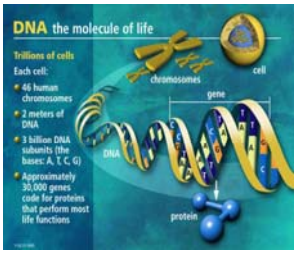
# Molekularna dijagnostika genetičkih bolesti i Europska mreža za kvalitetu molekularne dijagnostike

**Jadranka Sertić, Hana Ljubić, Ana Merkler**

HAZU, Odbor za primjenjenu genomiku, 16.listopada 2017.
Molekularna genetika – novosti u dijagnostici i terapiji

## Molekula života – DNA
Humani genom


Francis Collins,2003.

https://www.slideshare.net/nirmalajosephine1/biology-form-5-chapter-5-53-a-dna

1917–2017 100 godina Medicinskog fakulteta Sveučilišta u Zagrebu

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*We now know how God wrote the book of life*

*But do we know how to read the book ?*

**Bill Clinton 2003.**

https://deista.wordpress.com/2012/03/13/deismo-y-division-deista/

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# Precizna medicina – nacionalni prioritet


**President Obama, 2015.**

https://www.nih.gov/allofus-research-program/precision-medicine-initiative-storify-collection

# 100,000 British Genomes

**A new initiative lead by the UK's National Health Service aims to sequence the genomes of as many as 100,000 patients, a project that will cost £100 million.**

**David Cameron, 2012.**

https://citas.in/autores/david-cameron/o=new

**"The UK will sequence 100,000 genomes from patients with cancer or rare disease"**

***The aims are to:***

- ***improve diagnosis of patients with rare disease***
- ***increase understanding of tumour variants that predict therapeutic response to targeted cancer drugs***
- ***accelerate uptake of genomic medicine in the NHS***
- ***stimulate and enhance UK industry and investment in genomics***

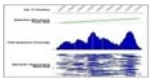
# DNA sekvenciranje

***gel-$P_{32}$***

tisuću bp / dan

***kapilarna elektroforeza***

milijun bp / dan

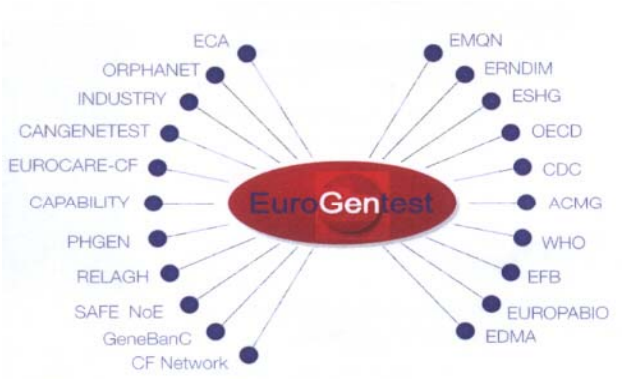


**sekvenciranje sljedeće generacije**

bilijun bp / dan

➢ **Hibridizacijske tehnike**

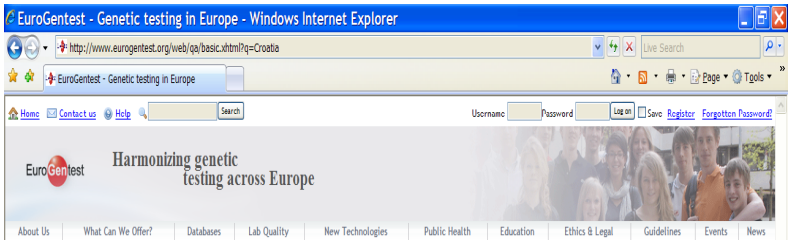
# Europska mreža za kvalitetu molekularne dijagnostike




https://www.eurogentest.org

# Harmonizacija genetičkog testiranja u Europi

https://eurogentest.org/



## Croatia

### Legal and regulatory background

In Croatia molecular genetic testing is regulated by national legislation. The legislation covers laboratory medicine in general, however there are no national guidelines or recommendations for MGT. Guidelines from EMQN are recommended.

### Licensing, certification, accreditation

A licence is required to operate a MGT laboratory. Certification and accreditation are not mandatory.

### Availability of EQA

Participation in EQA is not required by national legislation/guidelines.
There is no national EQA provider for MGT in Croatia.

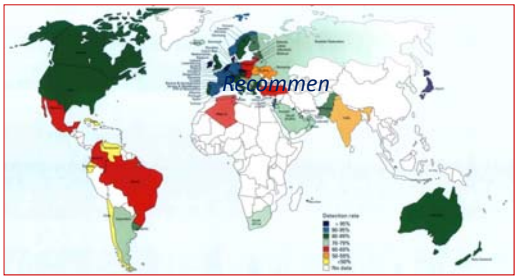
https://www.eurogentest.org

## 1991. molekularna dijagnostika u Hrvatskoj

### Cistična fibroza – monogenska bolest

Zergollern Lj, Stavljenić-Rukavina A, Barišić I, Sertić J.
*F508 deletion in Croatian cystic fibrosis patients*.
**Acta Med Croat 1992;46:181-184.**

## WHO: *The Molecular Genetic Epidemiology of Cystic Fibrosis*




Dequeker E, Cuppens H, Dodge J, Estivill X, Goossens M, Pignatti PF,et al. *Recommendations for quality improvement in genetic testing for cystic fibrosis. European Concerted Action on Cystic Fibrosis.* **Eur J Hum Genet.2000;8:2-24.**

## WHO - Pojavnost CFTR mutacija

**Table 1** Mutations found at a frequency higher than 1% in Europe[5-7]

| Country | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Albania | dF508 (70.0) | | | | | | | | | | | | | |
| Austria | dF508 (63.7) | G542X (2.1) | R1162X (1.9) | G551D (1.1) | | | | | | | | | | |
| Belarus | dF508 (63.0) | N1303K (2.7) | G542X (2.1) | W1282X (1.7) | | | | | | | | | | |
| Belgium | dF508 (75.5) | N1303K (2.9) | G542X (2.7) | W1282X (1.5) | S1251N (1.3) | 1717-1G>A (1.1) | | | | | | | | |
| Bulgaria | dF508 (61.0) | N1303K (409) | G542X (3.6) | R347P (2.0) | 1677delTA (2.0) | R1070Q (1.5) | W1282X (1.3) | G1244V+S912L (1.0) | | | | | | |
| Croatia | dF508 (64.5) | N1303K (3.6) | G542X (3.3) | G551D (1.1) | | | | | | | | | | |
| Cyprus | dF508 (46.7) | 1346P (16.7) | 1677delTA (6.7) | | | | | | | | | | | |
| Czechia | dF508 (71.6) | G551D (4.0) | N1303K (3.0) | G542X (2.2) | 1898+1G>A (2.0) | 2143delT (1.2) | CFTRdele2,3(21kb) (4.6) | | | | | | | |
| Denmark | dF508 (87.2) | 394delTT (1.9) | N1303K (1.0) | | | | | | | | | | | |
| Estonia | dF508 (54.0) | 394delTT (15.0) | | | | | | | | | | | | |
| Finland | dF508 (46.2) | 394delTT (28.8) | | | | | | | | | | | | |
| France | dF508 (66.8) | G542X (3.1) | 1717-1G>A (1.6) | N1303K (1.4) | | | | | | | | | | |
| Germany | dF508 (73.2) | R553X (2.7) | R347P (1.3) | G551D (1.3) | N1303K (1.2) | G542X (1.2) | 3849+10kbC>T (1.2) | CFTRdele2,3(21kb) (1.5) | | | | | | |
| Greece | dF508 (52.2) | 621+1G>T (4.5) | G542X (3.9) | N1303K (3.3) | 2183AA>G (1.8) | 2789+5G>A (1.8) | E822K (1.6) | R117H (1.2) | R344W (1.2) | 3272-26A>G (1.0) | R1158X (1.0) | G85E (1.0) | | |
| Hungary | dF508 (54.7) | G542X (2.2) | N1303K (1.0) | | | | | | | | | | | |
| Ireland | dF508 (72.7) | G551D (6.9) | R117H (2.0) | G542X (1.0) | | | | | | | | | | |
| Israel | dF508 (32.2) | W1282X (36.2) | G542X (5.4) | 3849+10kbC>T (4.6) | 405+1G>A (3.8) | N1303K (3.0) | Q359K+T360K (1.9) | S549R (1.1) | | | | | | |
| Italy | dF508 (51.1) | G542X (4.8) | N1303K (4.8) | 2183AA>G (2.7) | R1162X (2.4) | 1717-1G>A (2.1) | W1282X (1.2) | R553X (1.2) | | | | | | |
| Latvia | dF508 (54.2) | 3849+10kbC>T (12.5) | N1303K (8.3) | W1282X (4.2) | | | | | | | | | | |
| Lithuania | dF508 (30.9) | R553X (4.0) | N1303K (2.4) | | | | | | | | | | | |
| Macedonia | dF508 (56.0) | G542X (3.3) | N1303K (2.6) | 621+1G>T (1.9) | 3849+10kbC>T (1.9) | 457TAT>G (1.3) | V1397E (1.3) | | | | | | | |
| North Africa | dF508 (32.0) | N1303K (10.2) | W1282X (8.2) | 711+1G>T (7.5) | G542X (4.8) | R1162X (2.7) | L227R (1.4) | S549R (1.4) | S549N (1.4) | S549I (1.4) | G551D (1.4) | S945L (1.4) | | |
| Northern Ireland | dF508 (68.0) | G551D (5.1) | R117H (4.1) | R560T (2.9) | G542X (2.2) | 621+1 G>T (2.2) | dI507 (1.7) | | | | | | | |
| Norway | dF508 (66.7) | 394delTT (4.2) | R117H (3.0) | G551D (1.2) | | | | | | | | | | |
| Poland | dF508 (52.9) | 3849+10kbC>T (2.6) | G542X (2.5) | N1303K (1.7) | 1717-1G>A (1.7) | R553X (1.0) | CFTRdele2,3(21kb) (2.0) | | | | | | | |
| Portugal | dF508 (52.3) | R1066C (3.5) | G542X (2.3) | R334W (2.0) | A561E (2.0) | 3272-26A>G (1.5) | N1303K (1.5) | | | | | | | |
| Rovmania | dF508 (77.0) | | | | | | | | | | | | | |
| Russia P | dF508 (45.0) | W1282X (2.1) | N1303K (1.6) | 1677delTA (1.6) | 2143delT (1.0) | CFTRdele2,3(21kb) (7.5) | | | | | | | | |
| Russia M | dF508 (54.5) | N1303K (2.6) | 2143delT (2.0) | 2184insA (2.0) | W1282X (2.0) | G542X (1.8) | 3849+10kbC>T (1.8) | CFTRdele2,3(21kb) (8.4) | | | | | | |
| Slovakia | dF508 (59.4) | G542X (5.6) | R553X (3.4) | N1303K (3.0) | R347P (1.7) | CFTRdele2,3(21kb) (4.6) | | | | | | | | |
| Spain | dF508 (54.4) | G542X (7.7) | N1303K (2.5) | 1811+1.6kbA>G (1.5) | R1162X (1.3) | 712-1G>T (1.1) | 1609delCA (1.0) | | | | | | | |
| Sweden | dF508 (73.3) | 394delTT (9.7) | 3659delC (3.0) | 1716insT (2.4) | 1338 (1.2) | | | | | | | | | |
| Switzerland | dF508 (43.2) | R553X (24.2) | 3905insT (14.7) | G542X (3.2) | 1717-1G>A (2.1) | K1200E (2.1) | | | | | | | | |
| The Netherlands | dF508 (74.4) | A455E (3.3) | 1717-1G>A (1.5) | G542X (1.3) | R553X (1.2) | | | | | | | | | |
| Turkey | dF508 (34.8) | N1303K (6.4) | 1677delTA (2.8) | E92X (2.8) | R347H (2.8) | G542X (2.8) | K68N (1.4) | 2043delG (1.4) | 2183AA>G (1.4) | | 2789+5G>A (1.4) | | | |
| Ukraine | dF508 (50.0) | | | | | | | | | | | | | |
| United Kingdom | dF508 (75.3) | G551D (3.1 | G542X (1.7) | | | | | | | | | | | |
| Yugoslavia | dF508 (66.3) | G542X (5.3) | | | | | | | | | | | | |

Sources: Dehan C., Doerk T., Graham C., Macek M. Jnr., Pacheco. P. (Personal communication, January 1999).

European Journal of Human Genetics

Recommendations for quality improvement E Dequeker et al

# KBC Zagreb, KZLD-LBL
# Molekularna dijagnostika genetičkih bolesti

**Genetičke bolesti**

Azoospermija (mikrodelecije kromosoma Y - AZF)
Charcot-Marie-Tooth tip CMT1B - (sekvenciranje *MPZ*)
Charcot-Marie-Tooth tip CMTX1 (sekvenciranje *GJB1*)
Cistična fibroza CF (*CFTR*)
Deficit alfa-1-antitripsina (*SERPINA1*)
Friedrichova ataksija FA (*FXN*)
Gilbertov sindrom (*UGT1A1*)
Huntingtonova koreja HD (*HTT*)
Miotonična distrofija tipa 1 MD1 (*DMPK*)
Miotonična distrofija tipa 2 MD2 (*CNBP*)
Mišićna distrofija DMD/BMD (*DMD*)
Monogenski dijabetes MODY (sekvenciranje *HNF1A*, *HNF4A* i *GCK*)
Multipla endokrina neoplazija - MEN1 (sekvenciranje *MEN1*)
Multipla endokrina neoplazija - MEN2 (sekvenciranje *RET*)
Nasljedna hemokromatoza (*HFE*)
Nasljedne neuropatije CMT/HNPP (*PMP22*)
Neutropenija (sekvenciranje *ELANE* i *HAX1*)
Shwachman-Diamondov sindrom (sekvenciranje *SBDS*)
Sindrom fragilnog kromosoma X FRAX (*FMR1*)
Spinalna mišićna atrofija SMA (*SMN1*, *SMN2*, *NAIP*)
Spinocerebelarne ataksije SCA tipa 1, 2, 3, 6 i 7 (*ATXN1, ATXN2, ATXN3, CACNA1A , ATXN7*)
Wilsonova bolest WB (sekvenciranje *ATP7B*)
X-vezana agamaglobulinemija (sekvenciranje *BTK*)
X-vezani hiper-IgM sindrom (sekvenciranje *CD40LG*)
X-vezani limfoproliferativni sindrom (sekvenciranje *SH2D1A*)

**Rizični čimbenici**

*ACE* - angiotenzin-konvertirajući enzim
*ADPN* - adiponektin
*APOB* - apolipoprotein B
*APOE* - apolipoprotein E
*GPIa* - glikoprotein Ia
*HP* - haptoglobin
*IL-6* - interleukin-6
*LPL* - lipoprotein-lipaza
*MTHFR* - metilentetrahidrofolat-reduktaza
*PPAR* - receptori za aktivator proliferacije peroksisoma

**Mitohondrijske bolesti**

MELAS (*MT-TL1*)
MERRF (*MT-TK*)
NARP (*MT-ATP6*)


❑ *Prof.dr.sc. Jadranka Sertić, spec.med.biokem.*
❑ *Dr.sc. Hana Ljubić, mag.biol.*
❑ *Dr.sc. Ana Merkler, mag.ing.bioproc.inž*
❑ *Domagoj Caban, mag.med.lab.diagn.*
❑ *Senka Škaro, bacc.med.lab.diagn.*
❑ *Ana Acman Barišić, bacc.med.lab.diagn.*
❑ *Karolina Petrović, zdrav.lab. tehničar*


# Molekularna dijagnostika - vanjska procjena kvalitete

EMQN The European Molecular Genetics Quality Network

RfB Referenzinstitut für Bioanalytik

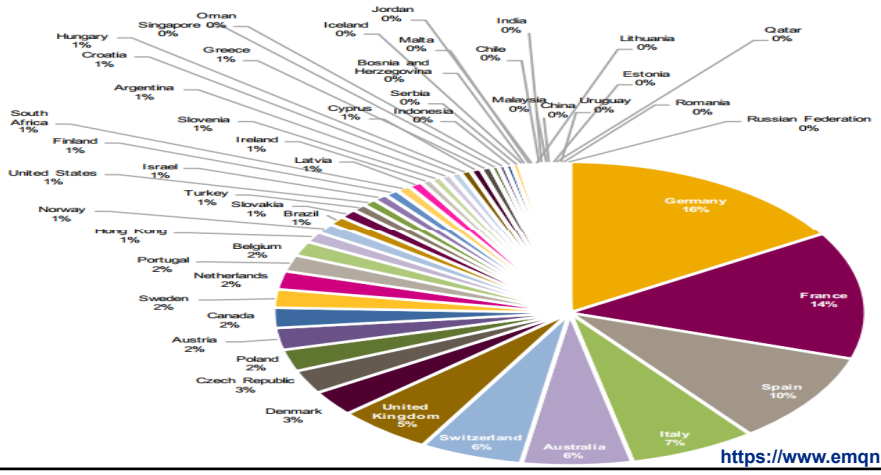
CF network

INSTAND

https://www.instand-ev.de/en.html
https://www.rfb.bio/cgi/switchLang?lang=en
https://www.emqn.org/

# EMQN -2016.

## 32 sheme za specifičnu bolest, 515 laboratorija




https://www.emqn.org

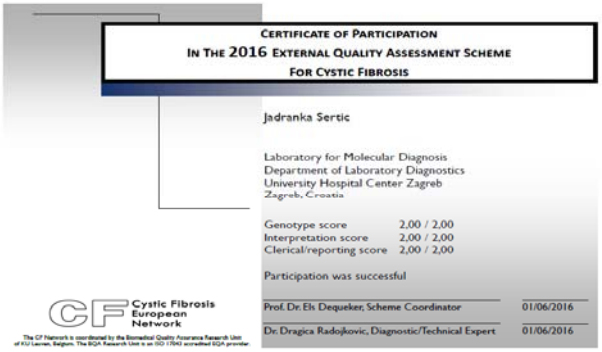
# CF Network

## KBC Zagreb: 1998. -

| Country | Number of laboratories that participated in the scheme in | | | Country | Number of laboratories that participated in the scheme in | | |
|---|---|---|---|---|---|---|---|
| | 2014 | 2015 | 2016 | | 2014 | 2015 | 2016 |
| Australia | 11 | 11 | 14 | The Netherlands | 8 | 8 | 7 |
| Austria | 3 | 5 | 4 | New Zealand | 1 | 1 | 2 |
| Belgium | 10 | 8 | 8 | Norway | 1 | 2 | 2 |
| Croatia | 2 | 2 | 1 | Oman | 0 | 0 | 1 |
| Cyprus | 1 | 1 | 1 | Poland | 6 | 5 | 6 |
| Czech Republic | 3 | 3 | 3 | Portugal | 2 | 2 | 3 |
| Denmark | 3 | 3 | 3 | Serbia | 1 | 1 | 1 |
| Estonia | 1 | 1 | 0 | Slovakia | 1 | 1 | 1 |
| Finland | 1 | 0 | 0 | Slovenia | 2 | 2 | 2 |
| France | 33 | 30 | 32 | South Africa | 0 | 1 | 2 |
| Germany | 47 | 40 | 34 | Spain | 15 | 15 | 14 |
| Greece | 5 | 5 | 4 | Sweden | 3 | 3 | 2 |
| Hungary | 4 | 3 | 4 | Switzerland | 15 | 15 | 15 |
| Ireland | 1 | 1 | 1 | Tunisia | 1 | 0 | 0 |
| Israel | 0 | 1 | 0 | Turkey | 0 | 0 | 3 |
| Italy | 26 | 20 | 21 | Ukraine | 1 | 1 | 0 |
| Jordan | 0 | 0 | 1 | United Kingdom | 2 | 2 | 2 |
| Latvia | 1 | 1 | 1 | United States | 2 | 1 | 2 |
| Lithuania | 1 | 0 | 0 | Uruguay | 0 | 1 | 1 |
| Macedonia | 1 | 0 | 1 | TOTAL | 215 | 198 | 199 |

www.emqn.org/

# Međunarodni certifikat



CERTIFICATE OF PARTICIPATION
IN THE 2016 EXTERNAL QUALITY ASSESSMENT SCHEME
FOR CYSTIC FIBROSIS

Jadranka Sertic

Laboratory for Molecular Diagnosis
Department of Laboratory Diagnostics
University Hospital Center Zagreb
Zagreb, Croatia

| | |
|---|---|
| Genotype score | 2,00 / 2,00 |
| Interpretation score | 2,00 / 2,00 |
| Clerical/reporting score | 2,00 / 2,00 |

Participation was successful

Prof. Dr. Els Dequeker, Scheme Coordinator 01/06/2016

Dr. Dragica Radojkovic, Diagnostic/Technical Expert 01/06/2016

CF Cystic Fibrosis European Network

The CF Network is coordinated by the Biomedical Quality Assurance Research Unit of KU Leuven, Belgium. The BQA Research Unit is an ISO 17043 accredited EQA provider.

## Ocjenitelji - EMQN za shemu Huntingtonova bolest

| Osoba | Država | Funkcija |
|---|---|---|
| **Peter Bauer** | **Njemačka** | Organizator sheme |
| **Monique Losekoot** | **Nizozemska** | Ocjenitelj |
| **Anniek Corveleyn** | **Belgija** | Ocjenitelj |
| **Sara Seneca** | **Belgija** | Ocjenitelj |
| **Hana Ljubić** | **Hrvatska** | Ocjenitelj |

Losekoot M, van Belzen MJ, Seneca S, Bauer P, Stenhouse SA, Barton DE., European Molecular Genetic Quality Network (EMQN).
*EMQN/CMGS best practice guidelines for the molecular genetic testing of Huntington disease.*
**Eur J Hum Genet. 2013;21(5):480-6.**

## Ocjenitelji - EMQN za shemu Miotonična distrofija tip 1

| Osoba | Država | Funkcija |
|---|---|---|
| **Morten Duno** | **Danska** | Organizator sheme |
| **Erik Jan Kamsteeg** | **Nizozemska** | Ocjenitelj |
| **Ana Merkler** | **Hrvatska** | Ocjenitelj |

Kamsteeg EJ, et al.
*Best practice guidelines and recommendations on the molecular diagnosis of myotonic dystrophy types 1 and 2.*
**Eur J Hum Genet. 2012;20(12):1203-8.**

TREAT-NMD Neuromuscular Network

**www.treat-nmd.eu**

## Međunarodni registar bolesnika s neuromuskularnim bolestima

Hrvatska

**DMD 47**
**BMD 18**
**SMA 48**

**Nina Barišić. KBC Zagreb**

Merkler A, Kelecic J, Tjesic-Drinkovic D, Baric I, Vukovic J, Sarnavka V. et al.
*Molecular Diagnostics of Severe Congenital Neutropenia and Shwachman-Diamond Syndrome in Seven Croatian Families.*
**J Clin Immunol. 2014;34 (Suppl 2):363.**

Merkler A, Richter D, Kelecic J, Ljubic H, Caban D, Sertic J.
*Genetic basis of primary immunodeficiencies in Croatian patients.*
**Eur J Hum Genet. 2013;21 (Suppl 2): 237.**

## Nove mutacije utvrđene u genima za primarne imunodeficijencije

**BTK**

c.1349+1G>C

c.831_839+2delAATGTATGAGT

c.974+4A>G

**ELANE**

c.213C>G, p.Cys71Trp

**HAX1**

c.737A>C, p.Asp246Ala

Ljubić H, Kalauz M, Telarović S, Ferenci P, Ostojić R, Noli MC, Lepori MB, Hrstić I, Vuković J, Premužić M, Radić D, Grubelić Ravić K, Sertić J, Merkler A, Acman Barišić A, Loudianos G, Vucelić B.
*ATP7B Gene Mutations in Croatian Patients with Wilson Disease.*
**Genetic Testing and Molecular Biomarkers. 2016;20:112-7.**

## Nove mutacije utvrđene u genu ATP7B

c.3079G>A, p.Asp1027Asn

c.3088G>A, p.Gly1030Ser

c.4295C>T, p.Ser1432Phe

Ana Merkler

*Genske mutacije u nasljednim demijelinizirajućim polineuropatijama Charcot-Marie-Tooth tipa 1 u stanovništva Republike Hrvatske.*

**Doktorski rad, 2017. Zagreb: Sveučilište u Zagrebu Prirodoslovno-matematički fakultet.**

## Nove mutacije utvrđene u genima za nasljedne neuropatije

**PMP22**

c.2T>C, p.Met1Thr

c.59_64delTCGTCT, p.Phe20_Val21del

**GJB1**

c.86T>C, p.Phe29Ser

c.422T>C, p.Phe141Ser

c.529G>A, p.Val177Met

## Multifaktorske bolesti / genetička heterogenost

- **Funkcijska genomika i proteomika rizičnih čimbenika ateroskleroze**
- **Uloga genskih i biokemijskih biljega u razvoju ateroskleroze i cerebrovaskularnog inzulta**
- **Uloga genskih i biokemijskih biljega u razvoju monogenskog dijabetesa**

## Ateroskleroza: koncept atero-upale

*"počima u djetinjstvu"*

DISLIPIDEMIJA

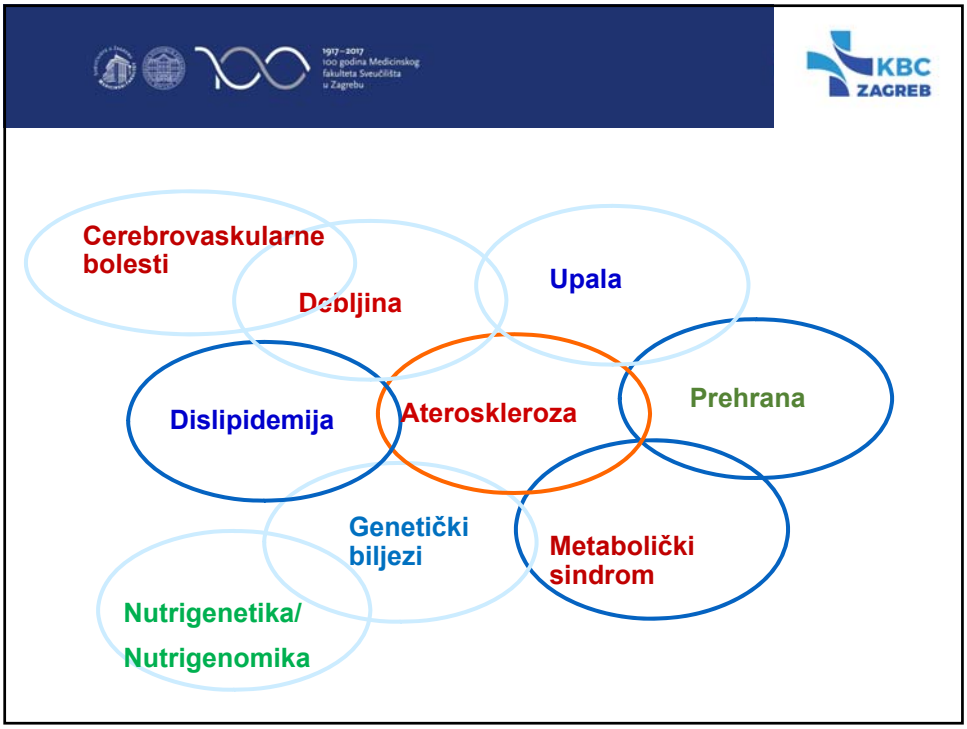
DEBLJINA

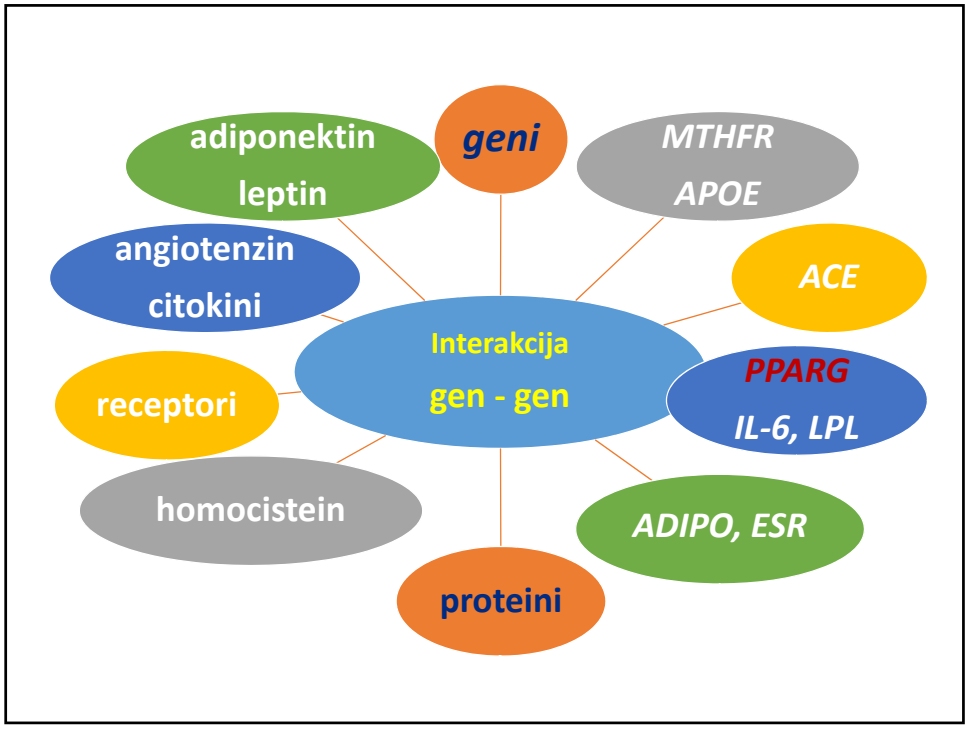
PREHRANA

ŠEĆERNA BOLEST

HIPERTENZIJA

AUTOIMUNOST



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BMC Res Notes. 2009 Oct 5;2:203. doi: 10.1186/1756-0500-2-203.

**Variants of ESR1, APOE, LPL and IL-6 loci in young healthy subjects: association with lipid status and obesity.**

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**Abstract**

**FINDINGS:** BMI was increased (>25) in 22% of young healthy subjects. Increased cholesterol values (>5.0 mmol/L) were found in 23% of subjects, LDL-C (>3.0 mmol/L) in 23%, triglycerides (>1.7 mmol/L) in 11% of subjects. We found statistically significant differences in subjects' weight (p = 0.015), BMI (p = 0.023), and waist-hip ratio (WHR) (p = 0.015) in regard to their diet type; subjects with Mediterranean diet had the lowest values compared to those on continental and mixed diet. Significant associations were found for: LPL genetic polymorphic variant and abdominal obesity (p = 0.013), APO epsilon4 allele and hypercholesterolemia (p = 0.003), and ESR1-TA long allele and hypercholesterolemia (p = 0.011).

**BACKGROUND:** Human obesity is a multifactorial syndrome influenced also by genetic factors. Among gene variants found to be involved in body weight regulation and development of obesity, particular attention has been paid to polymorphisms in genes associated with obesity-related metabolic disorders. We explored the association of genetic polymorphisms of: estrogen receptor alpha (ESR1-TA repeats); interleukin-6 (IL-6 G-174C); apolipoprotein E (APO epsilon2, epsilon3, epsilon4); lipoprotein lipase Pvu II (LPL P+/-), with clinical variables: gender, age, body mass index (BMI), diet type and biological variables: triglycerides, cholesterol, HDL-C, LDL-C, CRP, homocysteine, urate, and glucose in 105 healthy young subjects (20-35 yrs) of Croatian origin.

**METHODS:** Genotyping of IL-6, LPL was performed by PCR-RFLP, of APOE by real-time PCR, and of ESR1 by PCR and capillary electrophoresis. Association analyses were performed of alleles and genotypes with biological variables.

**CONCLUSION:** ESR-1, LPL, and APO E genetic polymorphic variants could represent predictive genetic risk markers for obesity-related metabolic disorders in young healthy subjects. Mediterranean type of diet is also an important protective factor against abdominal obesity.

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# Geni i okoliš: redoslijed po važnosti??

*"Geni pune revolver, no okoliš povlači okidač."*

*Elliot P. Joslin,*

Genet Test Mol Biomarkers. 2014 Jan;18(1):32-40. doi: 10.1089/gtmb.2013.0344. Epub 2013 Nov 7.

## Interaction of genetic risk factors confers increased risk for metabolic syndrome: the role of peroxisome proliferator-activated receptor γ.

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**Abstract**

**AIM:** The aim of the study was to estimate the influence of interactions between peroxisome proliferator-activated receptor γ (PPARγ) and target genes lipoprotein lipase (LPL), interleukin 6 (IL6), angiotensin converting enzyme (ACE), and angiotensin II type 1 receptor (AT1R) on metabolic syndrome (MetSy) and its traits.

**METHODS:** The study included 527 participants (263 with MetSy and 264 controls). Genotyping of PPARγ Pro12Ala, LPL PvuII (-/+), IL6 -174G>C, ACE I/D and AT1R 1166A>C was performed using polymerase chain reaction-restriction fragment length polymorphism-based methods.

**RESULTS:** Interaction between PPARγ Pro12Ala and LPL Pvu(-/+) improved prediction of MetSy over and above prediction based on a model containing no interactions (χ(2)=7.22; df=1; p=0.007). In the group of participants with PPARγ Pro12Ala or Ala12Ala genotypes, those with the LPL Pvu (-/+) or (+/+) genotype had greater odds for MetSy (odds ratio OR=5.98; 95% confidence interval CI: 1.46-24.47, p=0.013). Interaction between PPARγ Pro12Ala and IL6 -174G>C improved prediction of high fasting blood glucose (χ(2)=13.99; df=1; p<0.001). PPARγ Ala12 variant was found protective in patients with IL6 -174GG genotype (OR=0.10; 95% CI: 0.02-0.57, p=0.01), while in the case of IL6 -174C allele carriers, for PPARγ Ala12 carriers, larger odds for high glucose levels compared with Pro12 variant were observed (OR=2.39; 95% CI: 1.11-5.17, p=0.026). Interactions of PPARγ and ACE were significant for BMI. In the group with ACE DD genotype, those with PPARγ Pro12Ala or Ala12Ala genotype have greater odds for obesity (OR=9.98; 95% CI: 1.18-84.14, p=0.034).

**CONCLUSIONS:** PPARγ gene variants can, in interaction with some of its target genes, modulate physiological processes leading to the development of MetSy.

PMID: 24200052 DOI: 10.1089/gtmb.2013.0344

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Gene. 2015 Apr 15;560(2):200-4. doi: 10.1016/j.gene.2015.02.003. Epub 2015 Feb 7.

**PPARγ and IL-6 - 174G>C gene variants in Croatian patients with ischemic stroke.**

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4 University of Zagreb School of Medicine, Zagreb, Croatia. Electronic address: tena.lovric@vip.hr.
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6 University of Zagreb School of Medicine, Zagreb, Croatia; University Hospital Centre Zagreb, Clinic for Cardiovascular Diseases, Zagreb, Croatia. Electronic address: davor.milicic@mef.hr.

**Abstract**

**AIM:** Etiology of ischemic stroke (IS) is multifactorial and includes interaction of genetic and environmental factors. Different genes, their polymorphisms, host susceptibility, and inflammation processes play a role in IS development. The aim of this study was to evaluate the effect of PPAR-γ and IL-6 gene variants on IS onset.

**MATERIAL AND METHODS:** A total of 301 subjects (144 males, 157 females) participated in the study, 114 patients with IS and 187 healthy controls.

**RESULTS:** Statistically significant predictors of IS were male gender (OR 7.13, 95% CI 2.92-17.39, p<0.001), hypertension (OR 7.82. 95% CI 2.53-24.19, p<0.001), lowered HDL cholesterol (OR 8.20, 95% CI 2.41-27.94, p=0.001), elevated C-reactive protein (OR 5.26, 95% CI 1.92-14.41) and IL-6 -174 GC (OR 2.44 95% CI 1.01-5.91, p=0.0048) genotype. Males, compared to females, had 7 times higher odds for stroke. IL6 -174G/C genotype increased the odds for IS for 2.4 times. PPARγ was not statistically significantly associated with stroke.

**CONCLUSION:** We can point to the IL-6 -174G>C polymorphisms as candidate gene marker and risk factor for the prediction of ischemic stroke.



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# KBC Zagreb, KZLD-LBL
# Molekularna dijagnostika genetičkih bolesti

## Genetičke bolesti

**Azoospermija (mikrodelecije kromosoma Y - AZF)**
**Charcot-Marie-Tooth tip CMT1B - (sekvenciranje *MPZ*)**
**Charcot-Marie-Tooth tip CMTX1 (sekvenciranje *GJB1*)**
**Cistična fibroza CF (*CFTR*)**
**Deficit alfa-1-antitripsina (*SERPINA1*)**
**Friedrichova ataksija FA (*FXN*)**
**Gilbertov sindrom (*UGT1A1*)**
**Huntingtonova koreja HD (*HTT*)**
**Miotonična distrofija tipa 1 MD1 (*DMPK*)**
**Miotonična distrofija tipa 2 MD2 (*CNBP*)**
**Mišićna distrofija DMD/BMD (*DMD*)**
**Monogenski dijabetes MODY (sekvenciranje *HNF1A*, *HNF4A* i *GCK*)**
**Multipla endokrina neoplazija - MEN1 (sekvenciranje *MEN1*)**
**Multipla endokrina neoplazija - MEN2 (sekvenciranje *RET*)**
**Nasljedna hemokromatoza (*HFE*)**
**Nasljedne neuropatije CMT/HNPP (*PMP22*)**
**Neutropenija (sekvenciranje *ELANE* i *HAX1*)**
**Shwachman-Diamondov sindrom (sekvenciranje *SBDS*)**
**Sindrom fragilnog kromosoma X *FRAX* (*FMR1*)**
**Spinalna mišićna atrofija SMA (*SMN1*, *SMN2*, *NAIP*)**
**Spinocerebelarne ataksije SCA tipa 1, 2, 3, 6 i 7 (*ATXN1*, *ATXN2*, *ATXN3*, *CACNA1A* , *ATXN7*)**
**Wilsonova bolest WB (sekvenciranje *ATP7B*)**
**X-vezana agamaglobulinemija (sekvenciranje *BTK*)**
**X-vezani hiper-IgM sindrom (sekvenciranje *CD40LG*)**
**X-vezani limfoproliferativni sindrom (sekvenciranje *SH2D1A*)**

## Rizični čimbenici

***ACE - angiotenzin-konvertirajući enzim***
***ADPN - adiponektin***
***APOB - apolipoprotein B***
***APOE - apolipoprotein E***
***GPIa - glikoprotein Ia***
***HP - haptoglobin***
***IL-6 - interleukin-6***
***LPL - lipoprotein-lipaza***
***MTHFR - metilentetrahidrofolat-reduktaza***
***PPAR - receptori za aktivator proliferacije peroksisoma***

## Mitohondrijske bolesti

***MELAS (MT-TL1)***
***MERRF (MT-TK)***
***NARP (MT-ATP6)***

Prof.dr.sc. Jadranka Sertić, spec.med.biokem.
Dr.sc. Hana Ljubić, mag.biol.
Dr.sc. Ana Merkler, mag.ing.bioproc.inž

Domagoj Caban, mag.med.lab.diagn.
Senka Škaro, bacc.med.lab.diagn.
Ana Acman Barišić, bacc.med.lab.diagn.
Karolina Petrović, zdrav.lab. tehničar

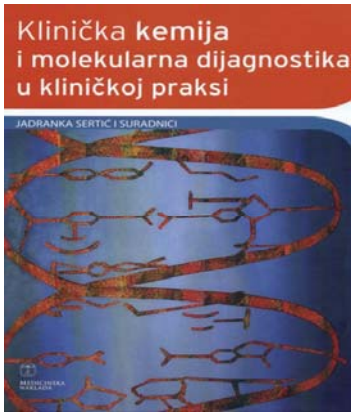
## Genski biljezi - rizični čimbenici

- *ApoE*
- *ApoB*
- *MTHFR*
- *HFE*
- *ATP7B*
- *A1AT*
- *ACE*
- *UGT*
- IL6
-

www.rfb.bio/cgi/switchLang?lang=en

## Molekularna dijagnostika

-




**Medicinska naklada**

https://www.medicinska naklada.hr

## Precizna dijagnostika - ciljana terapija

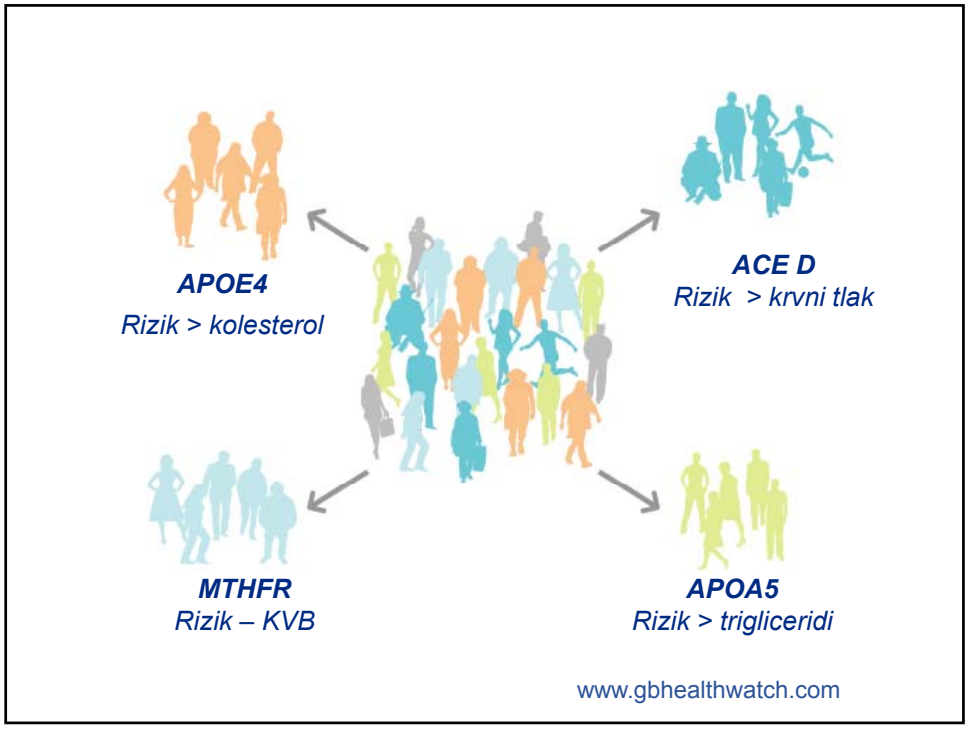
| |
|---|
| ➢ **Cistična fibroza –** *oligonukleotidi* |
| ➢ **Spinalna mišična atrofija –** *oligonukleotidi / neuroprotekcija* |
| ➢ **Multipla endokrina neoplazija tipa 2 –** *tireoidektomija* |
| ➢ **Monogenski dijabetes (precizni dijabetes) –** *inzulin/sulfonilurea* |
| ➢ ***MTHFR* –** folna kiselina<br>➢ ***Mikrodelecije kromosoma –*** *IVF* |

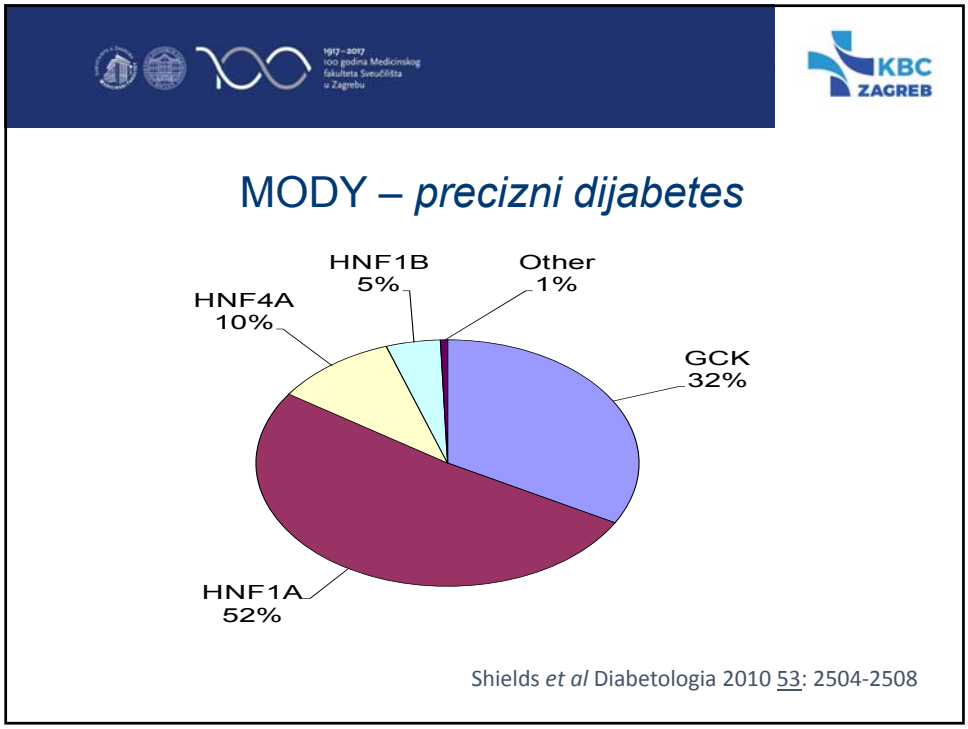
## Nutrigenetika */-omics*

*personalizirana prehrana*

### Interakcija gen – prehrana

- *ApoE*
- *APOA5*
- *ACE*
- *MTHFR*
- *IL-6*


APOE4
Rizik > kolesterol
ACE D
Rizik > krvni tlak
MTHFR
Rizik – KVB
APOA5
Rizik > trigliceridi
www.gbhealthwatch.com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KBC ZAGREB
MODY – precizni dijabetes
HNF1B 5%
Other 1%
HNF4A 10%
GCK 32%
HNF1A 52%
Shields et al Diabetologia 2010 53: 2504-2508

## Molekularna genetika – novosti u dijagnostici i terapiji


## Poruke za budućnost

- interdisciplinarnost
- analitička konsolidacija
- neinvazivno uzorkovanje
- pohranjivanje podataka, IT
- edukacija pacijenata
- nanotehnologije

# Hvala za pozornost!

**Prof.dr.sc. Jadranka Sertić**

Klinički bolnički centar Zagreb
Klinički zavod za laboratorijsku dijagnostiku

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