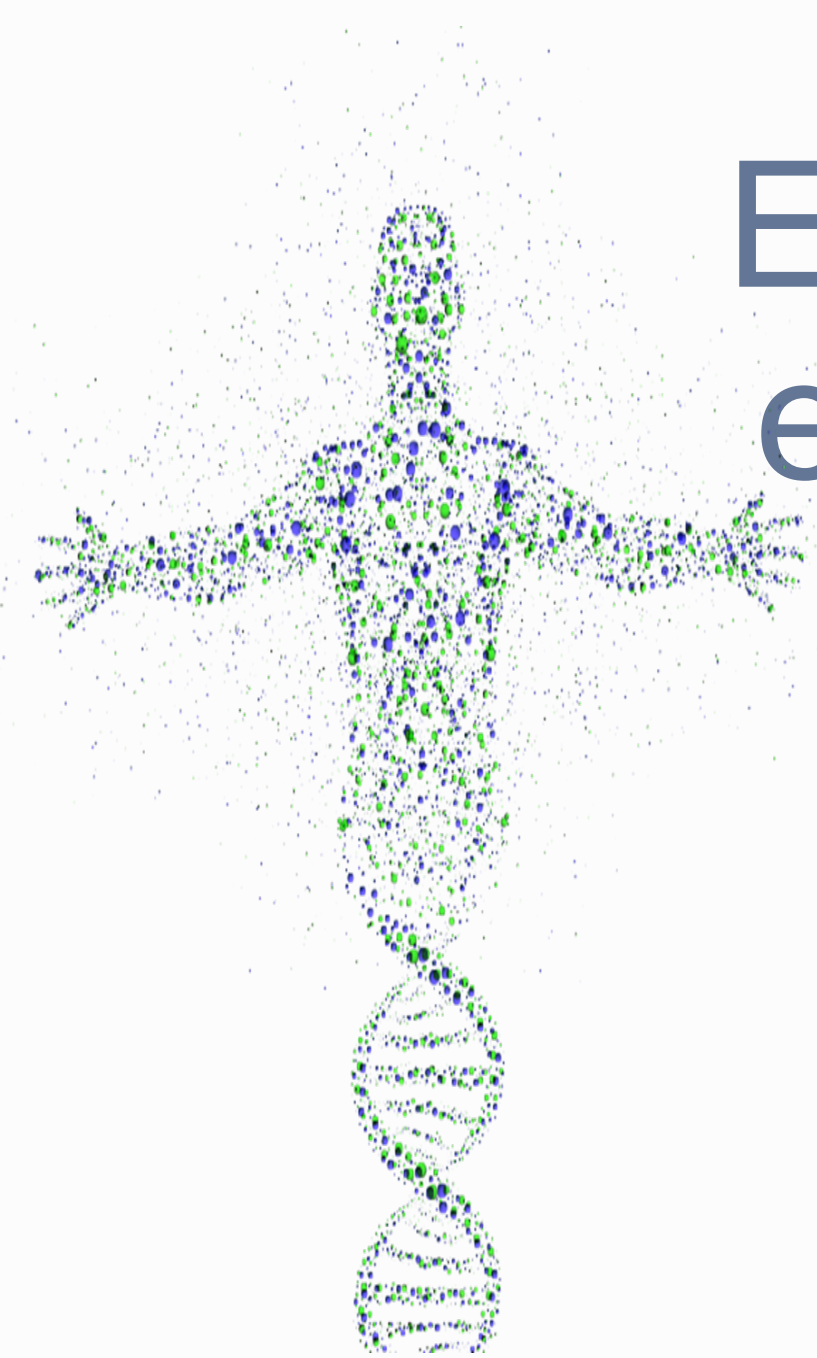


Epigenomika-raskrižje eksposoma i genoma

Martina Matovinović

KBC Zagreb

Epigenetika- Epigenomika

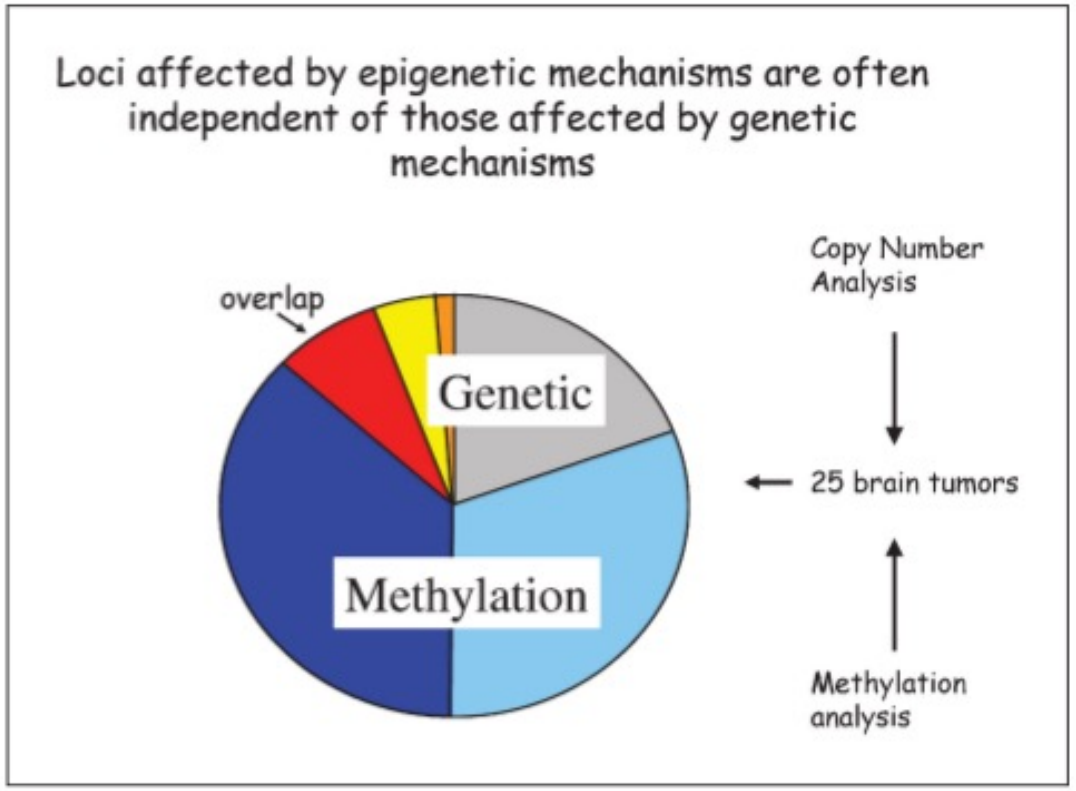
- 1942.g. pojam epigenetika osmislio je Waddington
- nasljedne promjene u funkciji gena koje moduliraju ekspresiju genotipa u određeni fenotip
- nasljednu i reverzibilnu promjenu funkcije gena

- epigenomika se bavi proučavanjem svih epigenetskih modifikacija koje utječu na genom
- globalna slika epigenomskih promjena u pojedinom genomu

Riggs AD et al. Introduction. Russo VEA, et al. Epigenetic mechanisms of gene regulation. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.1996.
Waddington CH. The epigenotype. Endeavour .1942. Barrero et al. Epigenetic mechanisms that regulate cell identity. Cell Stem Cell 2010.
Rakayan VK et al. Metastable epialleles in mammals. Trends Genet 2002.

Human Epigenome Project

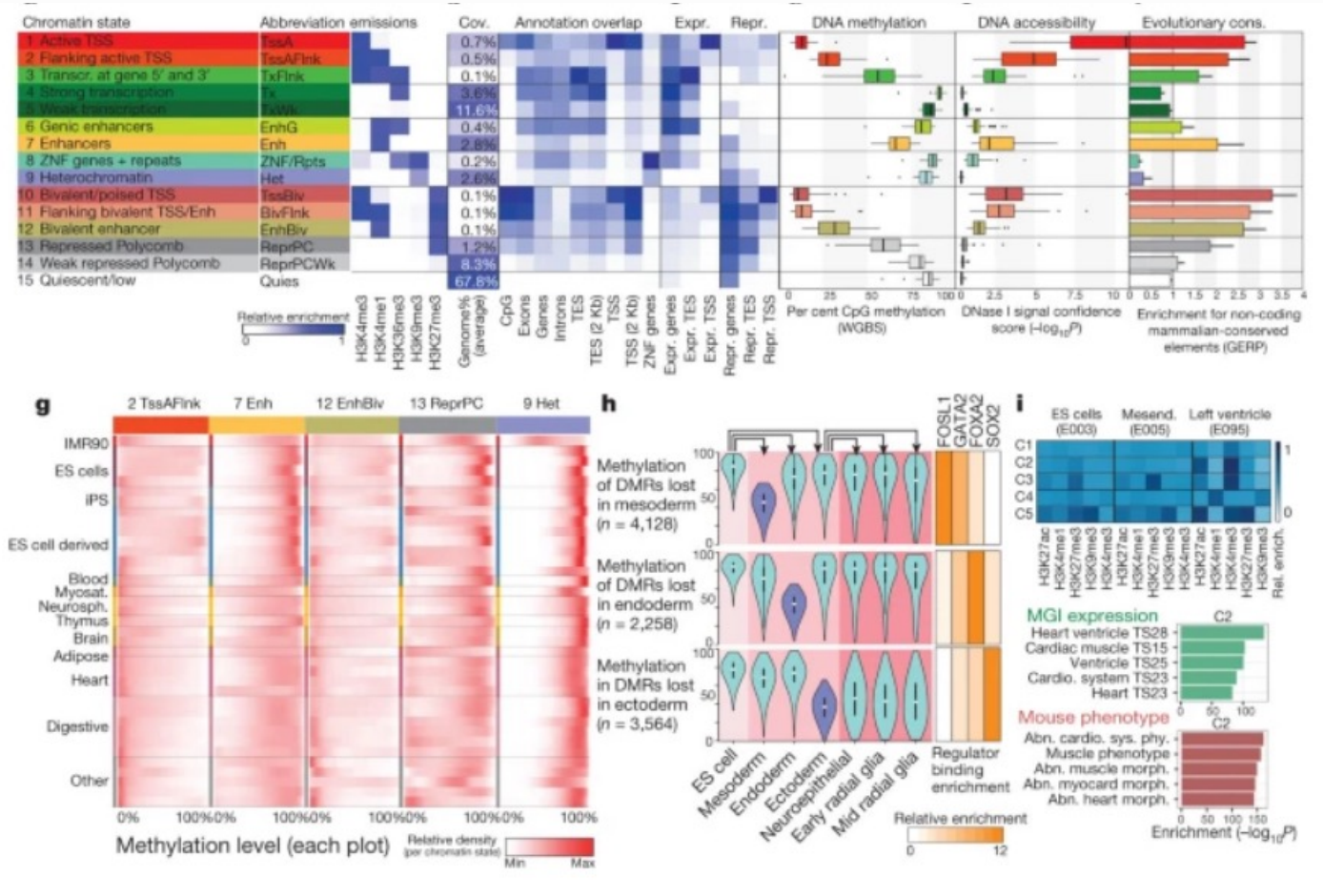
- epigenetički procesi kontroliraju ekspresiju ljudskog genoma
- usporedni doprinosi genetskih i epigenetskih mehanizama
- generirati karte metilacije za cijeli genom



A Blueprint for a Human Epigenome Project: The AACR Human Epigenome Workshop. Jones, P. & Martienssen, R. Cancer Research, 2005.

Ljudski epigenom

- epigenomske informacije
- razumijevanje regulacije gena, stanične diferencijacije i ljudske bolesti
- ispitivanjem zdravih i bolesnih tkiva identificirat će se specifične genomske regije koje su uključene u razvoj, tkivno specifično izražavanje, okoliš osjetljivost i patogeneza



Integrative analysis of 111 reference human epigenomes. Roadmap Epigenomics Concortium..Nature 2015.

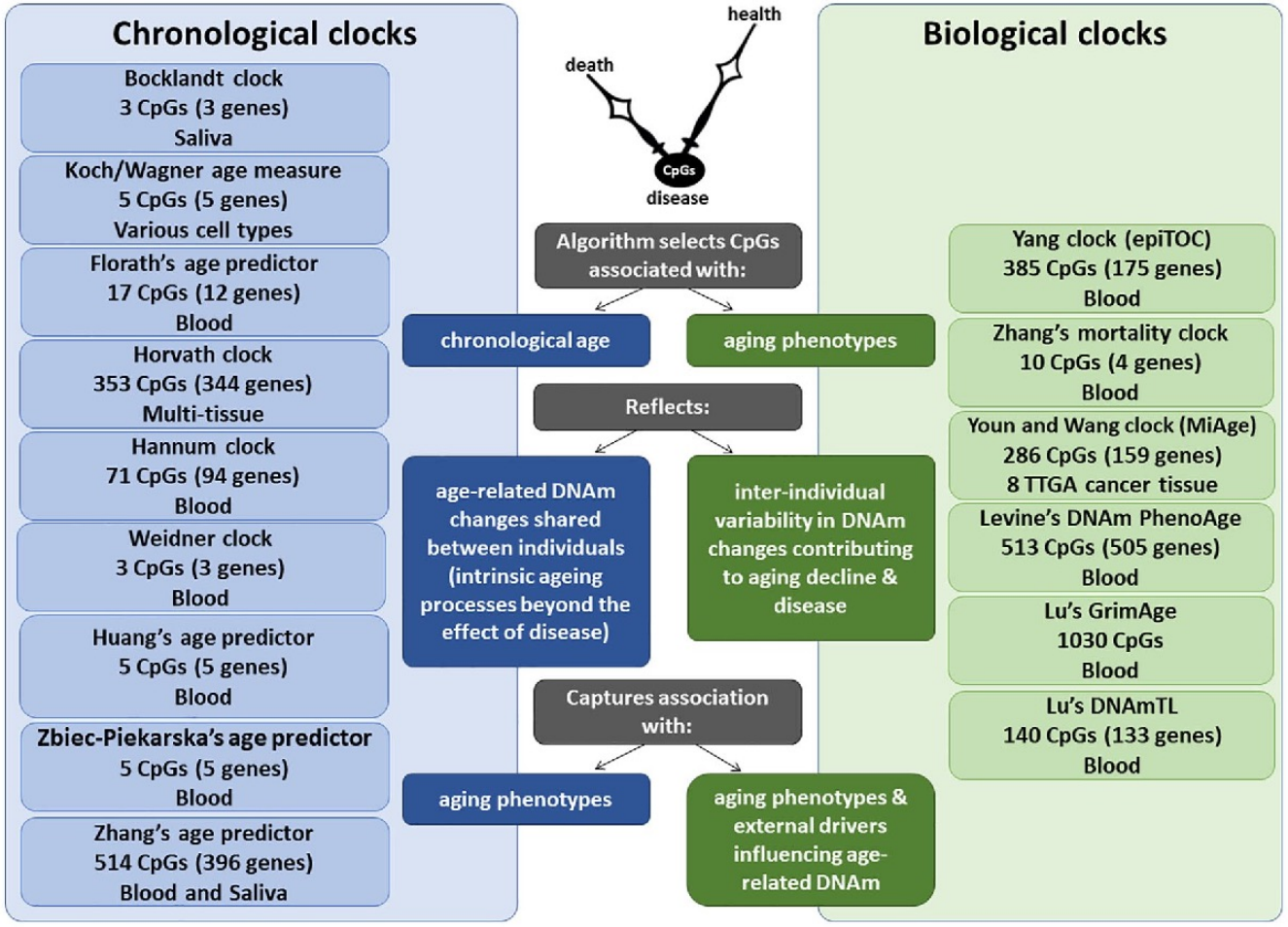
Epigenetski sat

- Horvath je proveo više od 4 godine prikupljajući javno dostupne podatke o metilaciji DNA i identificirajući prikladne statističke metode
- 2013.g.je analizira 353 pojedinačna CpG mjesta
- primjenjiv na širok spektar tkiva i tipova stanica
- omogućuje usporedbu starosti različitih tkiva istog subjekta
- identifikacija tkiva koja pokazuju ubrzanu starost zbog bolesti

Horvath S. DNA methylation age of human tissues and cell types. Genome Biology. 2013

Epigenetski satovi

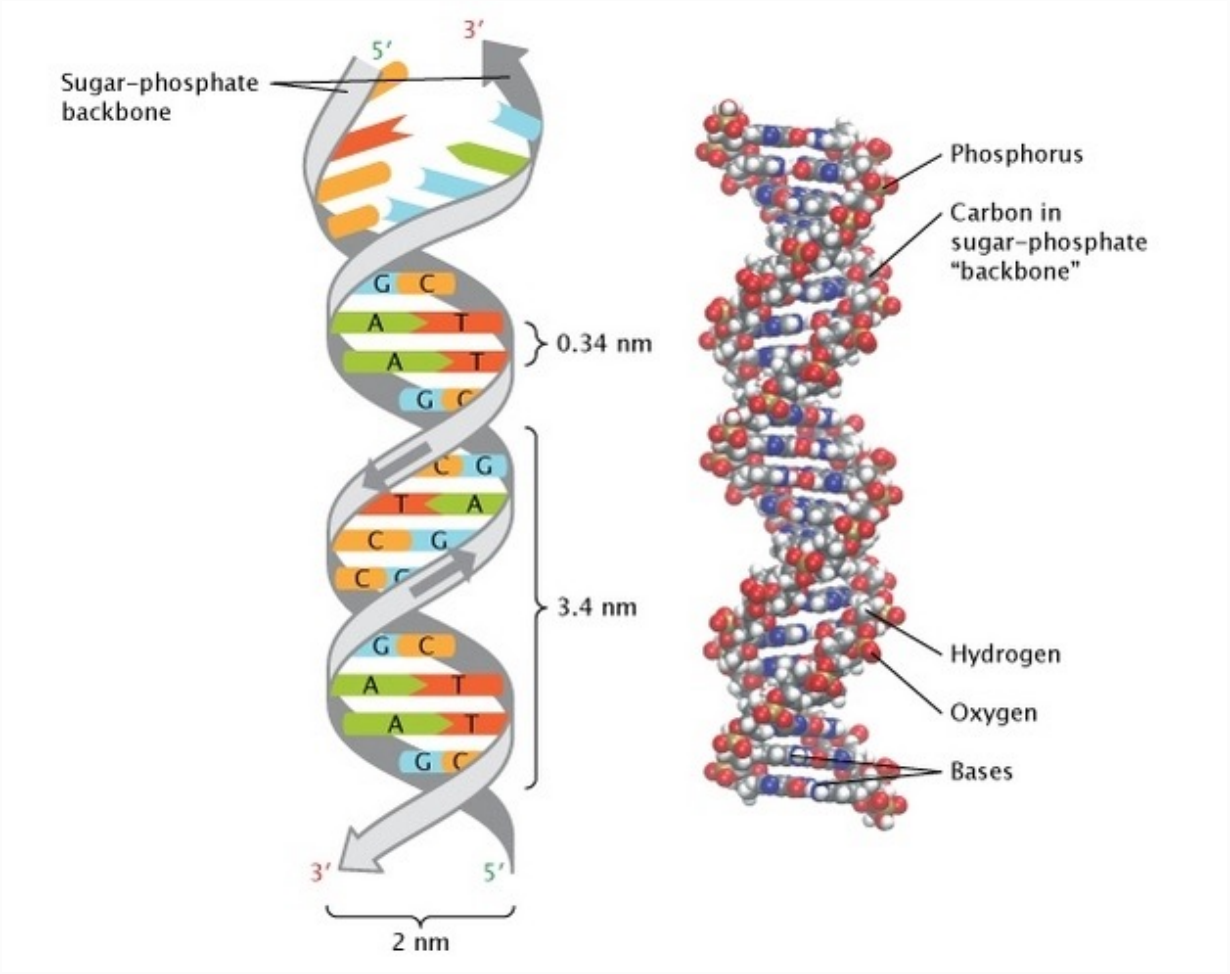
- više od 75% mjesta CpG je obično metilirano
- globalna DNAm opada s godinama
- može dovesti do gubitka kontrole transkripcije i uzrokovati ili pridonijeti štetnim učincima starenja



Unnikrishnan, A. et al. Revisiting the genomic hypomethylation hypothesis of aging. Ann. N. Y. Acad. Sci. 2018
Horvath S. DNA methylation age of human tissues and cell types. Genome Biology. 2013
Tessa Bergsma, E. Rogaeva. DNA Methylation Clocks and Their Predictive Capacity for Aging Phenotypes and Healthspan, . Neuroscience insights 2020,

Genom

- potpuna genetska informacija sadržana u DNA
- 1953.g. Watson i Crick objavili su da je DNA polimer oblika dvostruke uzvojnice
- ne mijenja se jer u protivnom bi se kodirana nasljeđena svojstva degradirala



Pray, L. Discovery of DNA structure and function: Watson and Crick. Nature Education, 2008

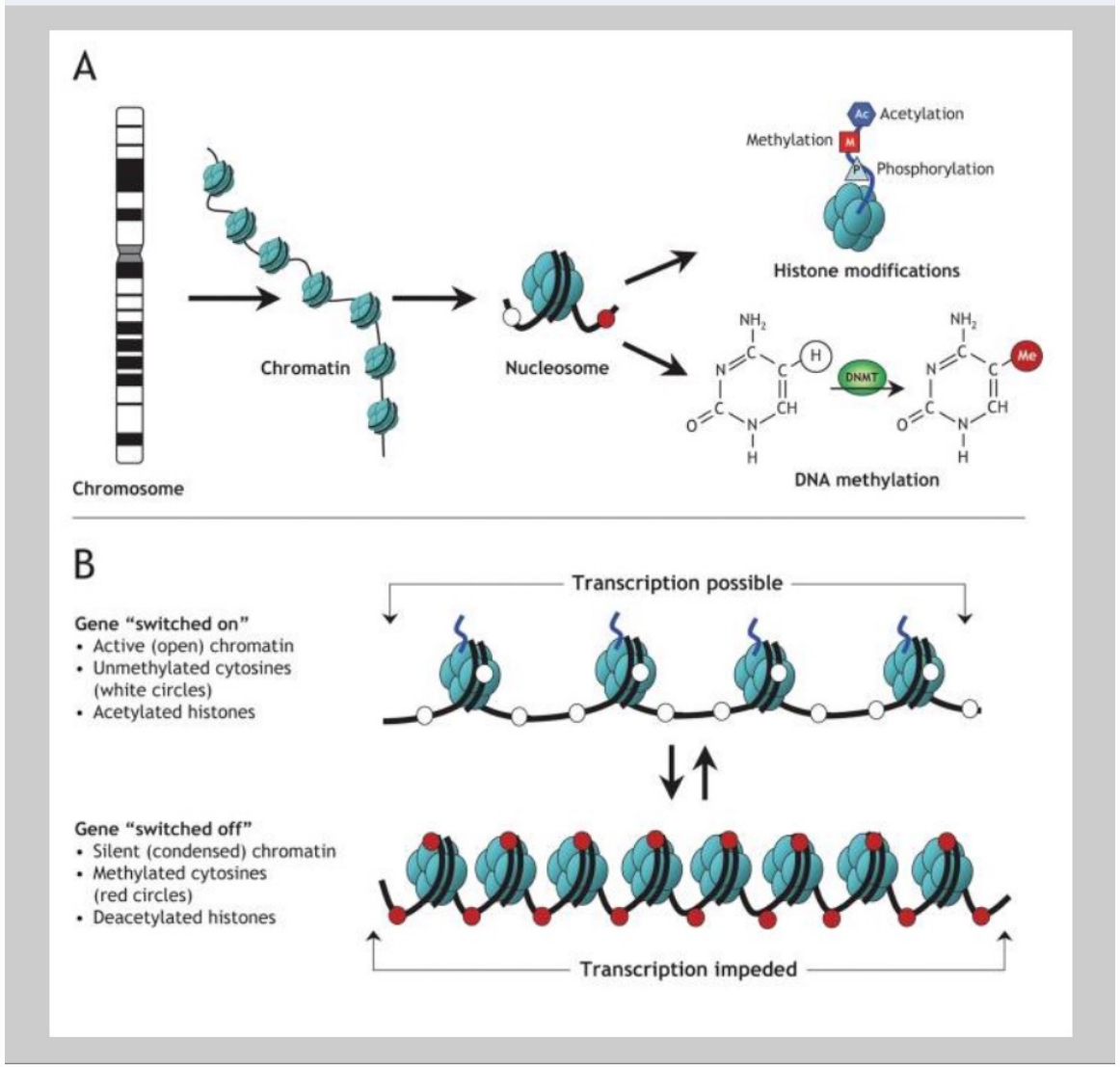
Eksposom

- 2005.g. molekularni epidemiolog Christopher Wild
- okarakterizirati i izmjeriti izloženosti okoliša tijekom cijelog životnog vijeka
- procjenjuje se da oko 70-90% rizika za razvoj bolesti se može objasniti okolišnim, a ne genetskim čimbenicima

Complementing the genome with an "exposome": the outstanding challenge of environmental exposure measurement in molecular epidemiology.*Wild CP.Cancer Epidemiol Biomarkers Prev. 2005*
Epidemiology. Environment and disease risks.*Rappaport SM, Smith MT. Science. 2010*
The Exposome: Understanding the Effect of the Environment on Our Health.2020

Osnovni mehanizmi epigenetike

- epigenetika je proučavanje nasljednih promjena u funkciji gena koje ne mijenjaju sekvencu DNK
- epigenetski mehanizmi pružaju "dodatni" sloj transkripcijske kontrole koji regulira način ekspresije gena

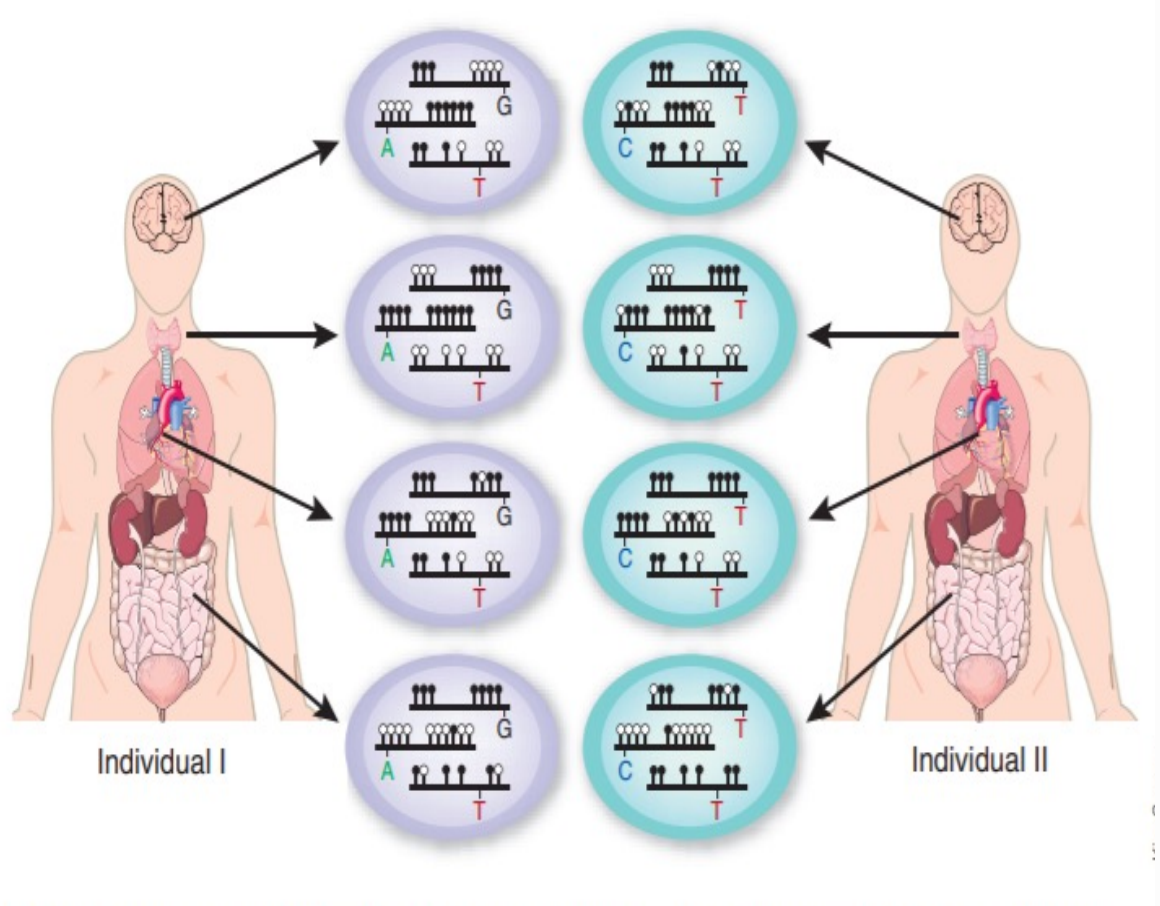


Epigenetics and human disease: translating basic biology into clinical applications.David Rodenhiser and Mellissa Mann. CMAJ.2006.

Tkivno specifični uzorak metilacije

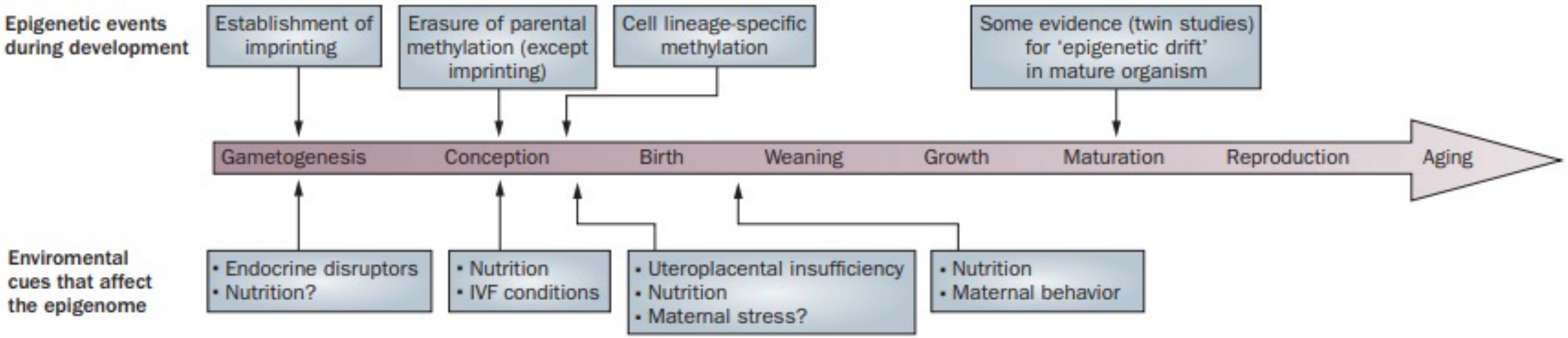
- epigenetska heterogenost među pojedincima
- podskup uzoraka metilacije DNA unutar stanice karakteristični su za taj tip stanice

- cjelokupna dosljednost u tkivno specifičnoj metilaciji DNA uzoraka
- varijacije u tim obrascima postoje među različitim pojedincima



Toward a human epigenome.Romulo M Brena et al. Nature Genetics.2006.

Ekposom-epigenom-genom



Gluckman P.D. et al. Epigenetic mechanisms that underpin metabolic and cardiovascular diseases. Nat. Rev. Endocrinol. 2009.

Pothranjenost majki tijekom prekoncepcije, koncepcije i trudnoće-utjecaj eksposoma (prehrana) na epigenom i fenotip u djece

- Nizozemska zima gladi (od 11/1944. do 5/1945.)
- blokirana je bila opskrba hrane
- smanjio se znatno prosječni dnevni unos hrane 1000-500kcal/osobi 69% ugljikohidrati (krumpir-kruh), 19 % masti i 12% bjelančevina
- trudnice trebaju otprilike oko 1800-2400 kcal/dan

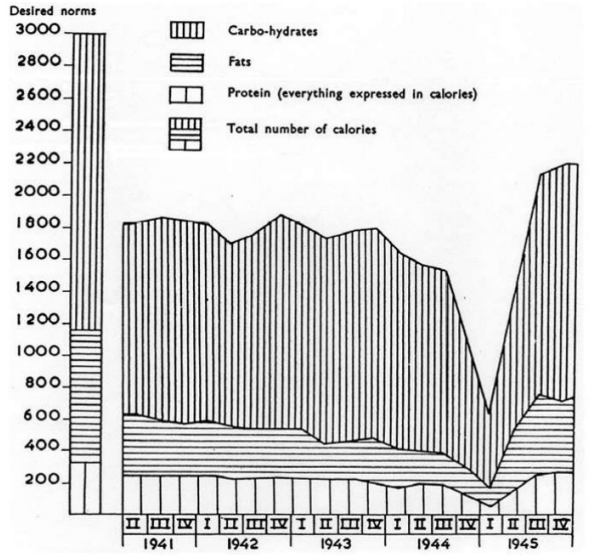


Table 3. Timing of famine exposure during gestation, *IGF2* DMR methylation, and birth weight

	Periconceptional exposure	Late gestational exposure	All controls
n	60	62	122
Males, %	46.7	45.2	45.9
Mean age, years	58.1 (SD, 0.35)	58.8 (SD, 0.4)	57.1 (SD, 5.5)
Birth weight, g	3612 (SD, 648)	3126 (SD, 408)	—
IGF2 DMR methylation			
Average	0.488 (SD, 0.047)	0.514 (SD, 0.045)	0.517 (SD, 0.047)
$P_{\text{vs all controls}}$	1.5×10^{-5}	.69	
$P_{\text{interaction}}$			4.7×10^{-3}

P values were obtained using a linear mixed model and adjusted for age.

Lumey LH et al. Cohort profile: The Dutch Hunger Winter Families Study. Int J Epidemiol.2007.
Heijmans BT et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A*. 2008., Burger et al 1948

Epigenom-metilacija IGF2

- izloženost u ranoj fazi trudnoće povezana je sa 5,2 % nižom metilacijom u odnosu na neizložene srodnike

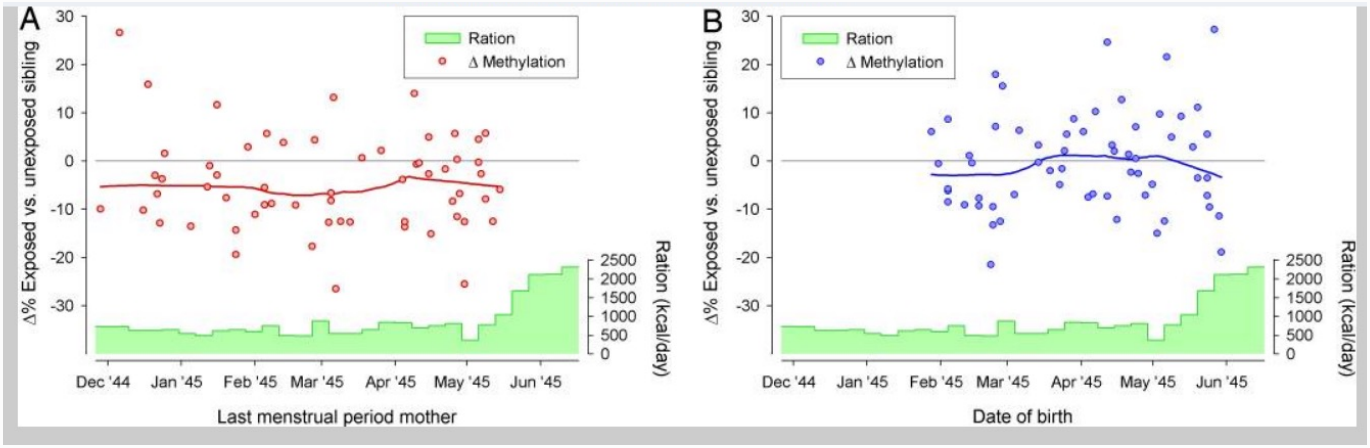


Table 1.

IGF2 DMR methylation among individuals periconceptionally exposed to famine and their unexposed, same-sex siblings

IGF2 DMR methylation	Mean methylation fraction (SD)				Relative change exposed	Difference in SDs	*P*
	Exposed (*n* = 60)		Controls (*n* = 60)				
Average	0.488	(0.047)	0.515	(0.055)	−5.2%	−0.48	5.9×10^{-5}
CpG 1	0.436	(0.037)	0.470	(0.041)	−6.9%	−0.78	1.5×10^{-4}
CpG 2 and 3	0.451	(0.033)	0.473	(0.055)	−4.7%	−0.41	8.1×10^{-3}
CpG 4	0.577	(0.114)	0.591	(0.112)	−2.3%	−0.12	.41
CpG 5	0.491	(0.061)	0.529	(0.068)	−7.2%	−0.56	1.4×10^{-3}

Table 3. Timing of famine exposure during gestation, *IGF2* DMR methylation, and birth weight

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Heijmans BT et al. Persistent epigenetic differences associated with prenatal exposure to famine in humans. *Proc Natl Acad Sci U S A*. 2008.

Eksposom-modifikacija epigenoma-fenotip

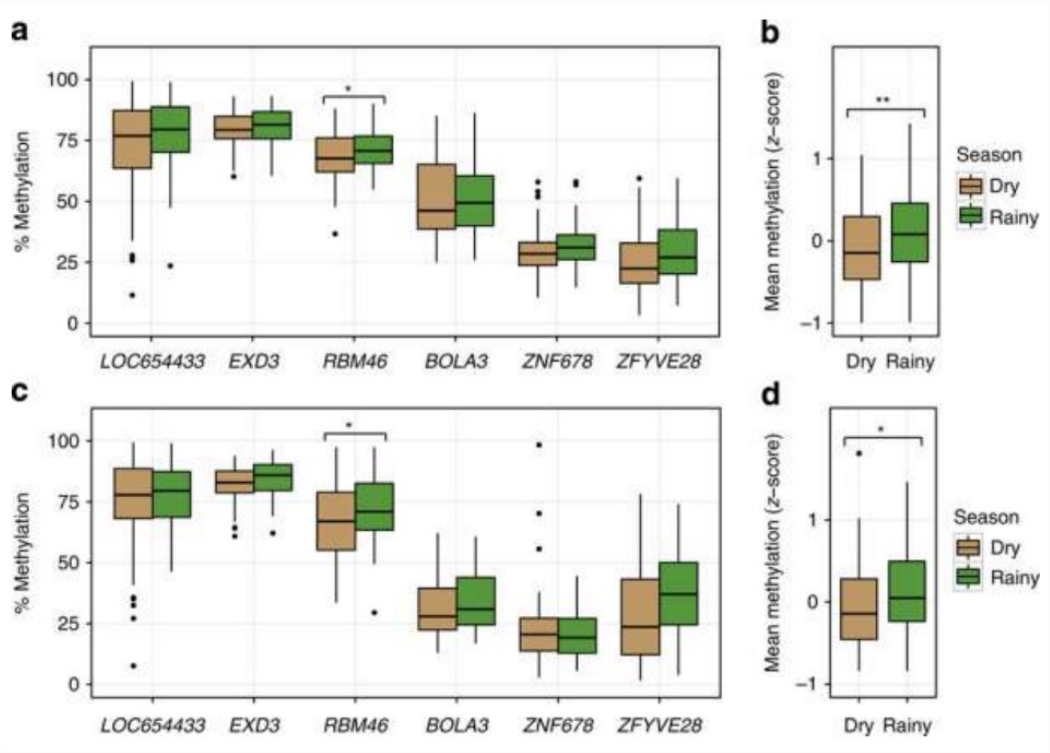
- osobe koje su bile izložene glađu u ranoj fazi trudnoće-DM2,pretilost, 2x veći rizik oboljevanja od shizofrenije

- osobe koje su bile izložene glađu kasnijoj fazi trudnoće-češće povezana intolerancija na glukozu, DM2

Ravelli AC et al. Glucose tolerance in adults after prenatal exposure to famine. Lancet.1998.
Lumey LH et al. Food restriction during gestation and impaired fasting glucose or glucose tolerance and type 2 diabetes mellitus in adulthood: evidence from the Dutch Hunger Winter Families Study. Journal of Developmental Origins of Healthand Disease.2009.
Susser E et al. Schizophrenia after prenatal famine. Further evidence. Arch. Gen. Psychiatry 1996.

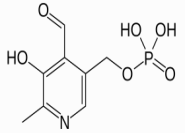
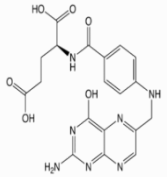
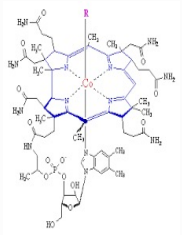
Prehrana- Epigenom

- u Gambiji se godišnje izmjenjuju kišno i suho razdoblje što uzrokuje velike razlike u prehrani
- kišna sezona- hrana je manje dostupna, ali je hrana bogatija nutijentima
- imale su više plazmatske koncentracije tvari koje su ključne u biokemijski put SAM (metionin, kolin, folat, vitamini B2, B6, B12, SAM)
- dojenčad su imala jaču metilaciju svih šest ispitanih gena
- suho razdoblje-hrana je više dostupna, ali manje bogata mikronutrijentima
- manja metilacija DNA



Dominguez-Salas et al. Maternal nutrition at conception modulates DNA methylation of human metastable epialleles.Nature communications. 2013.
Waterland et al. Season of conception in rural gambia affects DNA methylation at putative human metastable epialleles.PLoS Genet.2010.

Nutrijenti važni za sintezu S-Adenozil metionina (SAM)

Vitamin B 6 	**Izvor:** sjemenke suncokreta, pistacio, tuna, piletina, puretina, nemasna svinjetina, govedina, suhe šljive, banana, avokado, špinat
Vitamin B 9 	**Izvor:** grah, grašak, leća, špinat, brokula, šparoge, zeleno lisnato povrće, zelena salata, cjelovite žitarice, mango, naranče, avokado
Vitamin B 12 	**Izvor:** riba (skuša, losos, tuna, haringa, srdela, pastrva) morski plodovi, školjke, jaja, crveno meso, sir, mlijeko i mliječni proizvodi, obogaćene žitarice, proizvodi od soje

- Hipometilacija može biti povezana s manjkom nutrijenata važnih za proizvodnju donora metila
- utjecati na epigenetske modifikacije kromatina i naknadni fenotip

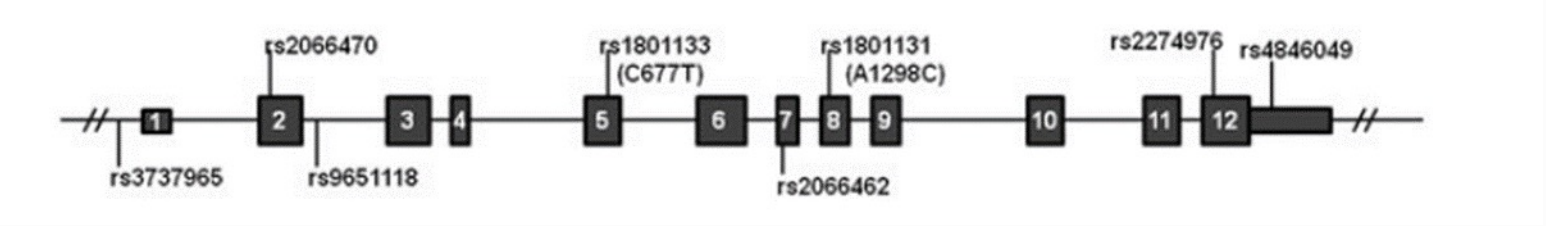
Rampersaud G.C. et.al., Folate key to optimizing health and reducing disease risk in elderly. J Am Coll Nuzt. 2003.
Houston M. The role of nutrition and nutraceutical supplements in the treatment of hypertension, Word J Cardiol. 2014.

Manjak folata

- vitamin B_9 ima važnu ulogu u ljudskom zdravlju i bolesti
- važan je za sintezu DNA, interkonverziju aminokiselina, različite metilacijske reakcije, za normalnu staničnu diobu
- nedostatak folata povezan je s nekoliko kongenitalnih malformacija, kao što su defekti neuralne cijev, kongenitalne srčane mane, orofacijalni rascjepi, komplikacije povezane s trudnoćom, kardiovaskularne bolesti, razne psihijatrijske bolesti i tumori

Medicines associated with folate-homocysteine-methionine pathway disruption. *Vidmar M, Grželj J, Mlinarič-Raščan I et. al. Arch Toxicol. 2019.*
Folate Deficiency and Gene Polymorphisms of MTHFR, MTR and MTRR Elevate the Hyperhomocysteinemia Risk. *Li WX et al. Clin Lab. 2017.*
Effects and safety of periconceptional folate supplementation for preventing birth defects. *De-Regil LM et al. Cochrane Database Syst Rev. 2010.*

Polimorfizmi *MTHFR* gena



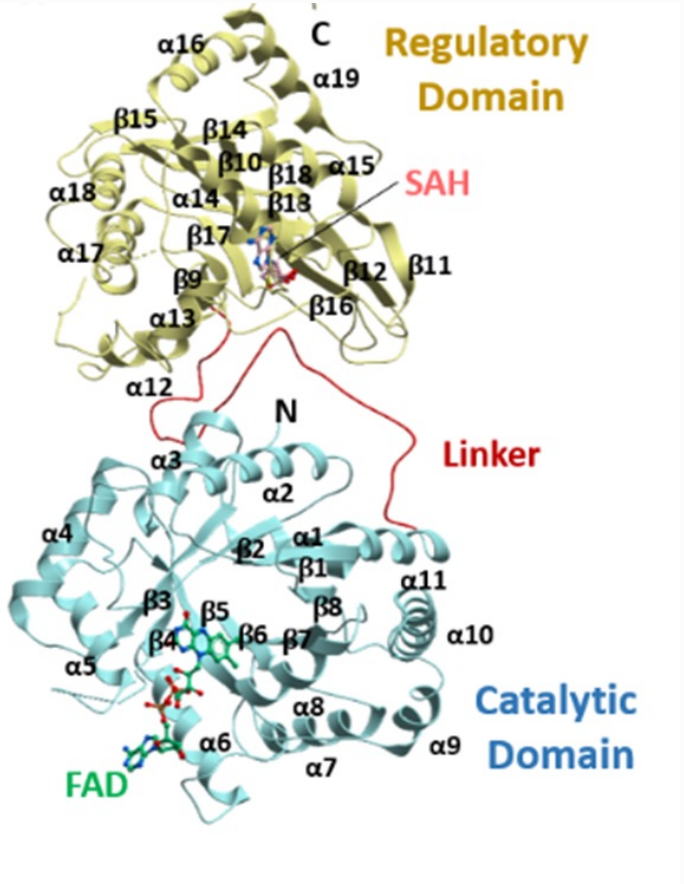
- *MTHFR* gen se sastoji od 12 egzona i smješten je na 1 kromosomu

- 1988.g. Kang i kolege su identificirali varijantu MTHFR

MTHFR C677T: C → T na nukleotidu 677→ alanin u valin konverzija u proteinu na poziciji 222

Smanjena je ezimska aktivnost enzima

MTHFR A1298C: A → C na nukleotidu 1298→ alanin u glutamat konverzija u proteinu na poziciji 429



Kang, S et al. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. Am. J. Hum. Genet. 1988.
www.thesgc.org/tep/mthfr
Spellicy et al. Folate Metabolism Gene 5,10-Methylenetetrahydrofolate Reductase (MTHFR) Is Associated with ADHD in Myelomeningocele Patients. Plos One 2012.
Goyette P. et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR).Mamm. Genome. 1998. https://

MTHFR C677T gen-polimorfizmi

- distribucija T alela je najviša među europskom populacijom
- Europa 24.5%–43.8%
- Azija 2.5%–36%
- Afrika 4.9%–9.1%

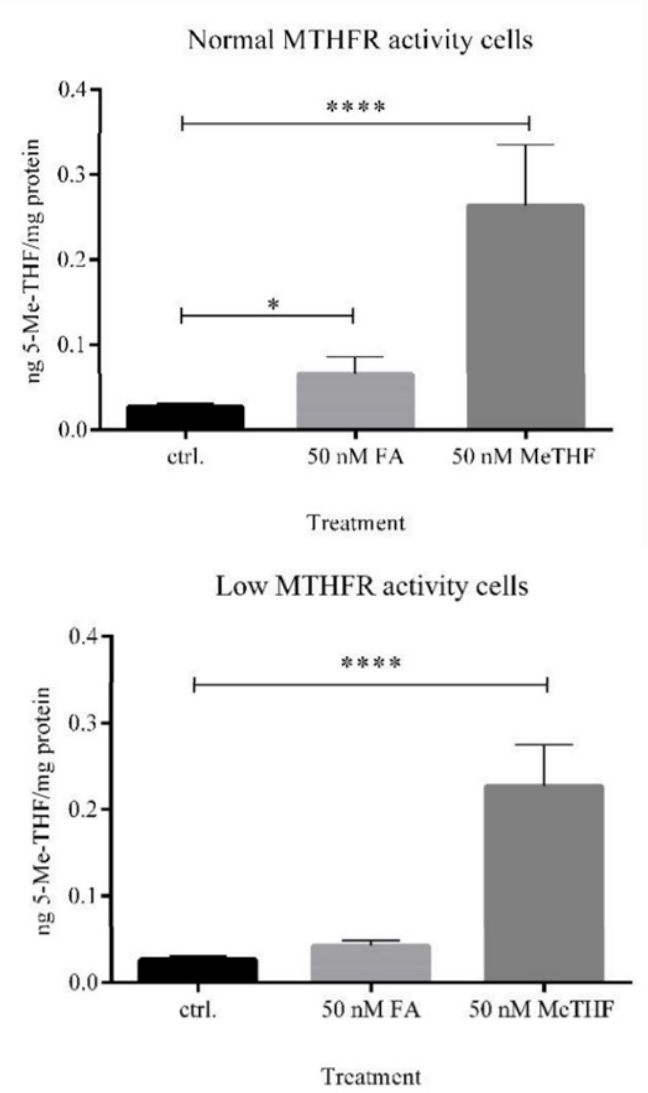
- MTHFR C677T polimorfizam je udružen s višim koncentracijama homocisteina i nižih vrijednosti folata

L. D. Botto and Q. Yang, "5, 10-methylenetetrahydrofolate reductase gene variants and congenital anomalies: a huge review," *American Journal of Epidemiology*, vol. 151, no. 9, pp. 862–877, 2000
Husemoen et al. "MTHFR C677T genotype and cardiovascular risk in a general population without mandatory folic acid fortification," *European Journal of Nutrition*, 2014
Junli Song et al. **Polymorphism of MTHFR C677T Gene and the Associations with the Severity of Essential Hypertension in Northern Chinese Population. International Journal of Hypertensio. 2020.**

Važnost izbora terapije ovisno o MTHFR polimorfizmu

- limfoblastoidnih staničnih linija (LCL)s niskom i visokom aktivnošću MTHFR enzima
- dodatak FA doveo je do 2,5 x povećanje 5-Me-THF u stanicama s normalnom aktivnošću MTHFR
- nije bilo povećanja nakon dodatka FA u stanicama s niskom MTHFR aktivnošću
- dodatak 5-Me-THF, dovodi do 10 x povećanja unutarstanične razine ovog metabolite

- važnost izbora terapije temeljene na nutrigenenomici i farmakogenetici



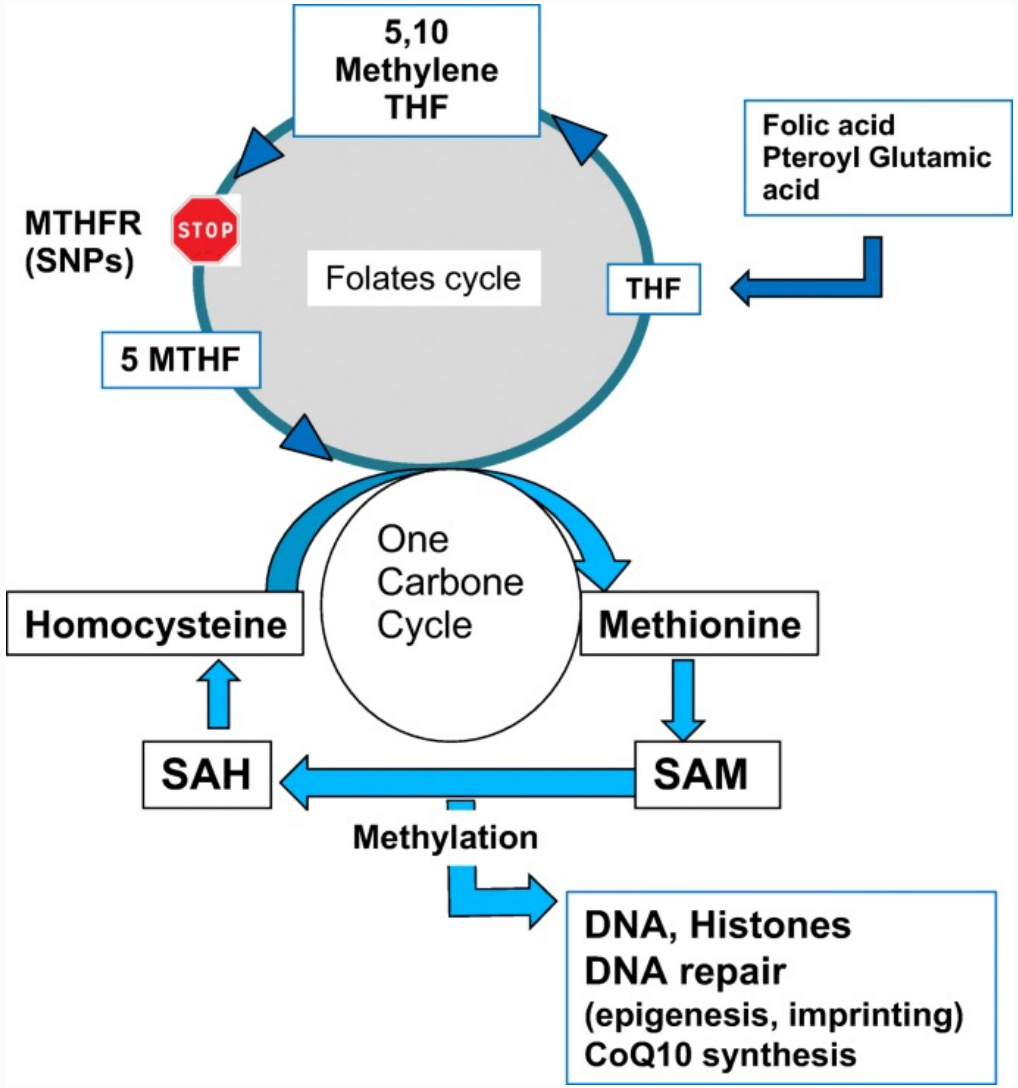
Vidmar Golja et al. Folate Insufficiency Due to MTHFR Deficiency Is Bypassed by 5-Methyltetrahydrofolate. J Clin Med. 2020.

MTHFR polimorfizam- Ciklus folne- donor SAM-metilacija

- polimorfizam MTHFR
- nedovoljna sinteza 5MTHF
- smanjeni SAM

- pojava u cirkulaciji nemetabolizirane folne kiseline-UMFA, sindroma nemetabolizirane folne kiseline

- može imati negativne učinke



Edouard Servy, Yves Menezo. Clinical Obstetrics, Gynecology and Reproductive Medicine. 2017.
Huang i sur. 2016

UMFA –nemetabolizirana folna kiselina

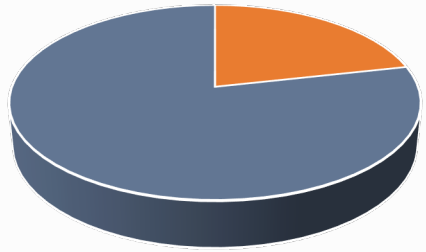
- obogaćivanje hrane folnom kiselinom i suplementacija s folnom kiselinom dovodi do porasta UMFA
- otkrivena je u gotovo svim uzorcima seruma američke djece i odraslih, u krvi pupkovine novorođenčadi i serumu 4-dnevne dojenčadi, u majčinom mlijeku kanadskih žena

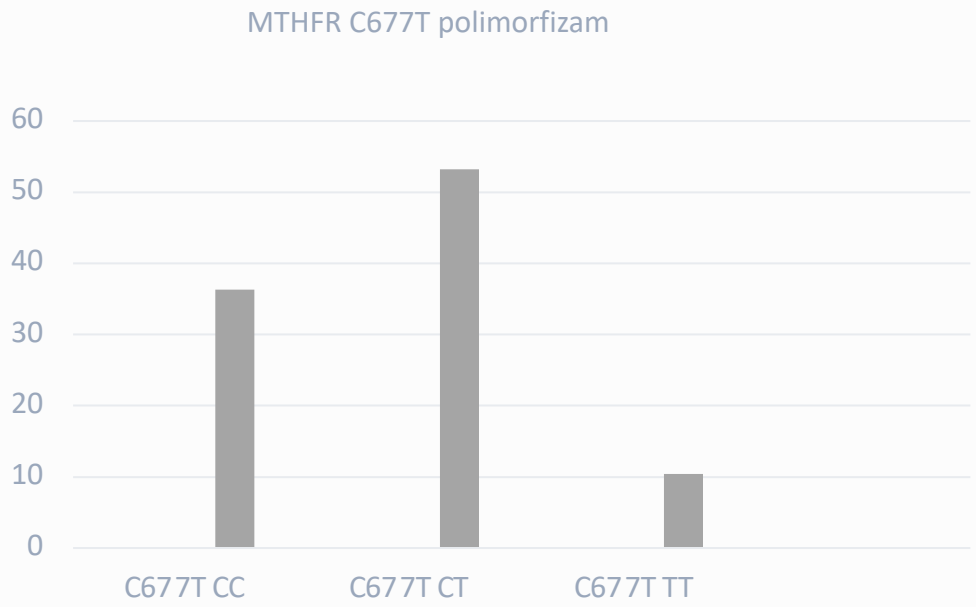
Pfeiffer CM et al. Unmetabolized folic acid is detected in nearly all serum samples from US children, adolescents, and adults. J Nutr. 2015
Page R. et al. Total folate and unmetabolized folic acid in the breast milk of a cross-section of Canadian women, The American Journal of Clinical Nutrition, 2017.

Naše malo istraživanje....
Polimorfizmi MTHFR C677T

- 154 osobe
- 48 godina
- BMI 38kg/m2
- M 22%
- Ž 78%,

- 36,3% C/C
- 53,2% C/T
- 10,39% TT





Matovinović M, Sertić J., Rubelj I, I sur. Personal data

Table 1

Frequency of MTHFR genotype in the Croatian population of healthy volunteers, and patients with coronary artery disease (CAD) and carotid stenosis (CS)

MTHFR genotype	Group			
	Healthy volunteers ($n=298$)	CAD+CS ($n=342$)	CS ($n=95$)	CAD ($n=247$)
CC	45 (134)	46 (158)	46.3 (44)	46.2 (114)
TC	49 (146)	45 (155)	46.3 (44)	44.9 (111)
TT	6 (18)	9 (29)	7.4 (7)	8.9 (22)
p-value		NS	NS	NS
OR (95% CI)		1.4 (0.78–2.65)	0.33 (0.13–0.79)	1.1 (0.56–2.03)
Allele frequency C/T	69/31	69/31	69/31	69/31
p-value		NS	NS	NS

TABLE 1

DISTRIBUTION OF THE C677T ALLELE OF THE MTHFR GENE IN 228 CROATIAN VOLUNTEERS

Gender	Number	Genotype			Allele frequency (%)		Frequency of TT homozygosity (%)		Frequency of CT (%)		Frequency of CC (%) homozygosity	
		TT	CT	CC	Frequency	95% confidence interval	Frequency	95% confidence interval	Frequency	95% confidence interval	Frequency	95% confidence interval
Male	175	18	79	78	32.9	27.9, 37.8	10.3	5.8, 14.8	45.1	37.8, 52.5	44.6	37.2, 51.9
Female	53	3	23	27	27.4	18.9, 35.9	5.7	*	43.4	30.1, 56.7	50.9	37.5, 64.4
Total	228	21	102	105	31.6	27.3, 35.9	9.2	5.4, 13.0	44.7	38.3, 51.2	46.1	39.6, 52.5

* Could not be assessed due to small sample

TABLE 2

DISTRIBUTION OF THE C677T ALLELE OF THE MTHFR GENE IN VARIOUS EUROPEAN POPULATIONS

Country	Total number	Genotype			Allele frequency (%)		Frequency of homozygosity (%)	
	Total	TT	CT	CC	frequency	95% confidence interval	Frequency	95% confidence interval
Britain/Wales *	1046	138	465	443	35.4	33.3, 37.4	13.2	11.1, 15.2
Croatia	228	21	102	105	31.6	27.3, 35.9	9.2	5.4, 13.0
France	133	13	70	50	36.1	30.1, 41.7	9.8	4.7, 14.8
Germany*	257	20	86	151	24.5	20.7, 28.1	7.8	4.5, 11.1
Ireland and Northern Ireland*	1309	141	568	600	32.5	30.7, 34.2	10.8	9.1, 12.5
Italy*	2053	370	1057	626	43.8	42.2, 45.3	18.0	16.4, 19.7
The Netherlands	503	45	234	224	32.2	29.3, 35.0	8.9	6.5, 11.4
Norway*	391	37	145	209	28.0	24.8, 31.1	9.5	5.0, 15.6
Sweden	126	13	50	63	30.2	24.3, 35.6	10.3	5.0, 15.6

*Pooled data from two or more studies.

228 osoba	298 osoba/342	154 osobe
Zdravi volonteri	Zdravi volonteri/CAD+CS	Osobe s pretilošću 22% M, 78% Ž
MTHFR CC 46%	45% / 46%	36,3%
MTHFR CT 44,74%	49% / 45%	53,2%
MTHFR TT 9,21%	6% / 9%	10,39%

Croatian population data for the C677T polymorphism in methylenetetrahydrofolate reductase: frequencies in healthy and atherosclerotic study groups. Žuntar et al. Clinica Chimica Acta. 2003.
5, 10-Methylenetetrahydrofolate Reductase (MTHFR) 677 CT Genetic Polymorphism in 228 Croatian Volunteers. Ivo Lovričević et al. Coll. Antropol. 2004
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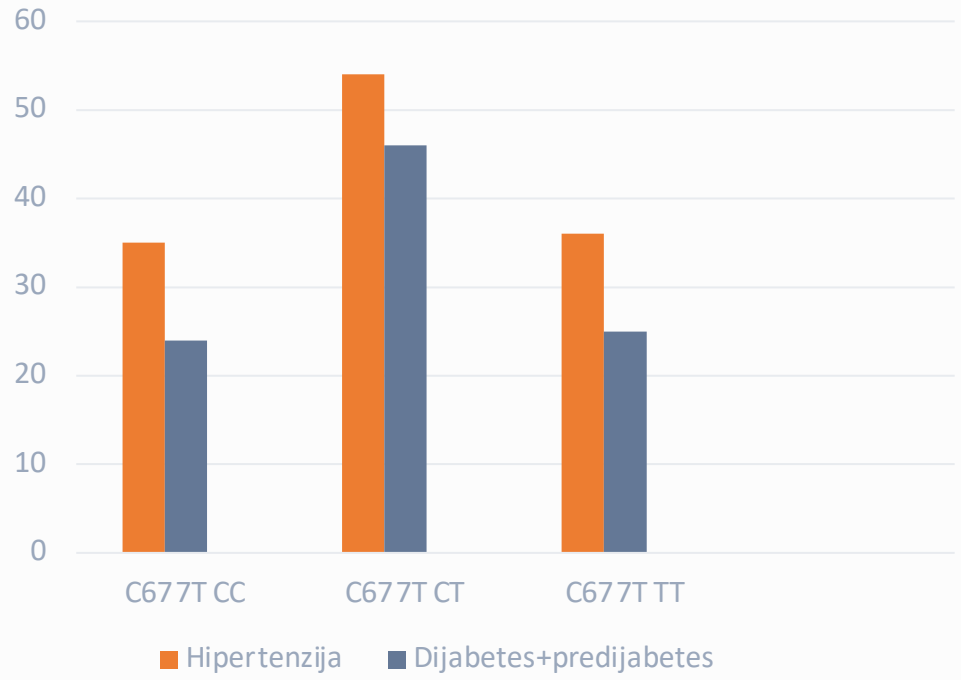
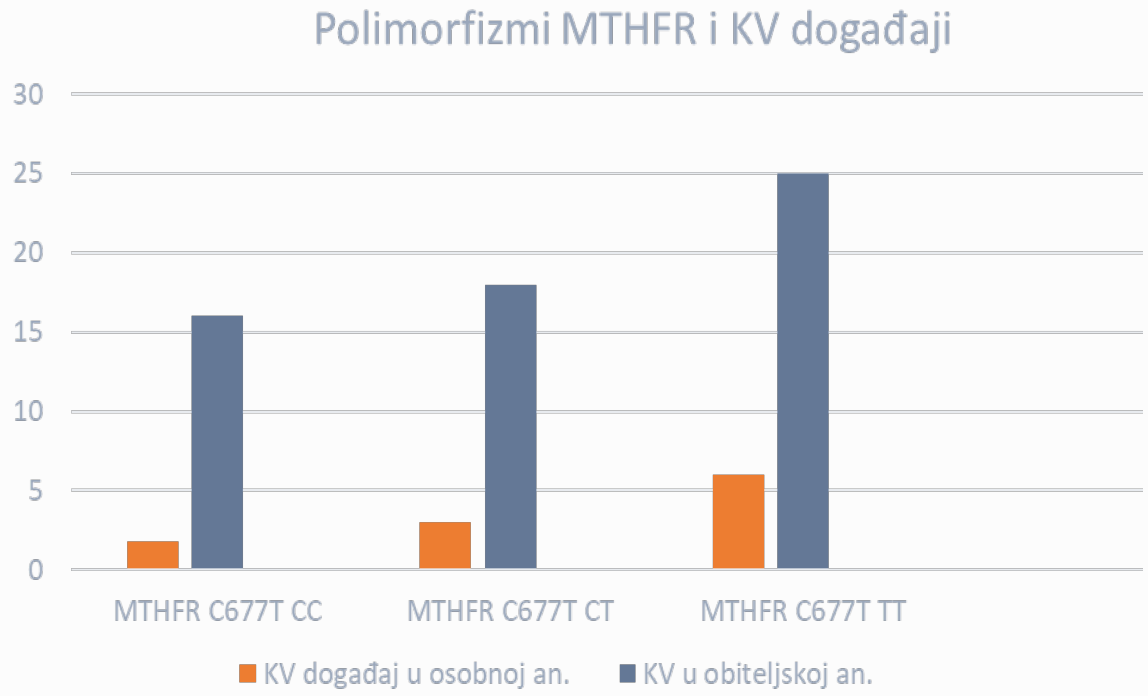
Polimorfizam MTHFR C677T i hipertenzija, dijabetes i predijabetes

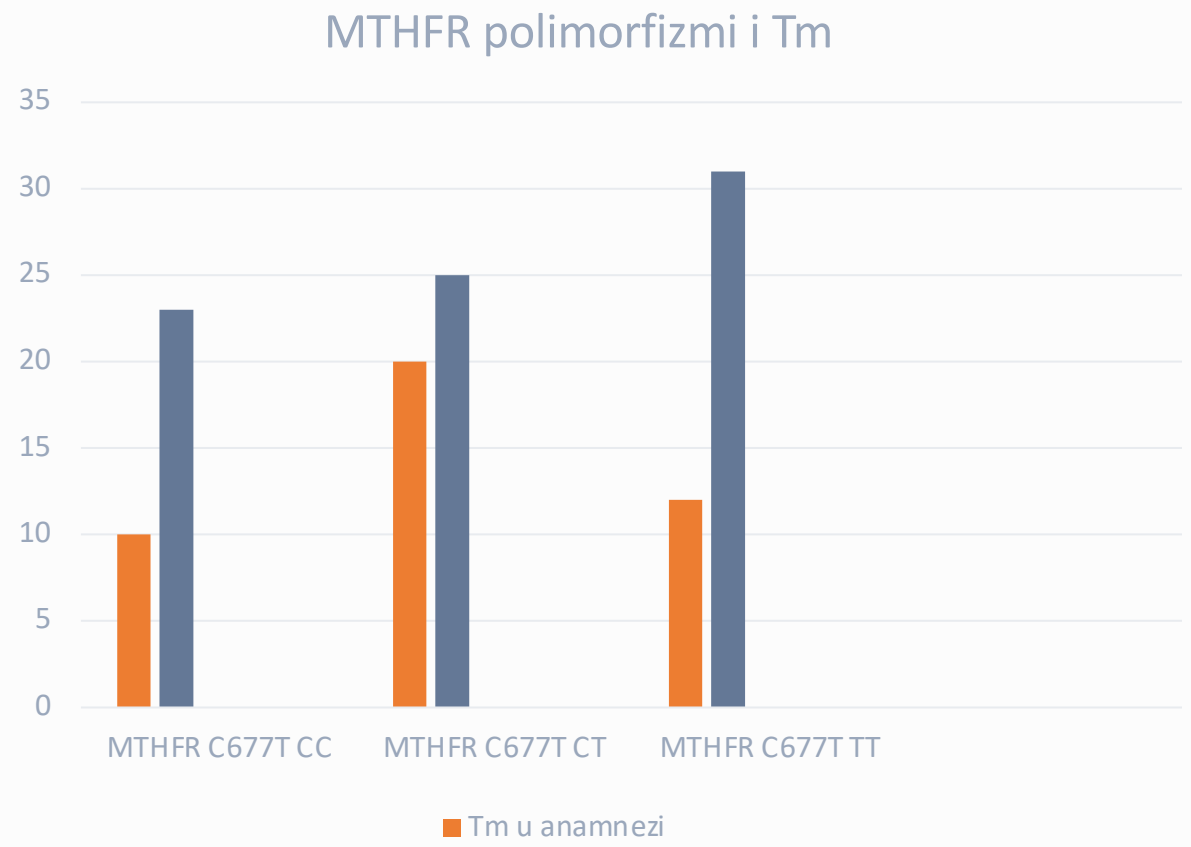


Polimorfizam MTHFR C667T i kardiovaskularni incidenti



Matovinović M., Sertić , Rubelj I. I sur. Personal data

Povezanost MTHFR polimorfizma i tumora u anamnezi



Matovinović M., Sertić J. , Rubelj I. i sur. Personal data

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Higher prevalence of *FTO* gene risk genotypes AA rs9939609, CC rs1421085, and GG rs17817449 and saliva containing *Staphylococcus aureus* in obese women in Croatia

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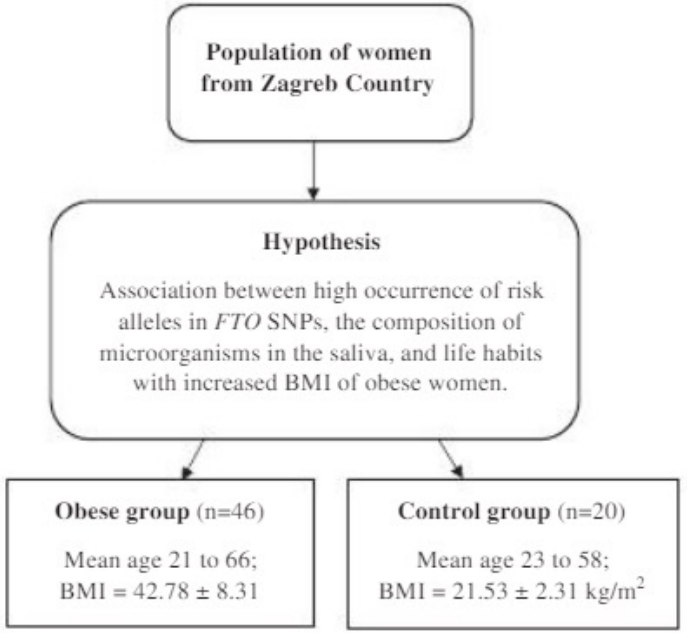
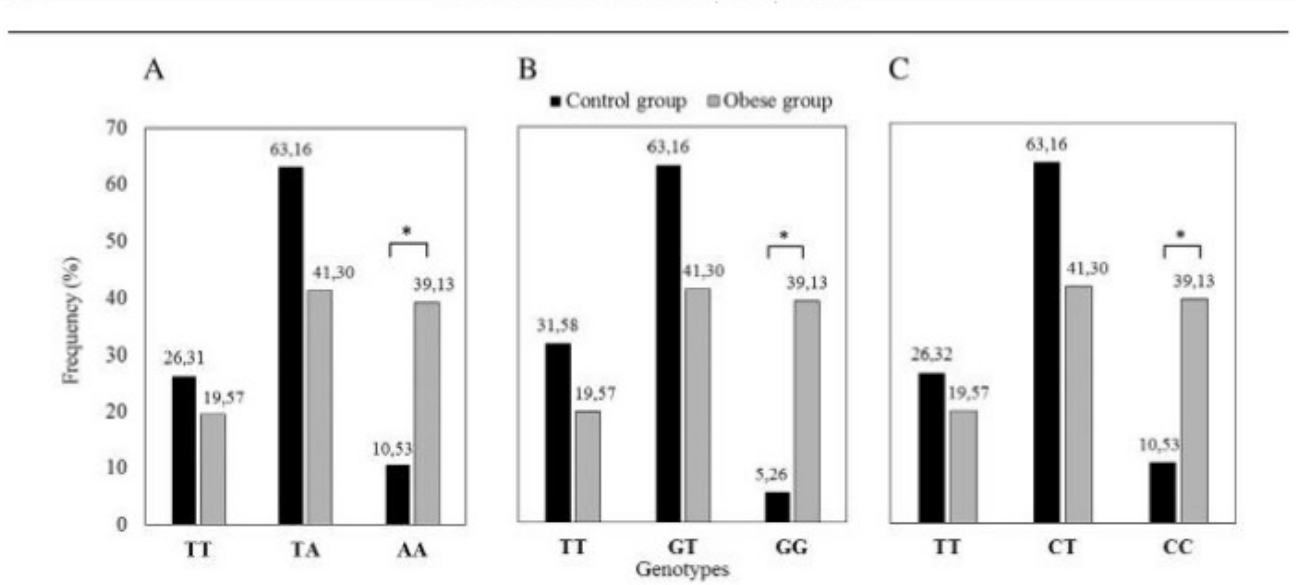
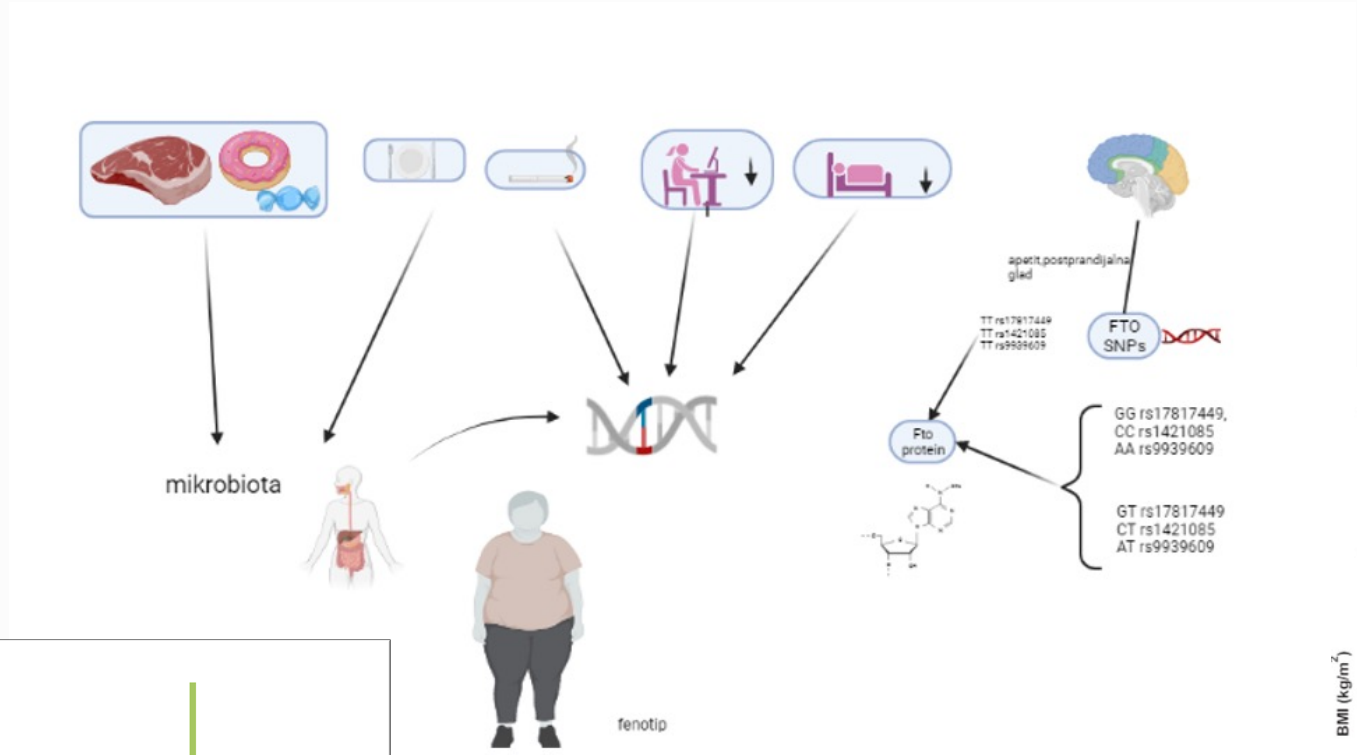
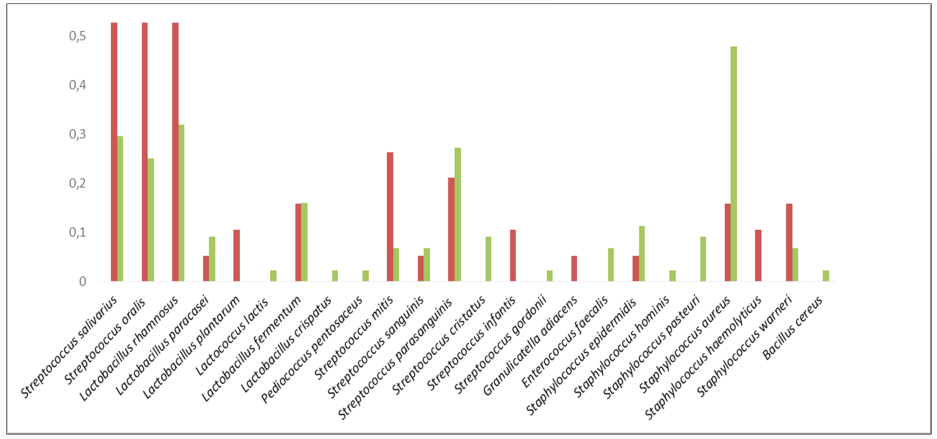
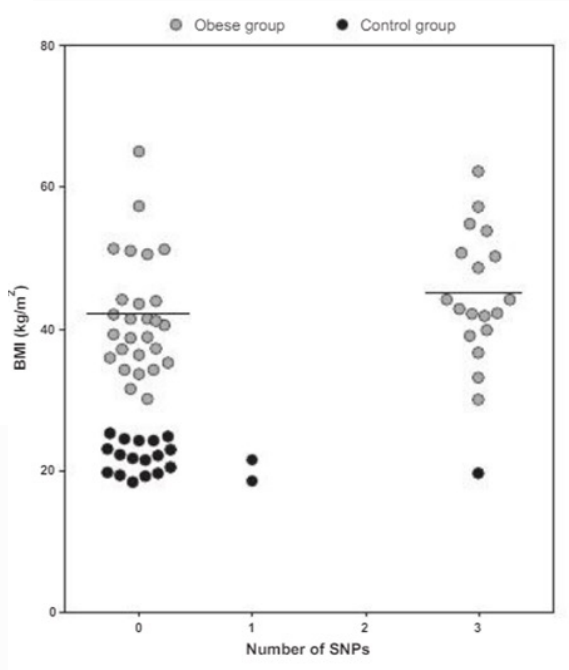




Polimorfizmi *FTO* gena u pretilih žena



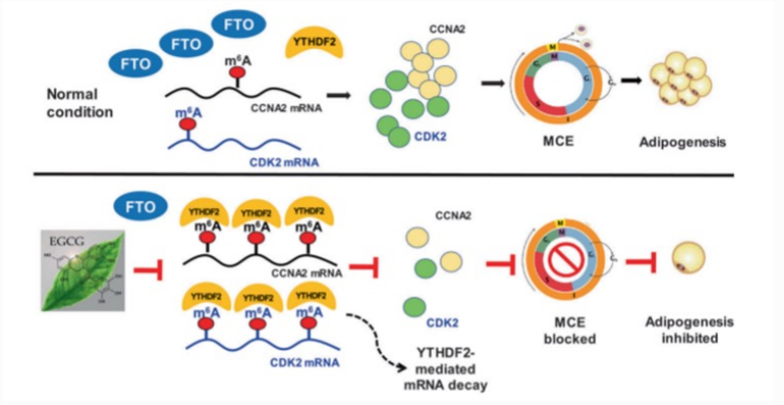




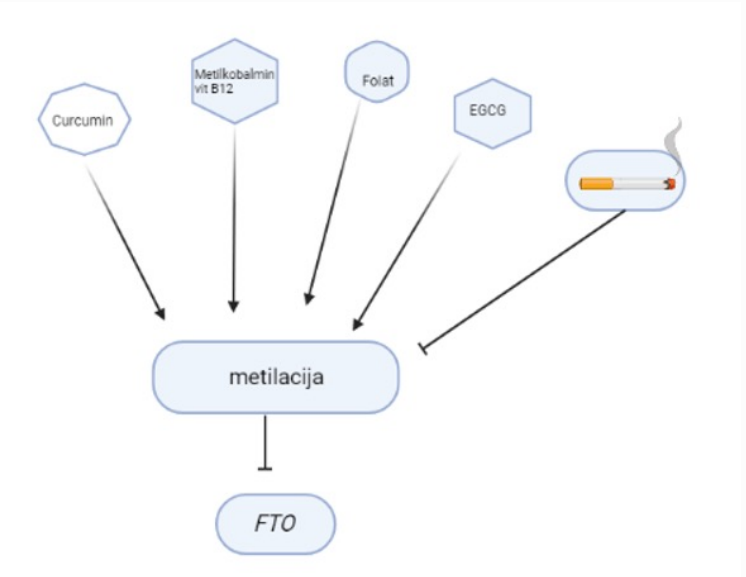
Higher prevalence of FTP gene risk genotypes AA rs9939609, CC rs1421085, and GG rs17817449 and saliva containing Staphylococcus aureus in obese women in Croatia. Huđek A et al. 2018.

Potencijalni učinci na ekspresiju *FTO* gena

- EGCG dovodi do povećane ukupne razine RNA m6A metilacije
- EGCG inhibira adipogenezu blokirajući mitotičku klonsku ekspanziju u ranoj fazi diferencijacije adipocita
- smanjuje ekspresiju FTO proteina,m6A demetilaza



- kravlje mlijeko povećava razinu BCAA što dovodi do prekomjerne ekspresije FTO gena
- supresiju DNMT što dovodi do hipometilacije
- fermentirani mliječni proizvodi suprotan učinak



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Yadav DK et al. Vitamin B12 supplementation influences methylation of genes associated with Type 2 diabetes and its intermediate traits.Epigenomics.2017.

Melnik BC. The Pathogenic Role of Persistent Milk Signaling in mTORC1- and Milk- MicroRNA-Driven Type 2 Diabetes Mellitus. Current Diabetes Reviews. 2015.

Budućnost...

- identifikacija genetske predispozicije za bolesti
- epigenomski sat
- odrediti i izračunati vanjske utjecaje

- individulano predviđanje rizika i strateškog planiranja preventivne skrbi i planiranja liječenja

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