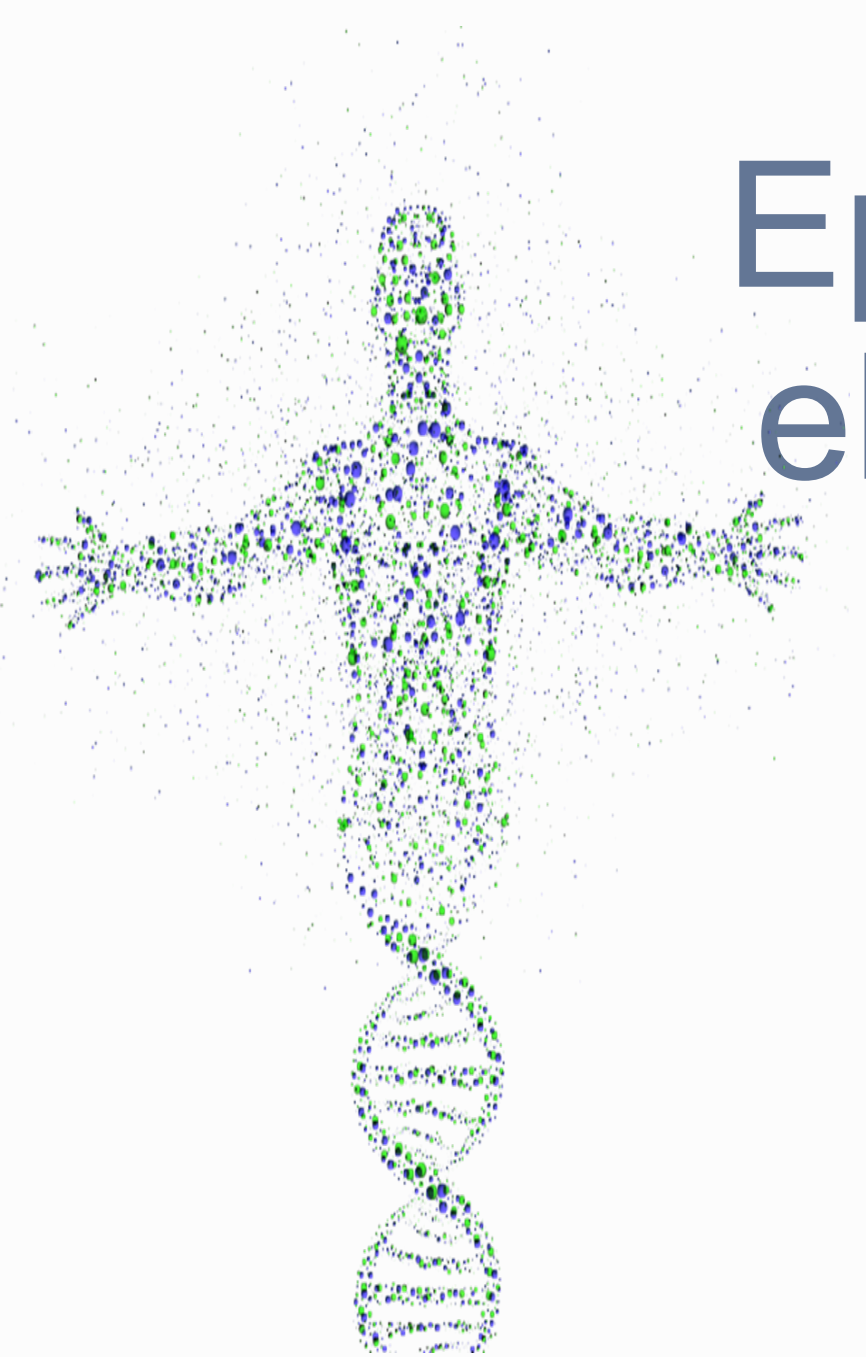


Epigenomika-raskrižje eksposoma i genoma

Martina Matovinović
KBC Zagreb

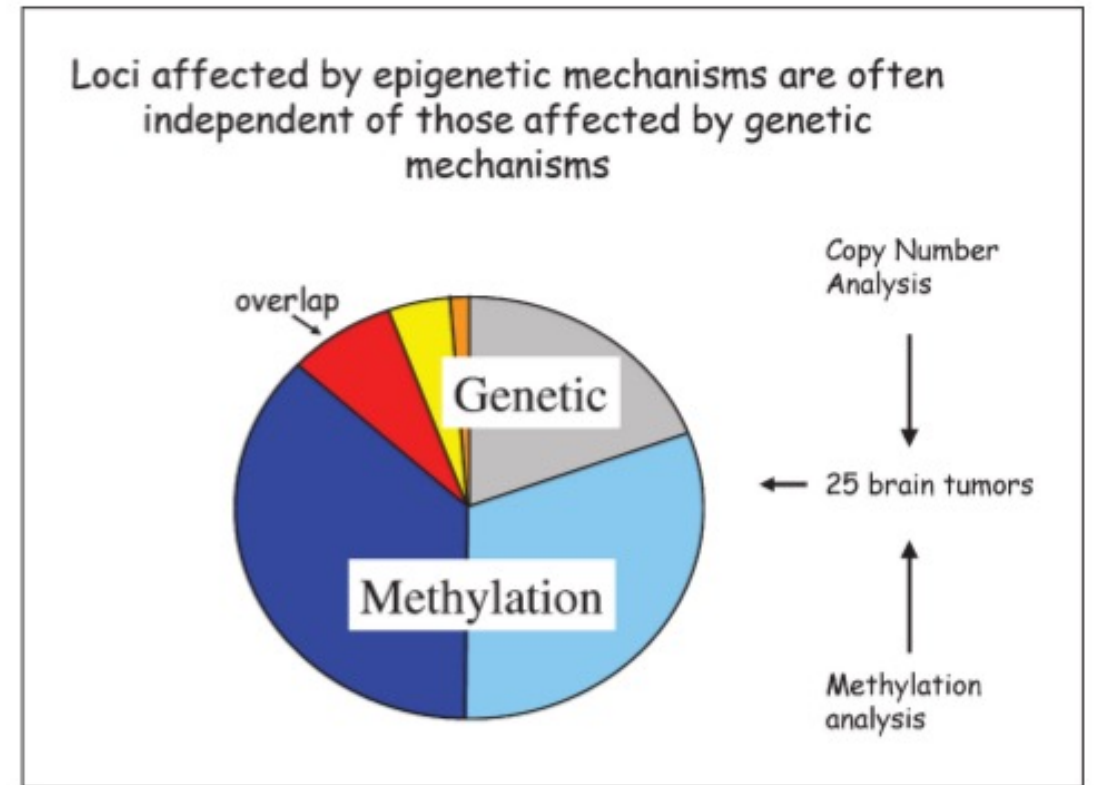


Epigenetika- Epigenomika

- 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

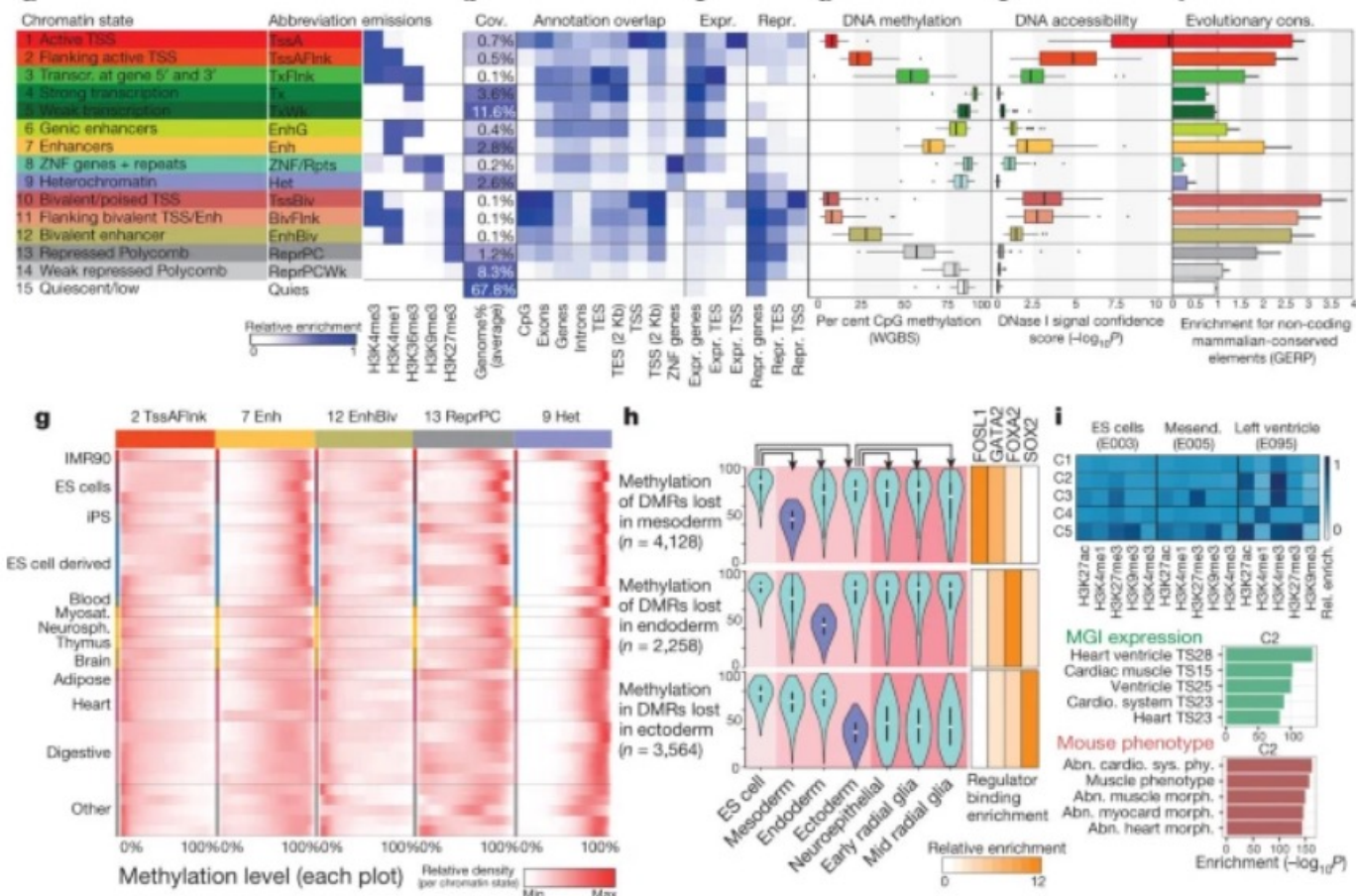
Human Epigenome Project

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



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



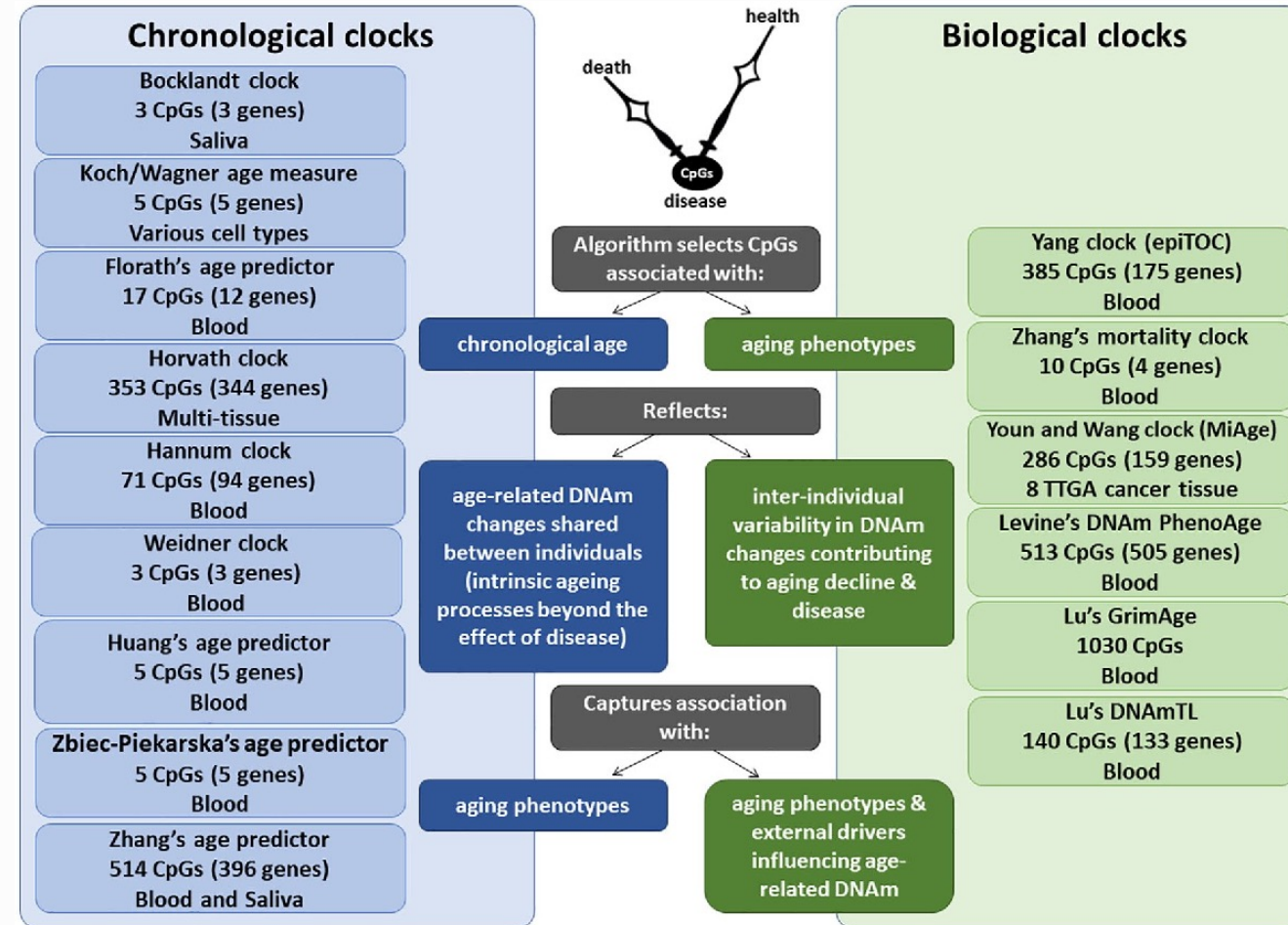
Epigenetski sat



- 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

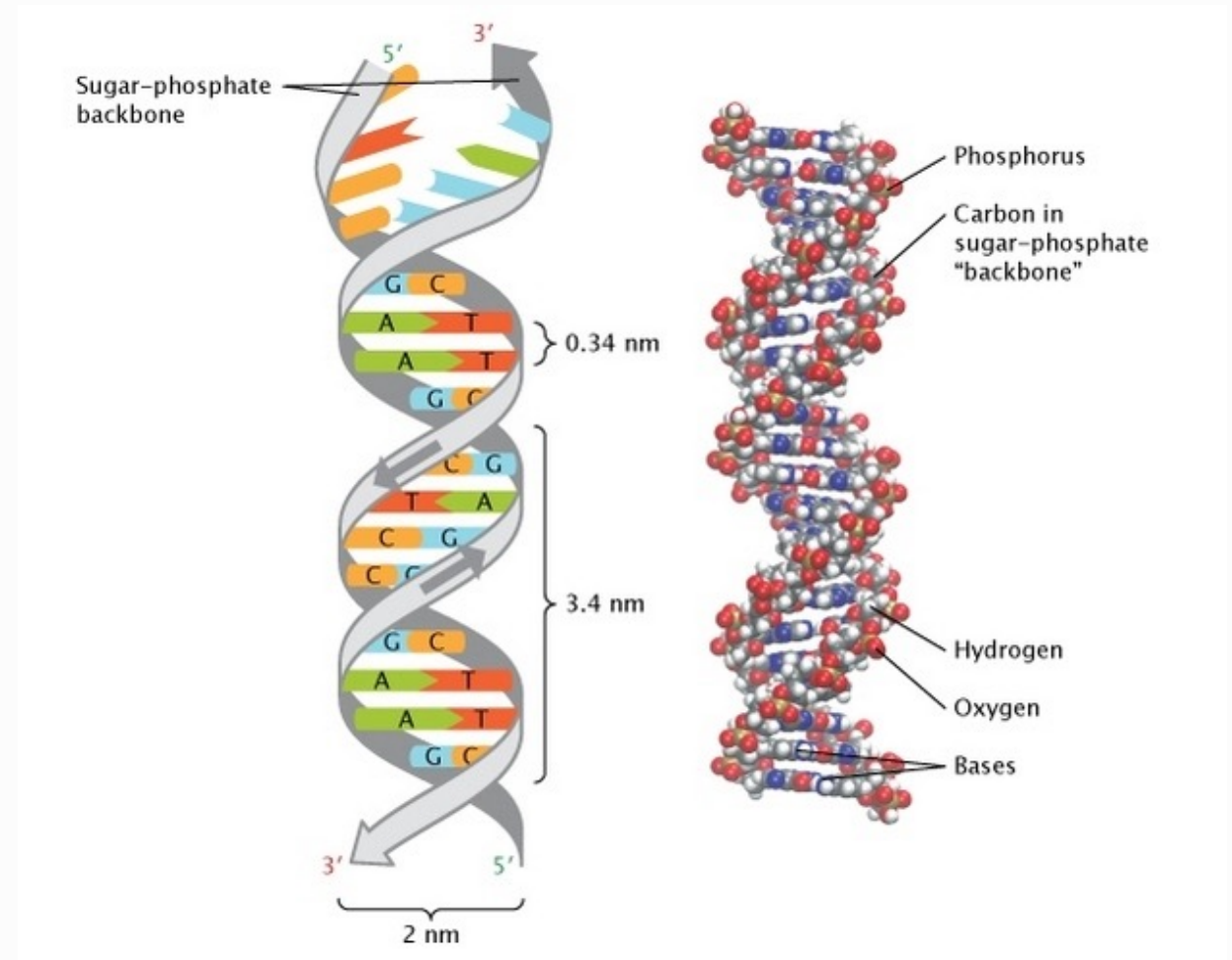
Epigenetski satovi

- 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



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 nasliedena svojstva degradirala



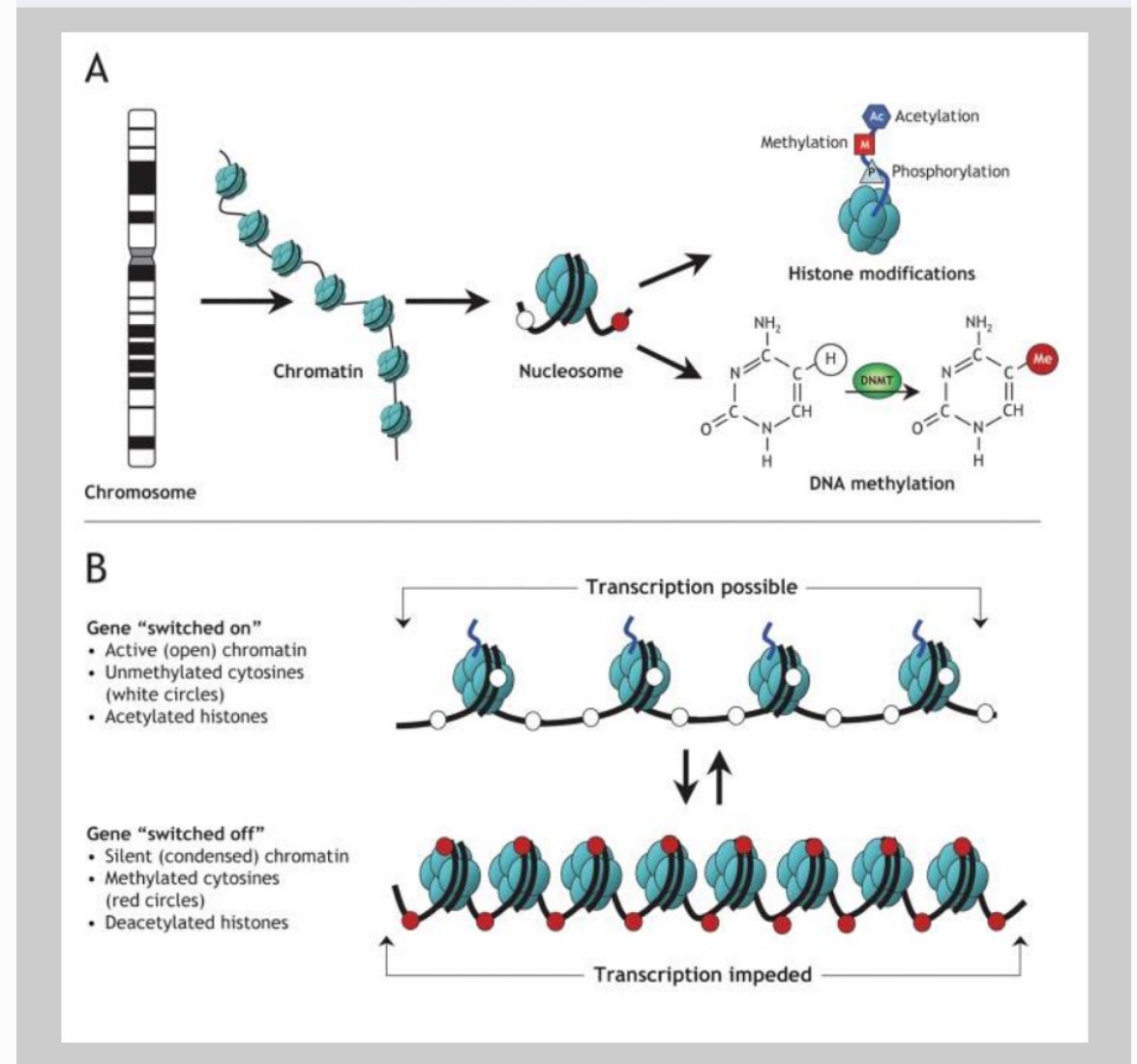
Eksposom

- 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



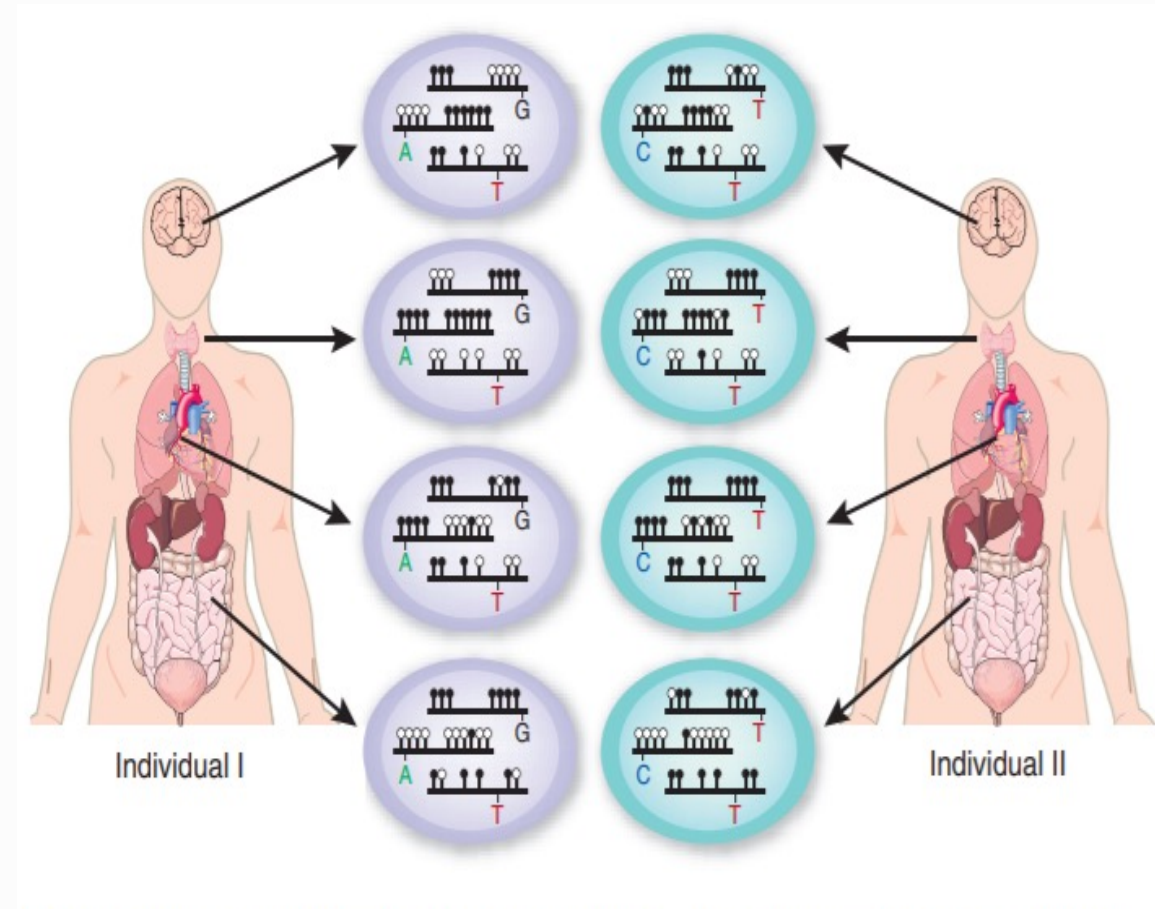
Osnovni mehanizmi epigenetike

- 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

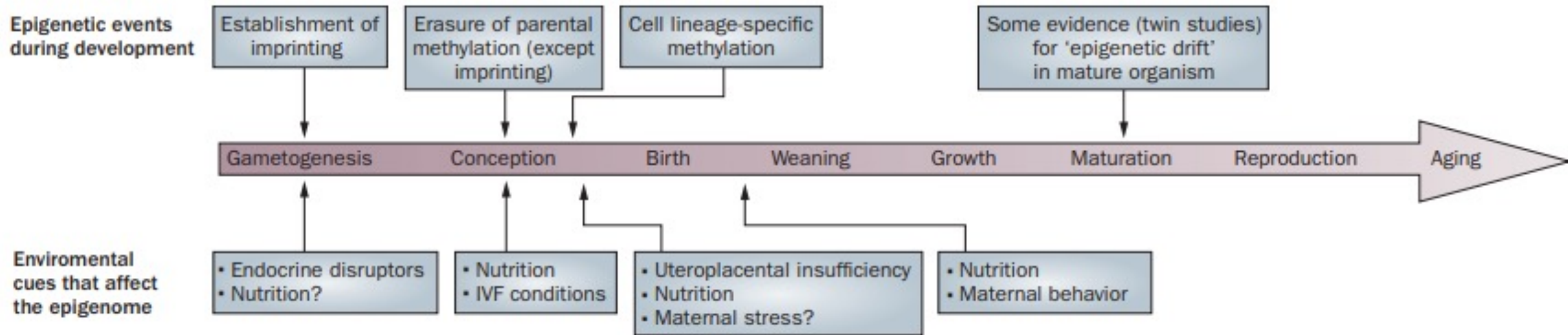


Tkivno specifični uzorak metilacije

- 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



Ekposom-epigenom-genom



Pothranjenost majki tijekom prekonceptije, 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

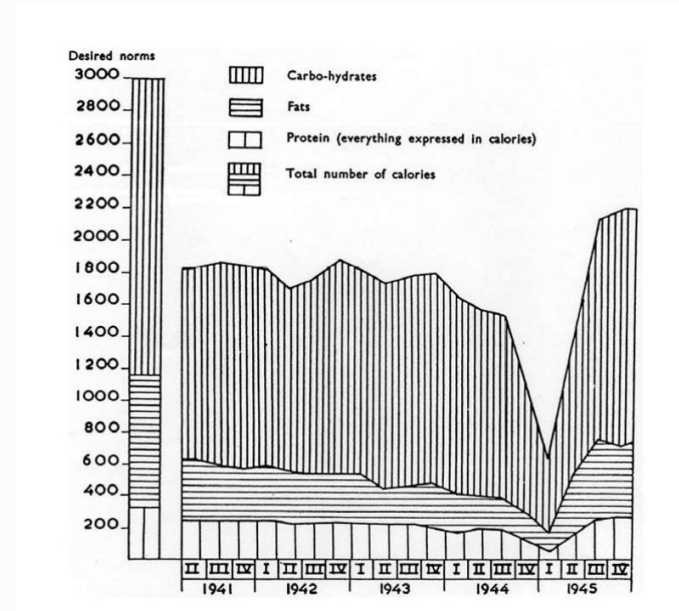


Table 3. Timing of famine exposure during gestation, IGF2 DMR methylation, and birth weight

	Periconceptual exposure	Late gestational exposure	All controls
<i>n</i>	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)
<i>P</i> _{vs all controls}	1.5×10^{-5}	.69	
<i>P</i> _{interaction}			4.7×10^{-3}

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

Epigenom-metilacija IGF2

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

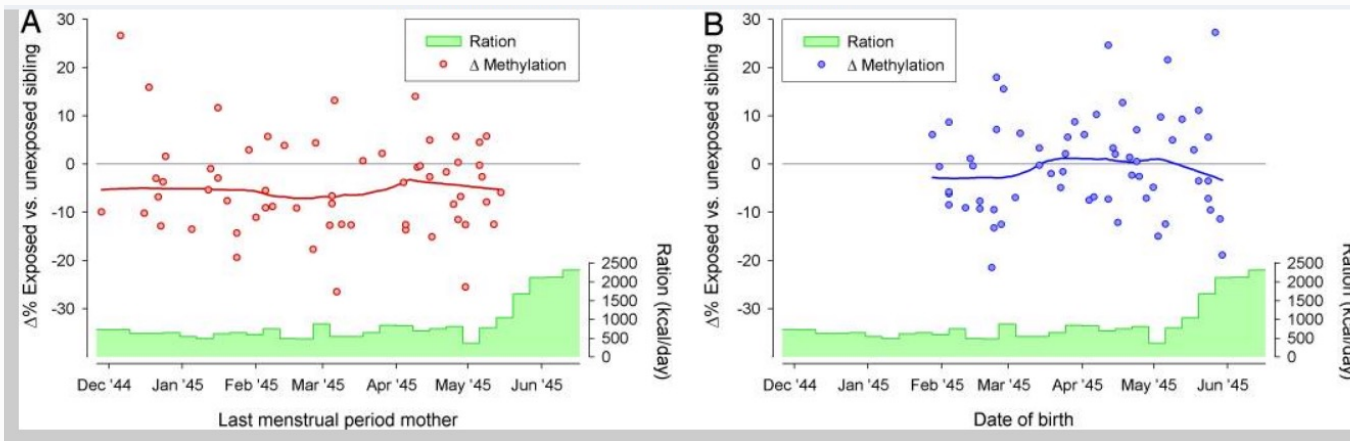


Table 1.

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

<i>IGF2</i> DMR methylation	Mean methylation fraction (SD)		Relative change exposed	Difference in SDs	<i>P</i>
	Exposed (<i>n</i> = 60)	Controls (<i>n</i> = 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

	Periconceptional exposure	Late gestational exposure	All controls
<i>n</i>	60	62	122
Males, %	46.7	45.2	45.9
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Birth weight, g	3612 (SD, 648)	3126 (SD, 408)	—
<i>IGF2</i> DMR methylation			
Average	0.488 (SD, 0.047)	0.514 (SD, 0.045)	0.517 (SD, 0.047)
<i>P</i> vs all controls	1.5×10^{-5}	.69	
<i>P</i> interaction			4.7×10^{-3}

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

Ekspozom-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

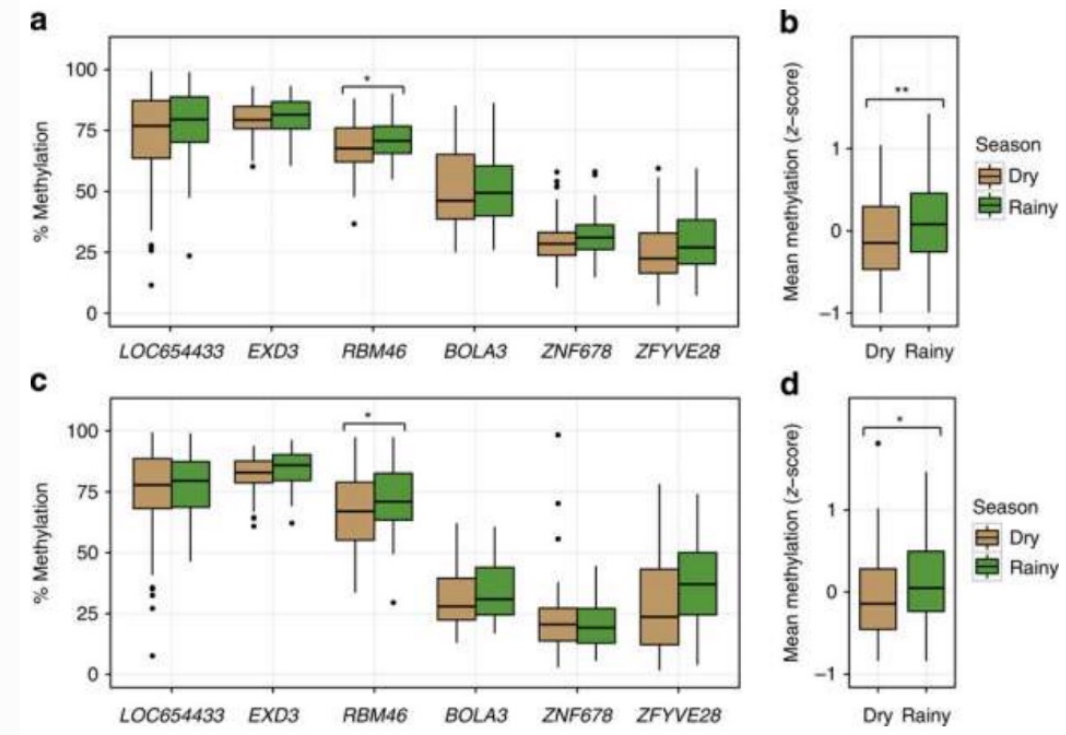
Ravelli AC et al. Glucose tolerance in adults after prenatal exposure to famine. Lancet.1998.

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 Health and Disease.2009.

Susser E et al. Schizophrenia after prenatal famine. Further evidence. Arch. Gen. Psychiatry 1996.

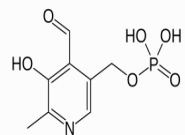
Prehrana- Epigenom

- 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



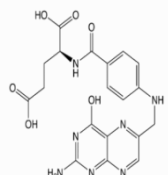
Nutrijenti važni za sintezu S-Adenozil metionina (SAM)

Vitamin B 6



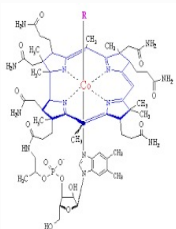
Izvor: sjemenke suncokreta, pistacio, tuna, piletina, puretina, nemasna svinjetina, govedina, suhe šljive, banana, avokado, špinat

Vitamin B 9



Izvor: grah, grašak, leća, špinat, brokula, šparoge, zeleno lisnato povrće, zelena salata, cjelovite žitarice, mango, naranče, avokado

Vitamin B 12



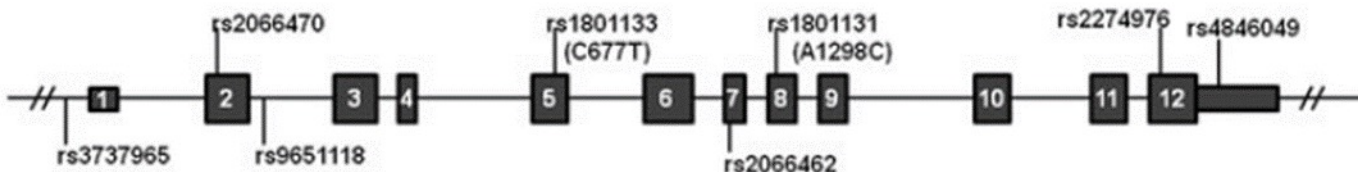
Izvor: riba (skuša, losos, tuna, haringa, srdela, pastrva) morski plodovi, školjke, jaja, crveno meso, sir, mlijeko i mliječni proizvodi, obogaćene žitarice, proizvodi od soje

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

Manjak folata

- vitamin B₉ 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

Polimorfizmi *MTHFR* gena

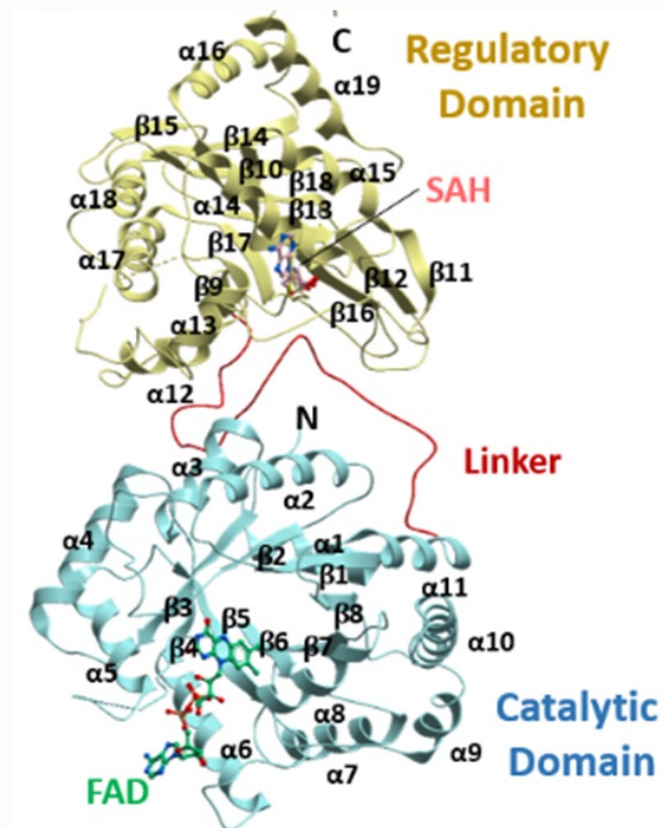


- *MTHFR* gen se sastoji od 12 egzona i smješten je na 1 kromosomu
- 1988.g. Kang i kolege su identificirali varijantu MTHFR

MTHFR C677T: C $\rightarrow$ T na nukleotidu 677 $\rightarrow$ alanin u valin konverzija u proteinu na poziciji 222

Smanjena je ezimska aktivnost enzima

MTHFR A1298C: A $\rightarrow$ C na nukleotidu 1298 $\rightarrow$ 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.

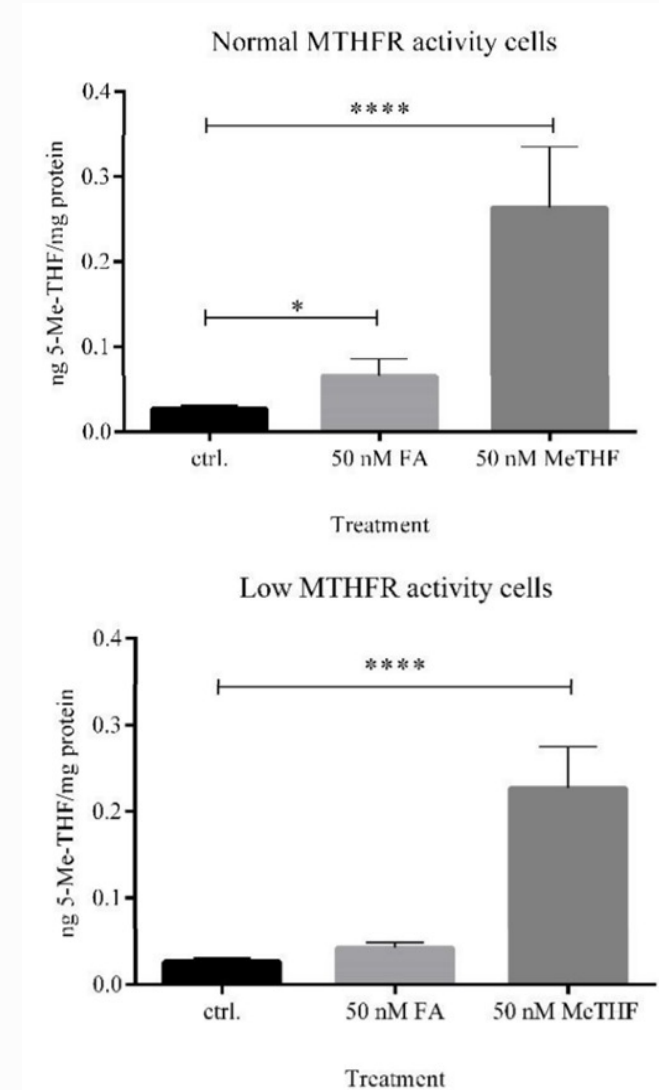
Goyette P. et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). Mamm. Genome. 1998. <https://doi.org/10.1007/s003350000000>

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

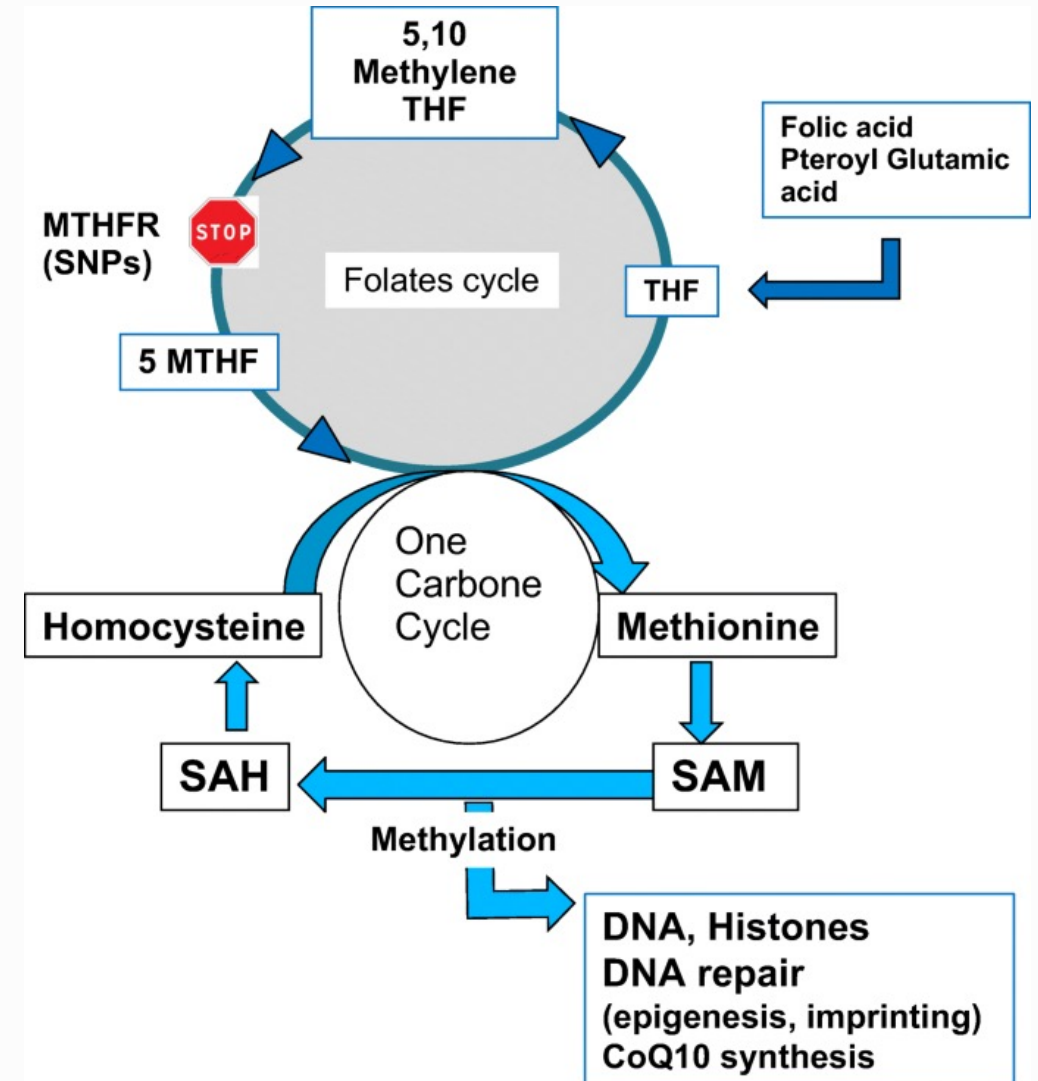
Važnost izbora terapije ovisno o MTHFR polimorfizmu

- 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



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



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

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

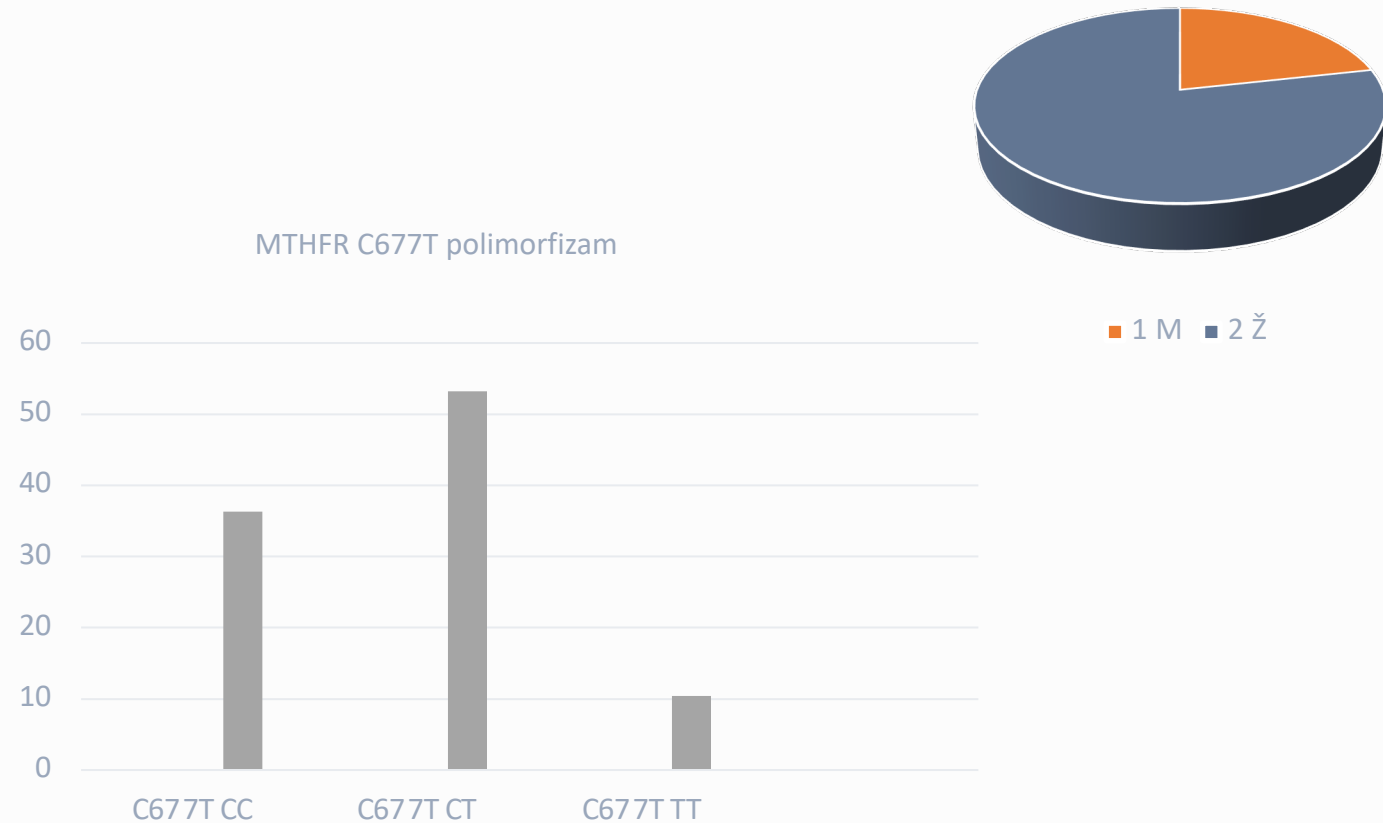


Table 1
Frequency of MTHFR genotype in the Croatian population of healthy volunteers, and patients with coronary artery disease (CAD) and carotid stenosis (CS)

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

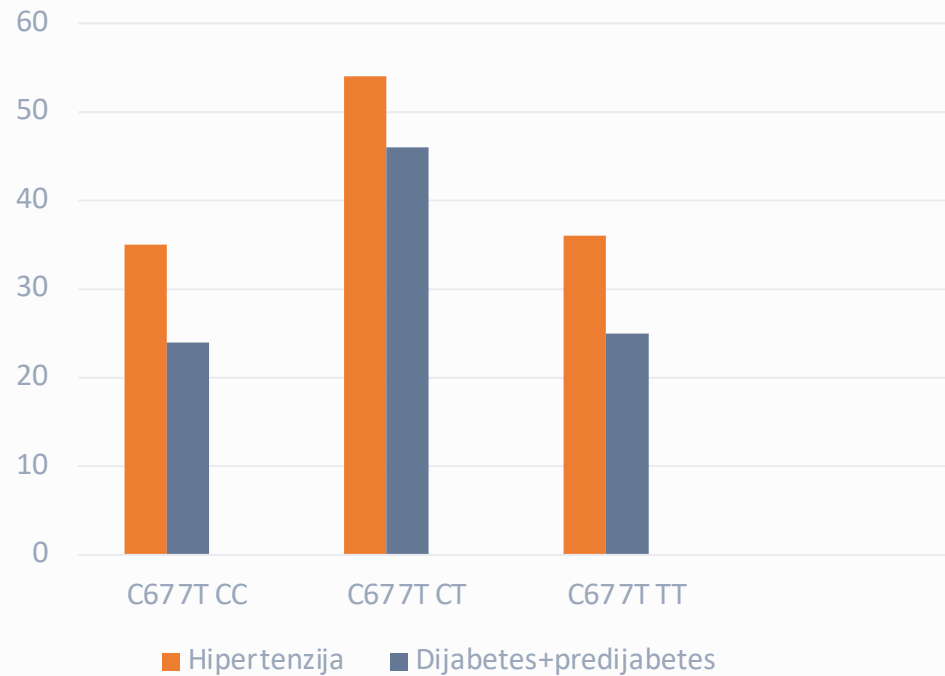
Country	Total number	Genotype			Allele frequency (%)		Frequency of homozygosity (%)		
	Total	TT	CT	CC	frequency	95% confidence interval	Frequency	95% confidence interval	
Britain/Wales *	1046	138	465	443	35.4	33.3, 37.4	13.2	11.1, 15.2	
Croatia	228	21	102	105	31.6	27.3, 35.9	9.2	5.4, 13.0	
France	133	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.

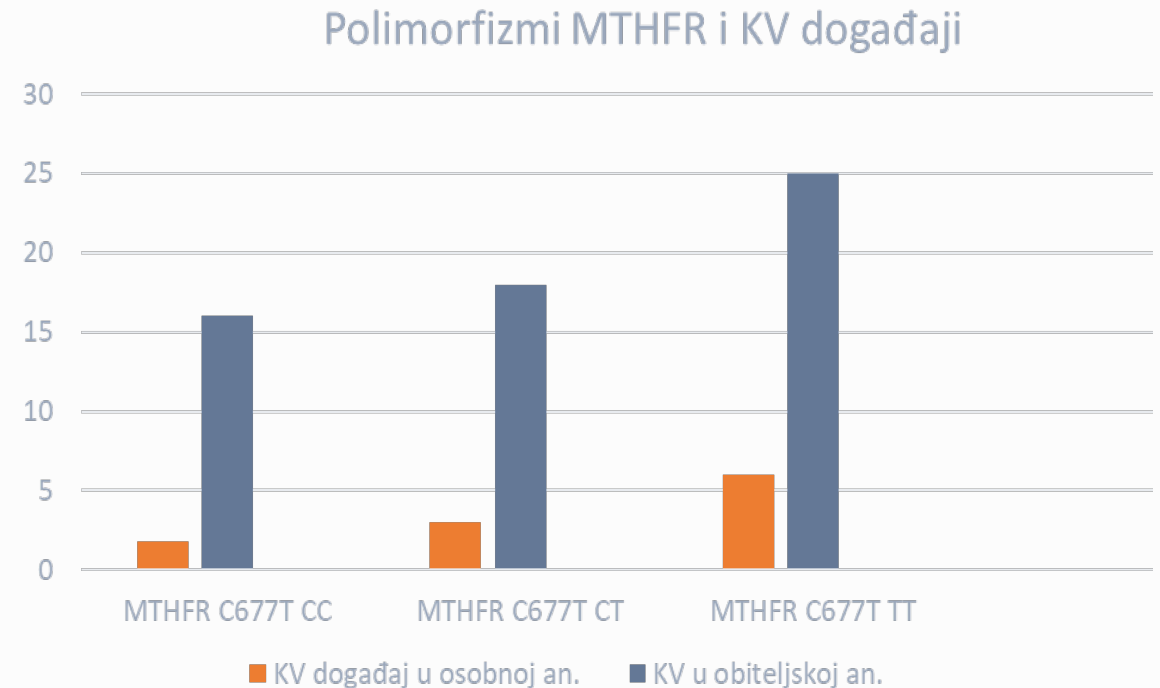
228 osoba	298 osoba/342	154 osobe
Zdravi volonteri	Zdravi volonteri/CAD+CS	Osobe s pretilošću 22% M, 78% Ž
MTHFR CC 46%	45% / 46%	36,3%
MTHFR CT 44,74%	49% / 45%	53,2%
MTHFR TT 9,21%	6% / 9%	10,39%

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
Sultana, T. et al. C677T genotypes in methyltetrahydrofolate reductase gene in student obesity. Journal of King Saud University - Science.2019.

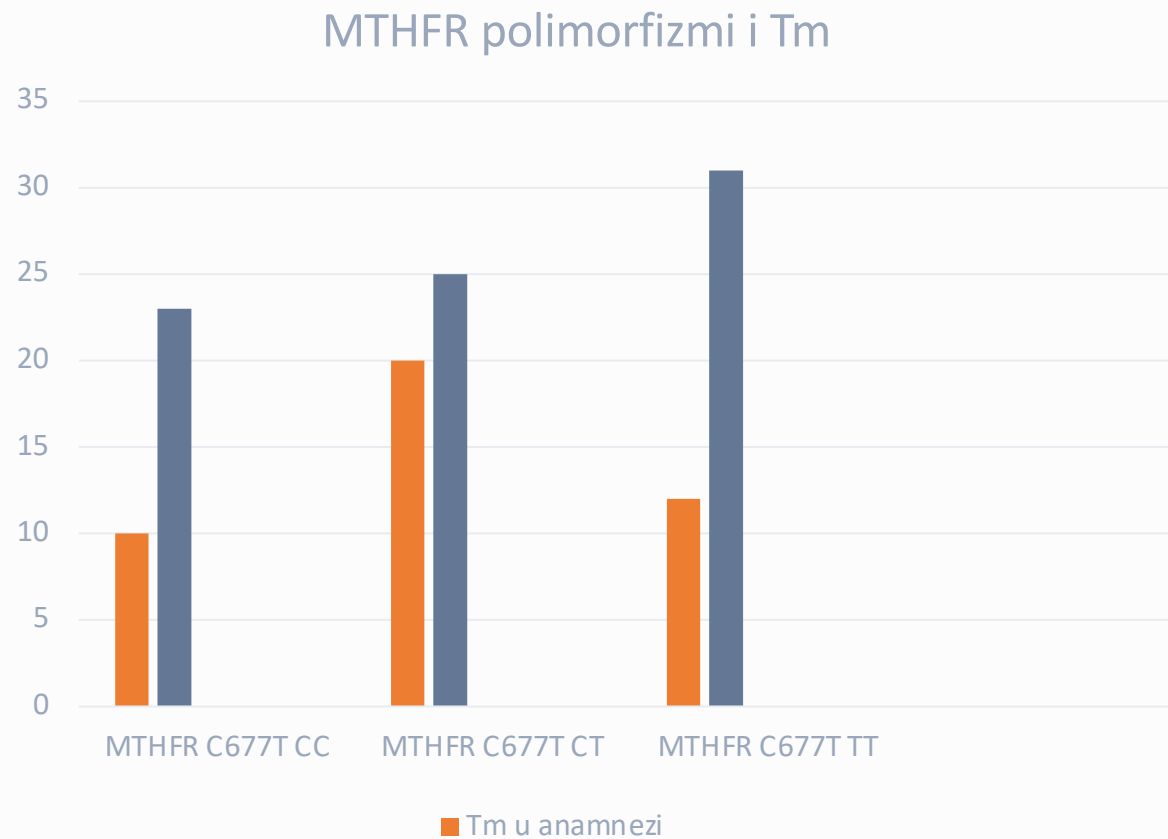
Polimorfizam MTHFR C677T i hipertenzija, dijabetes i predijabetes



Polimorfizam MTHFR C667T i kardiovaskularni incidenti



Povezanost MTHFR polimorfizma i tumora u anamnezi





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

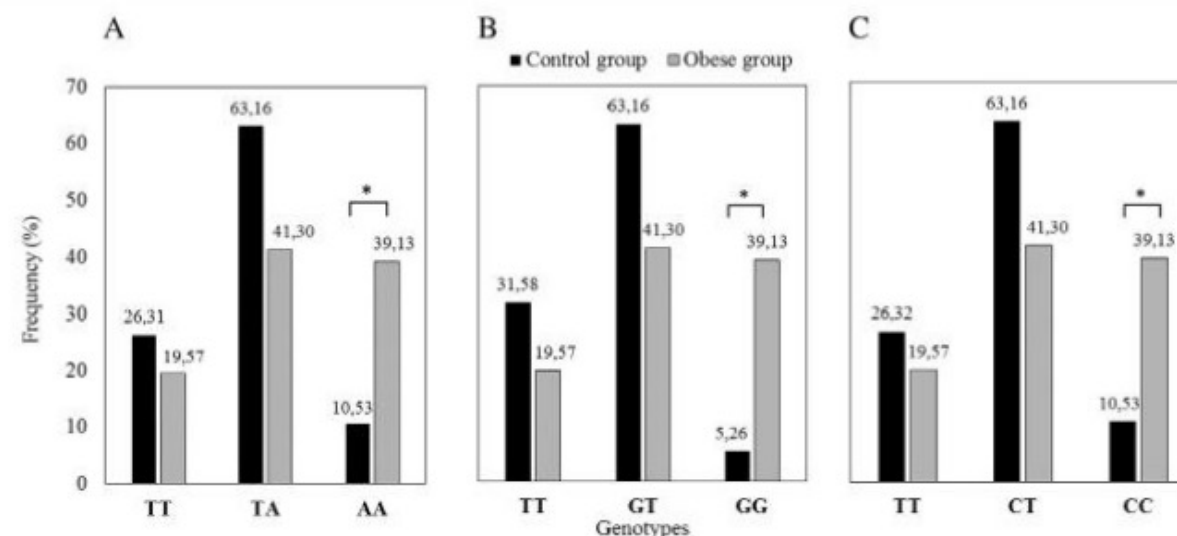
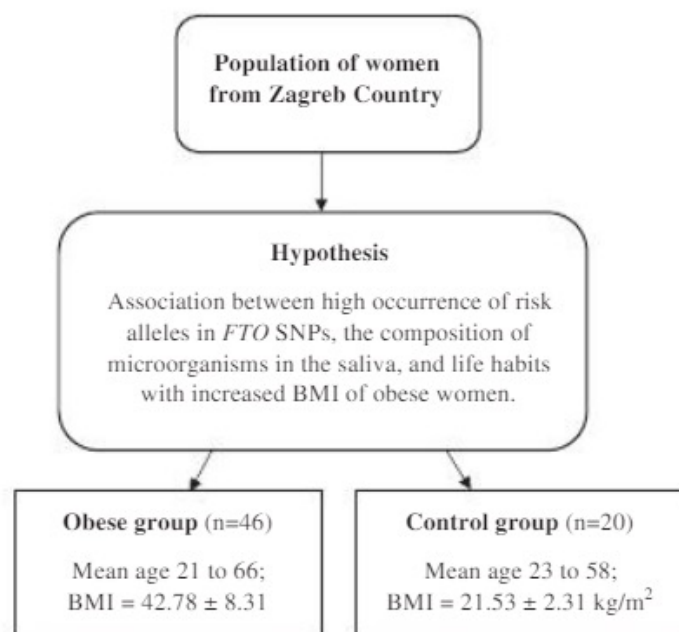
Ana Hudek^a, Lucija Škara^a, Barbara Smolkovič^a, Snježana Kazazić^b, Sanda Ravlić^b, Lucia Nanić^b, Martina Matovinović Osvatić^c, Jozo Jelčić^d, Ivica Rubelj^b, Višnja Bačun-Družina^{a,*}

^a Faculty of Food Technology and Biotechnology University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

^b Ruđer Bošković Institute, Bijenička cesta 54, 10000, Zagreb, Croatia

^c Department of Endocrinology, University Hospital Centre Zagreb, Kišpatičeva 12, 10000 Zagreb, Croatia

^d Endocrinology Practice PhD Jozo Jelčić, MD, Ulica grada Vukovara 284, 10000 Zagreb, Croatia



The figure illustrates the multifactorial etiology of obesity, showing the interplay of diet, microbiota, genetics, and lifestyle.

Top Diagram: Multifactorial Etiology

- Diet:** Represented by an icon of a steak and donut, leading to the **mikrobiota** (microbiome).
- Microbiota:** Represented by an icon of a human torso with the gut highlighted, leading to the **fenotip** (phenotype).
- Genetics:** Represented by a DNA double helix, influenced by **FTO SNPs** (FTO gene variants) and **FTO protein**. The FTO protein is shown with its chemical structure. The FTO gene is associated with the phenotype *aperit, postprandijalna glad* (hunger after eating).
- Lifestyle:** Represented by icons of a person sitting at a desk and a person in bed, both with downward arrows, indicating sedentary behavior and sleep patterns.

Bottom Left: Bacterial Abundance

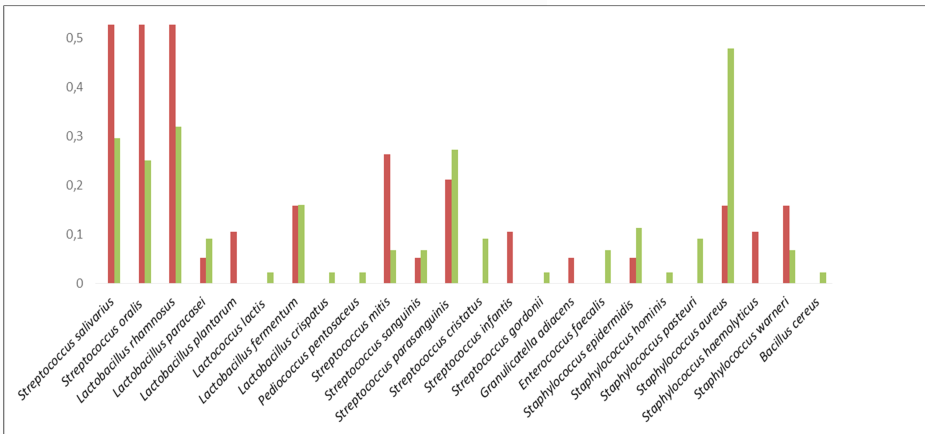
A bar chart showing the relative abundance of various bacterial species. The y-axis represents relative abundance (0 to 0.5). The x-axis lists bacterial species. Red bars indicate higher abundance, and green bars indicate lower abundance.

Bacterial Species	Relative Abundance (approx.)
<i>Streptococcus salivarius</i>	0.52
<i>Streptococcus oralis</i>	0.29
<i>Lactobacillus rhamnosus</i>	0.52
<i>Lactobacillus paracasei</i>	0.25
<i>Lactobacillus plantarum</i>	0.52
<i>Lactobacillus lactis</i>	0.05
<i>Lactobacillus fermentum</i>	0.10
<i>Pedococcus pentosaceus</i>	0.02
<i>Pedococcus pentosaceus</i>	0.16
<i>Streptococcus mitis</i>	0.02
<i>Streptococcus sanguinis</i>	0.27
<i>Streptococcus parvulus</i>	0.07
<i>Streptococcus anginosus</i>	0.07
<i>Streptococcus cristatus</i>	0.21
<i>Streptococcus infantis</i>	0.27
<i>Streptococcus infantis</i>	0.09
<i>Granulicatella adiacens</i>	0.10
<i>Enterococcus faecalis</i>	0.02
<i>Enterococcus faecalis</i>	0.05
<i>Staphylococcus epidermidis</i>	0.05
<i>Staphylococcus epidermidis</i>	0.11
<i>Staphylococcus hominis</i>	0.02
<i>Staphylococcus aureus</i>	0.09
<i>Staphylococcus aureus</i>	0.48
<i>Staphylococcus aureus</i>	0.15
<i>Staphylococcus aureus</i>	0.10
<i>Staphylococcus aureus</i>	0.07
<i>Bacillus cereus</i>	0.15
<i>Bacillus cereus</i>	0.07
<i>Bacillus cereus</i>	0.02

Bottom Right: BMI vs. Number of SNPs

A scatter plot showing BMI (kg/m²) on the y-axis (0 to 80) versus the Number of SNPs on the x-axis (0 to 3). The plot compares the **Obese group** (grey circles) and the **Control group** (black circles). The Obese group shows a higher BMI across all SNP counts, while the Control group shows a lower BMI.

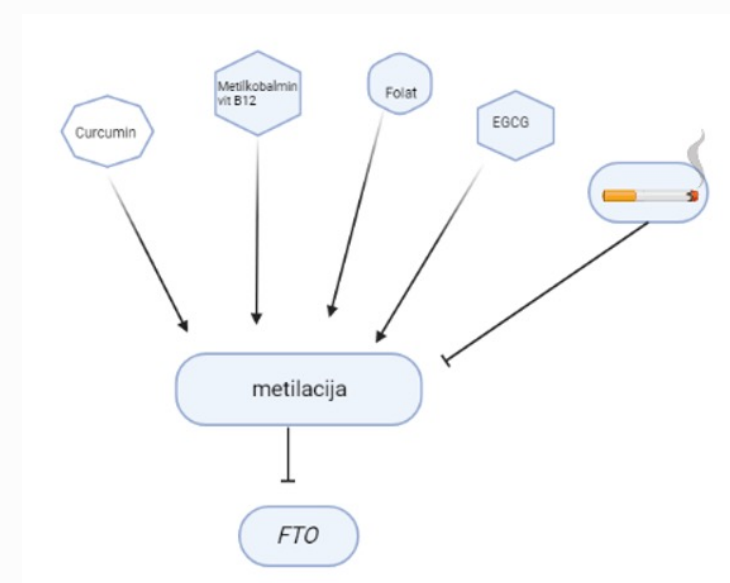
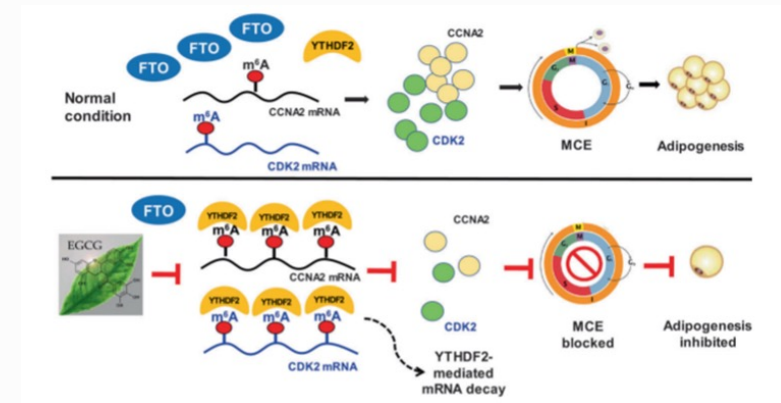
Number of SNPs	Obese group BMI (kg/m ²)	Control group BMI (kg/m ²)
0	30, 32, 35, 38, 40, 42, 45, 48, 50, 52, 55, 58, 60, 65, 68, 70, 72, 75, 78, 80	18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80
1	30, 32, 35, 38, 40, 42, 45, 48, 50, 52, 55, 58, 60, 62, 65, 68, 70, 72, 75, 78, 80	18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80
2	30, 32, 35, 38, 40, 42, 45, 48, 50, 52, 55, 58, 60, 62, 65, 68, 70, 72, 75, 78, 80	18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80
3	30, 32, 35, 38, 40, 42, 45, 48, 50, 52, 55, 58, 60, 62, 65, 68, 70, 72, 75, 78, 80	18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80



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



Yadav DK et al. Vitamin B₁₂ supplementation influences methylation of genes associated with Type 2 diabetes and its intermediate traits. Epigenomics. 2018

Ruifan Wu et al. Epigallocatechin gallate targets FTO and inhibits adipogenesis in an mRNA m⁶A-YTHDF2-dependent manner. Int J Obes (Lond). 2018

Melnik BC et al. Milk's Role as an Epigenetic Regulator in Health and Disease. Diseases. 2017 . Melnik BC. Milk: an epigenetic amplifier of FTO-mediated transcription? Implications for Western diseases. Journal of Translational Medicine. 2015

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

HVALA