

offset project CRO_A-00033 "Technology & Know-how Transfer in Metabolomics and Establishment of Latest Scientific Equipment in Zagreb"

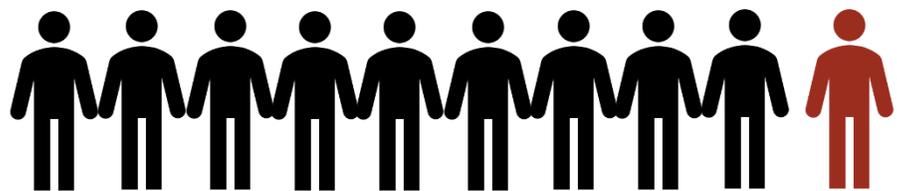


Genomski i glikanski biomarkeri
PTSP-a -GlikoGenPTSP
IP-2014-09-4289

METABOLOMSKA I GLIKOMSKA ISTRAŽIVANJA U PTSP-U

DR. SC. DUBRAVKA ŠVOB ŠTRAC, VIŠA ZNANSTVENA SURADNICA
LABORATORIJ ZA MOLEKULARNU NEUROPSIHIJATRIJU
INSTITUT RUĐER BOŠKOVIĆ

PTSP u društvu



Opća populacija 7-12%



Hrvatski branitelji 25-30%

značajno narušeno psihičko, socijalno i radno funkcioniranje

- agresivno ponašanje, zloraba alkohola i droga, suicidalnost

često prisutni i drugi psihopatološki komorbiditeti

- veliki depresivni poremećaj, anksiozni poremećaji, panični poremećaj, poremećaj ličnosti, alkoholizam, suicidalnost...

često prisutni somatski komorbiditeti

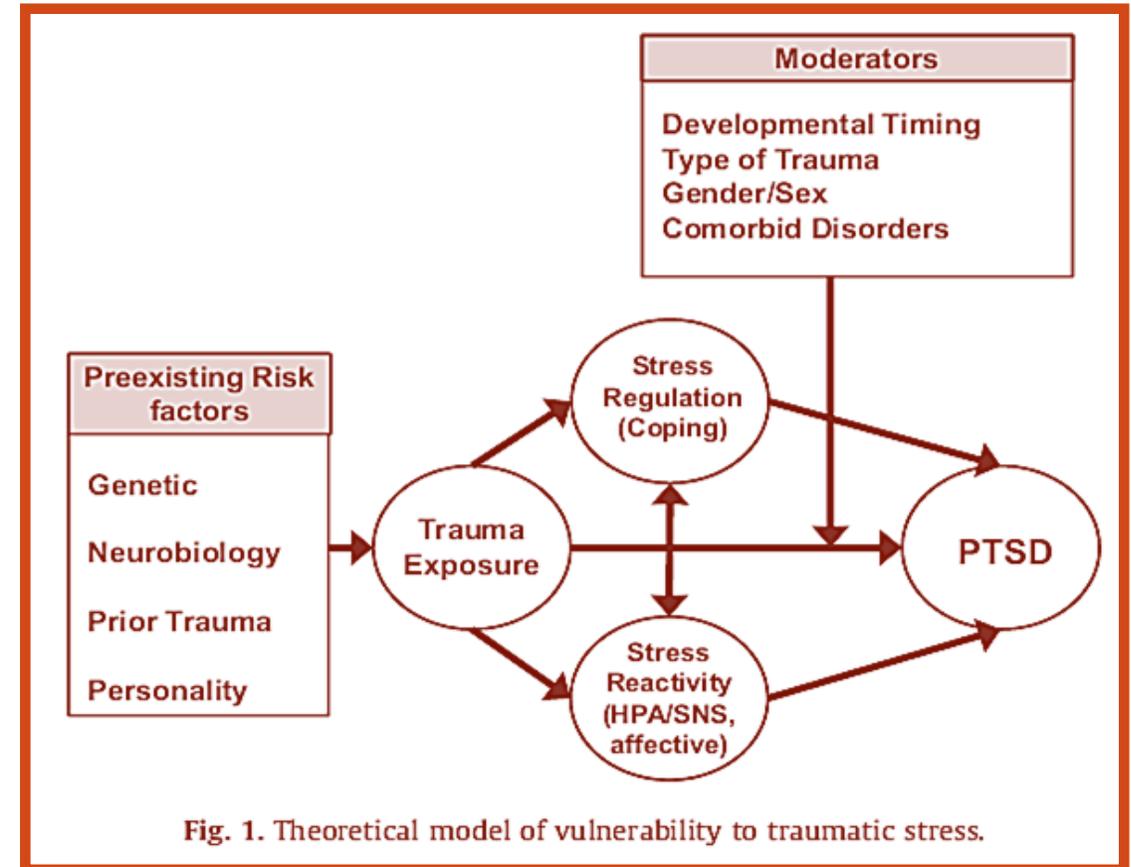
- kardiovaskularne bolesti, koronarna bolest srca, endokrine bolesti, metabolički sindrom, respiratorne bolesti, probavni poremećaji

40% oboljelih od PTSP-a ne pokazuje dobar odgovor na terapiju



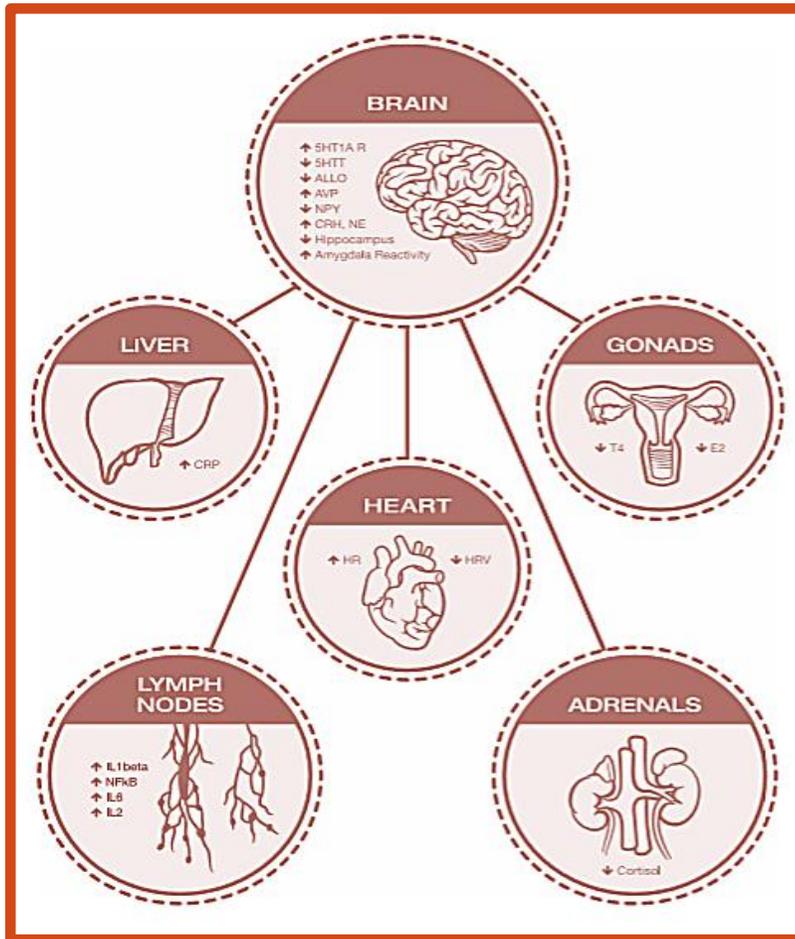
Rizični čimbenici za razvoj PTSP-a

- doživljaj traume
- težina i vrsta traume
- spol
- komorbidne bolesti
- genetski i epigenetski čimbenici
- neurobiološki i psihološki čimbenici
- okolišni čimbenici
- izloženost ranoj traumi



Neurobiološke i neuroendokrinološke promjene u PTSP-u

- kao odgovor na traumu amigdala stimulira simpatički živčani sustav i os HHN

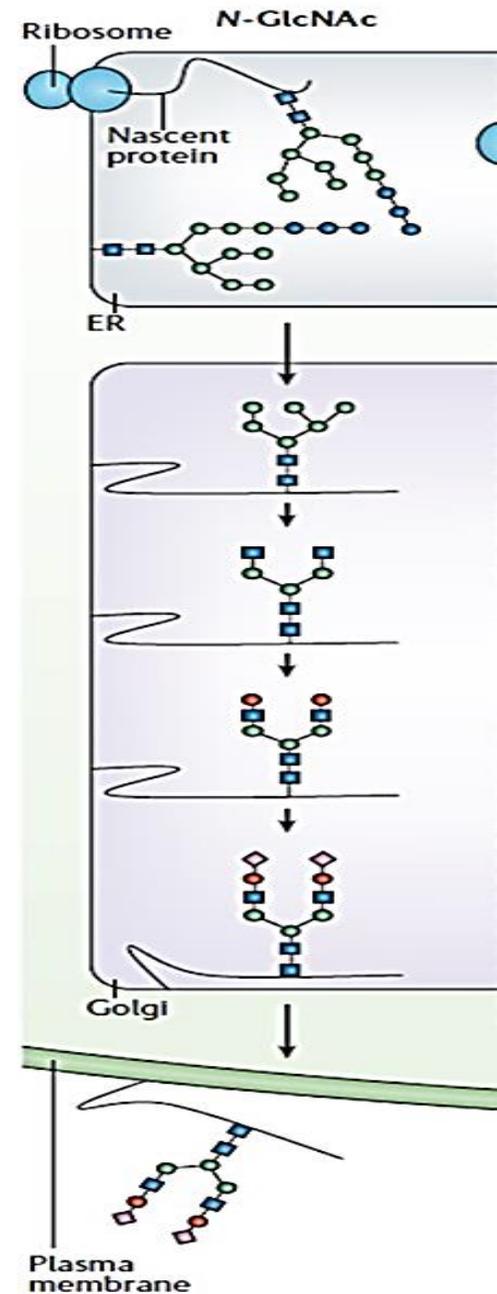
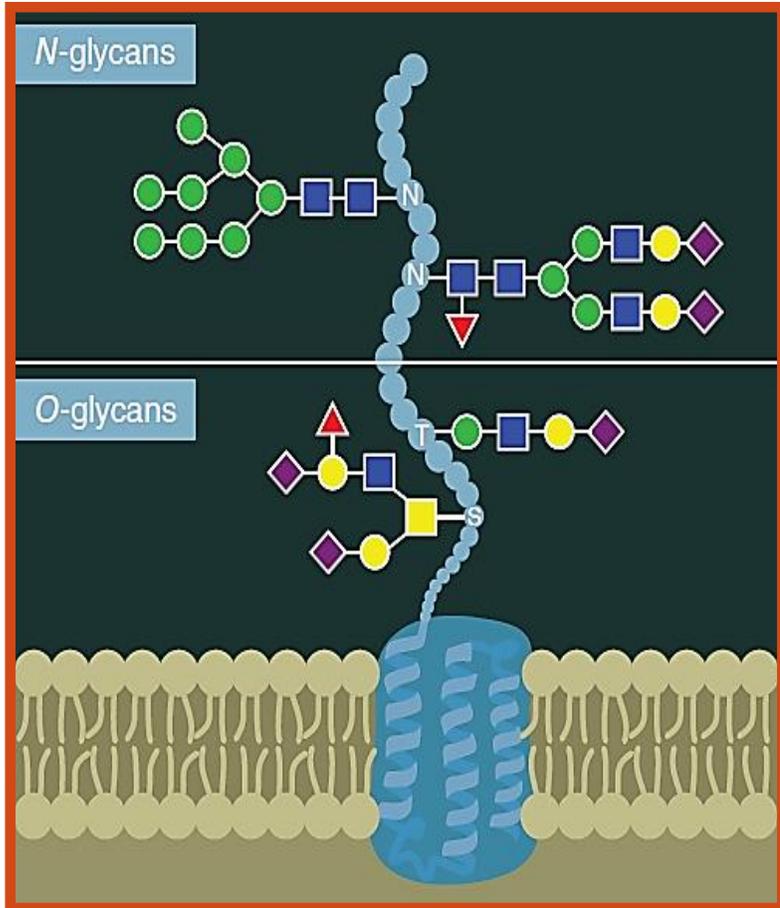


Poremećena neuroendokrinologija i os HHN

- ↑ CRF
- paradoksalno ↓ aktivnost osi HHN
- ↓ kortizol u 24h urinu
- kortizol u serumu
- hipersupresija nakon DST-a
- ↓ mehanizam negativne povratne sprege
- ↑ broj receptora za GK u limfocitima
- ↓ volumen hipokampusa
- teškoće pamćenja
- poremećaji neurotransmisije

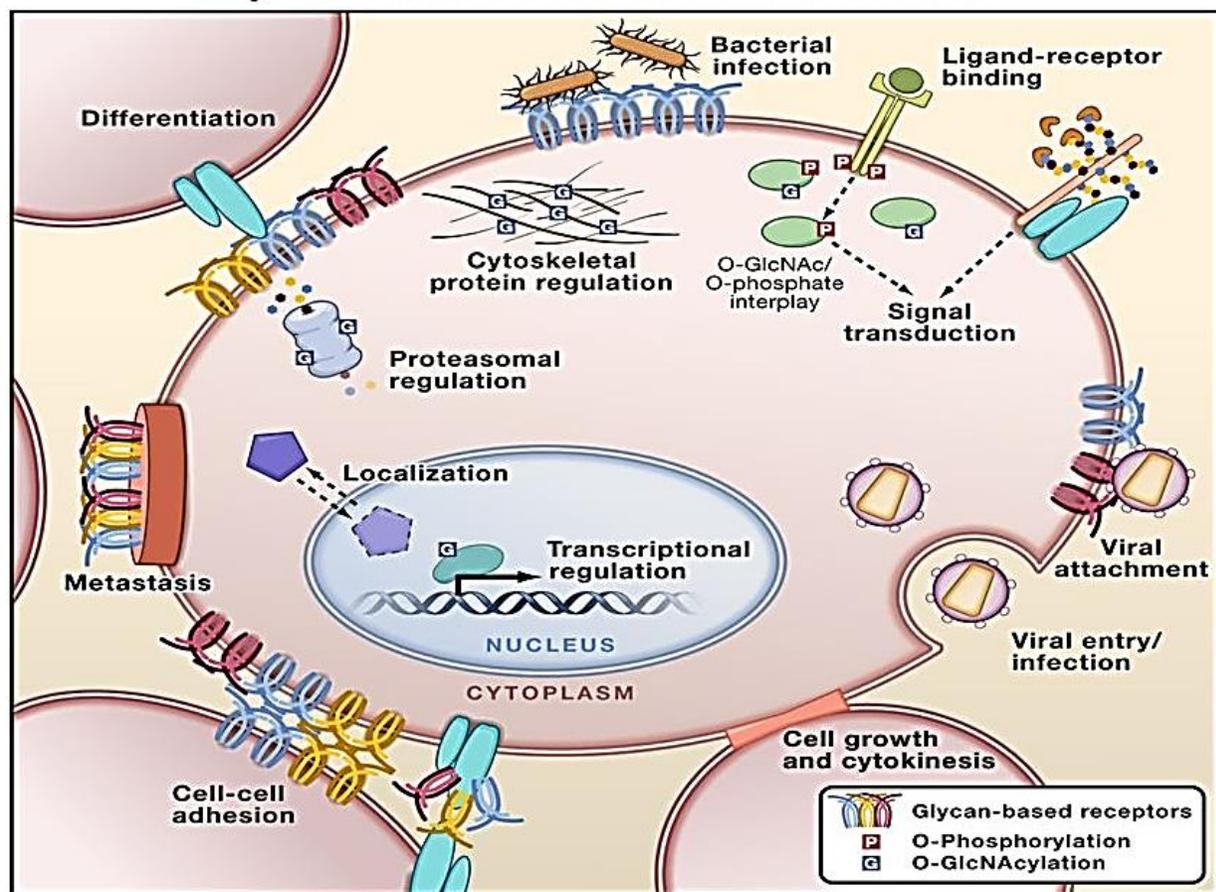
Glikani

- oligosaharidni lanci kovalentno vezani na polipeptide i lipide
- mijenjaju fizikalno-kemijska svojstva i biološku ulogu proteina
- modifikacija proteina u endoplazmatskom retikulumu i Golgijevom aparatu
- poveznica genetičke upute i okolišnih čimbenika → adaptacija
- N-glikani i O-glikani



Gotovo sve interakcije na površini stanice uključuju glikane

Glycans Permeate Cellular Functions



Cell 143, 672-676

Uloga glikana u:

- međustaničnoj komunikaciji
- signalizaciji
- smatanju proteina
- regulaciji ekspresije gena
- diferencijaciji
- razvoju
- upalnim procesima
- metastaziranju...

Promjene u glikozilaciji

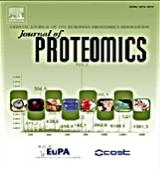
➤ u neuropsihijatrijskim poremećajima: ADHD, Alzheimerovoj bolesti, autizmu, shizofreniji...

JOURNAL OF PROTEOMICS 75 (2012) 5123–5139

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Review

Glycomics of pediatric and adulthood diseases of the central nervous system

Rita Barone^{a, 1}, Luisa Sturiale^{b, 1}, Angelo Palmigiano^b, Mario Zappia^c, Domenico Garozzo^{b,*}

Molecular & Cellular Proteomics 10.1

Research

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This paper is available on line at <http://www.mcponline.org>

Human Plasma Glycome in Attention-Deficit Hyperactivity Disorder and Autism Spectrum Disorders*

Nela Pivac^{‡, d}, Ana Knežević^{§, d}, Olga Gornik[§], Maja Pučić[¶], Wilmar Igl^{||}, Hilde Peeters^{**}, An Crepel^{**}, Jean Steyaert^{**}, Mislav Novokmet[¶], Irma Redžić[§], Matea Nikolac[‡], Vesna Novković Hercigonja^{‡‡}, Katarina Dodig Čurković^{§§}, Mario Čurković^{¶¶}, Gordana Nedić[‡], Dorotea Muck-Seler[‡], Fran Borovečki^{|||}, Igor Rudan^{ab} and Gordan Lauc^{¶¶}

Alzheimer's Disease

Serum: decrease of digalactosylated core- α -1,6-fucosylated biantennary glycan. Increase of α 1,3-fucosylated trigalactosylated glycans. [patent application publication, May 5, 2011].

CSF: altered levels of full-length Reelin and Reelin 180 kDa fragment. Abnormal glycosylation pattern of 180-kDa reelin [26]. Increase of sialylated O-glycans in Tyr10 of APP/A β glycopeptides [23]. Decrease of TTR brain-specific isoform [76]. Quantitative changes of apolipoprotein E, clusterin, α -1- β -glycoprotein and α -1-AAT. Decreased glycosylation of one specific α -1-antitrypsin isoform [80,82]. Decrease of WGA-reactive Tf glycoforms [88,89].

Idiopathic normal pressure hydrocephalus

CSF: increase of Tf-2 (serum type)/Tf-1 (brain-type) glycoforms ratios

Multiple sclerosis

Serum: modification of plasma acute-phase proteins glyco-isoforms

Schizophrenia

Serum: increase in male patients of tetraantennary tetrasialylated glycans bearing polylactosamine with A4G4LacS4 extension and triantennary trisialylated containing the SLe^x epitope [105].

CSF: decrease of bisecting and sialylated glycans levels.

ADHD Plasma: \uparrow in glycan groups GP11 and DG7 and \downarrow in GP12

Serum: increased antennary fucosylation of biantennary glycans and decreased levels of some complex glycans with three or four antennas

N-glikani u PTSP-u

OPEN

Citation: *Transl Psychiatry* (2013) **3**, e320; doi:10.1038/tp.2013.93
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www.nature.com/tp



ORIGINAL ARTICLE

N-glycosylation profiling of plasma provides evidence for accelerated physiological aging in post-traumatic stress disorder

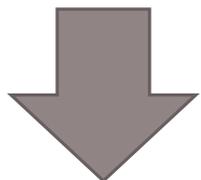
M Moreno-Villanueva^{1,7}, J Morath^{2,7}, V Vanhooren^{3,4}, T Elbert², S Kolassa⁵, C Libert^{3,4}, A Bürkle¹ and I-T Kolassa^{2,6}

- testirali 9 N-glikanskih struktura iz plazme na 13 ispitanika s PTSP-om, 9 ispitanika koji su bili izloženi traumi i 10 kontrolnih ispitanika
- koncentracija N-glikana se mijenja s dobi

GlycoAge Test: = $\log_{10}(\text{FA2}/\text{FA2G2})$
daje predodžbu o stvarnoj dobi ispitanika



PTSP

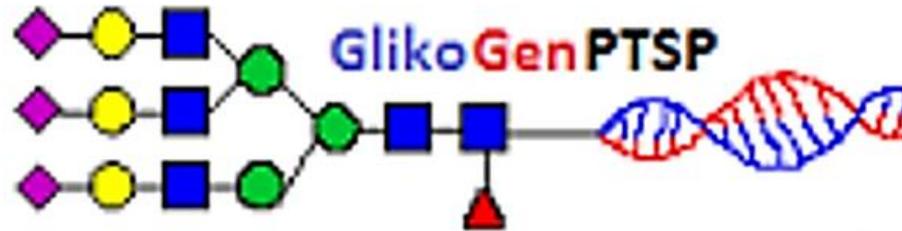


Peak1=FA2 - agalactosylated core-a-1,6-fucosylated biantennary

Peak6=FA2G2 - bigalactosylated core-a-1,6-fucosylated biantennary; NA2F

HrZZ projekt : Genomski i glikanski biomarkeri PTSP-a

GlikoGenPTSP (voditelj: prof. dr. Nela Pivac)



Cilj istraživanja:

- ispitati razlike u glikozilaciji kod osoba s dijagnosticiranim PTSP-om (N=350) i zdravih kontrolnih ispitanika (N=350) određivanjem N-glikana u plazmi i vezanih na IgG
- ispitati postoji li povezanost određenih genetskih polimorfizama i epigenetskih modifikacija s glikomskim promjenama i shodno tome povećanim rizikom za razvoj PTSP-a

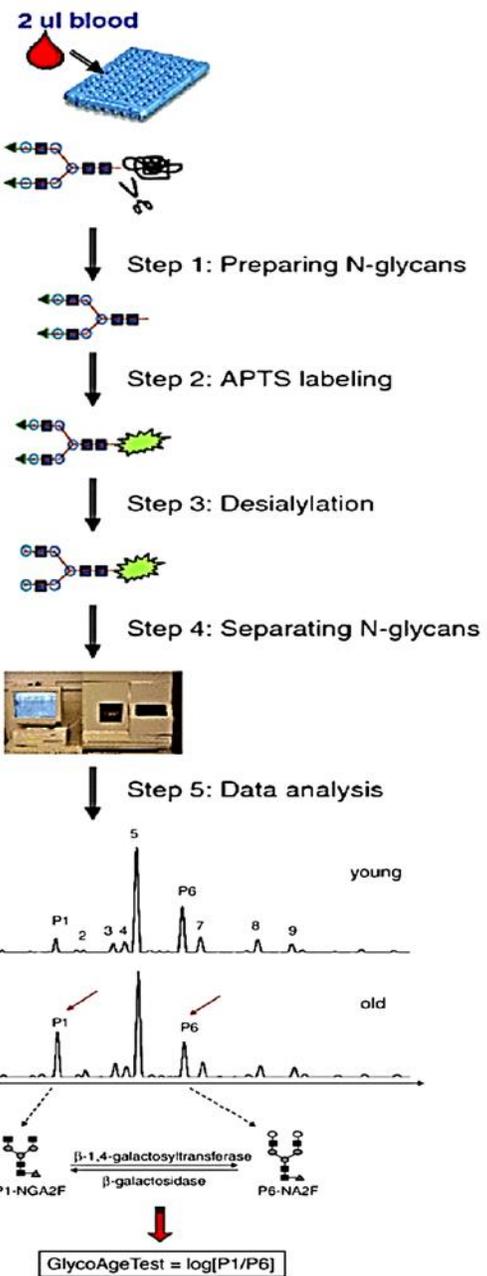
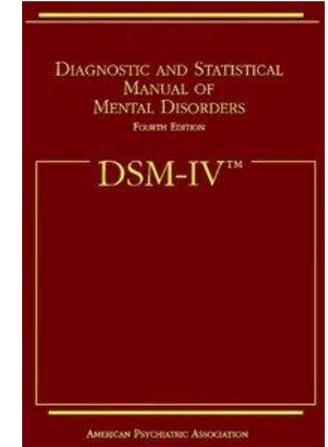
Materijali i metode

Ispitanici: Klinika za psihijatriju Vrapče, Zagreb

- osobe s PTSP-om (N=204): DSM-IV (SCID, CAPS)
- zdrave kontrole (N=134)
- ispitanici su muškarci (prosječna dob: 56 god.) hrvatskog podrijetla, koji nisu u međusobnom srodstvu

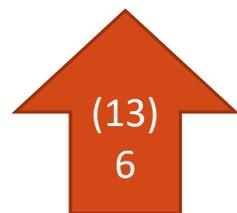
Obrada N-glikana: Laboratorij za glikobiologiju, Genos d.o.o, Zagreb

- visokotlačna tekućinska kromatografija (HPLC) fluorescentno obilježenih N-glikana za kvantitativnu analizu glikoma u plazmi i vezanog za IgG
- naša studija obuhvaća 39 N-glikanskih pikova iz plazme i 24 N-glikanska pika vezana za IgG



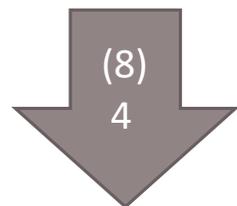
Rezultati

N-glikani u plazmi:

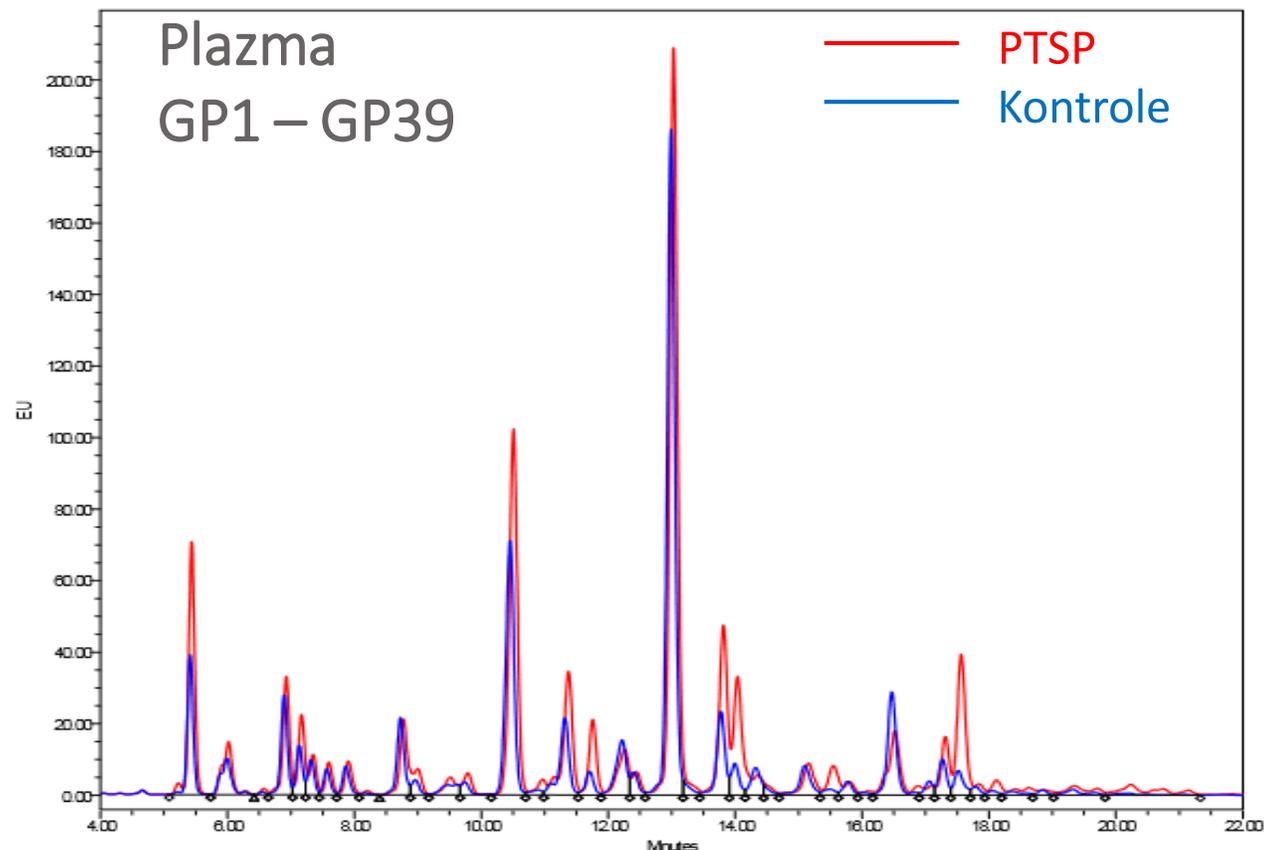


GP14, GP15, GP26, GP27, GP30,
GP32, GP33, GP34, GP35, GP36,
GP37, GP38, GP39

PTSP



GP4, GP5, GP10, GP13, GP16,
GP19, GP22, GP29



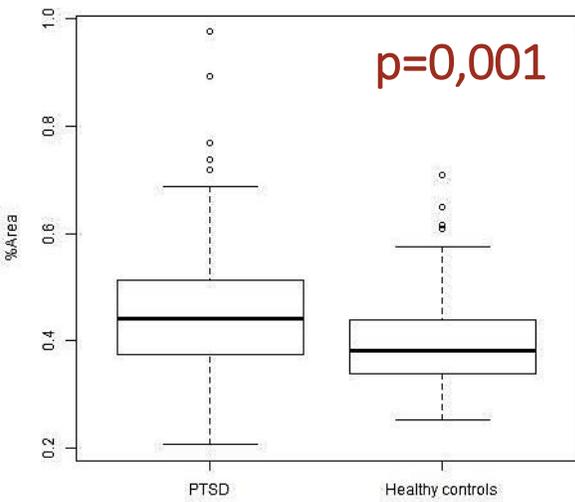
- snažan utjecaj dobi na razinu N-glikana → korekcija ($p=0,05$; korekcija na višestruko testiranje: $p=0,001^*$)

Rezultati

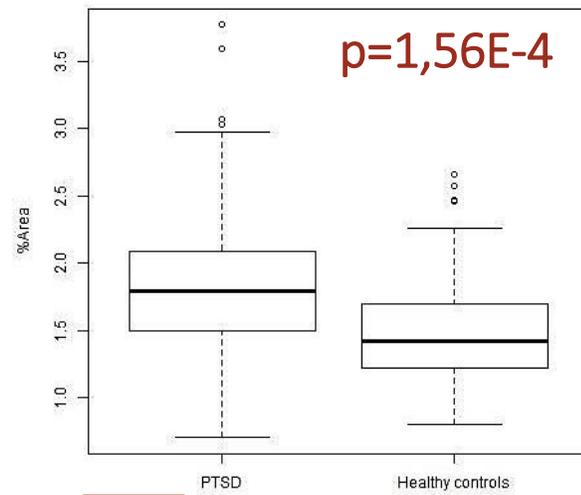
N-glikani u plazmi:



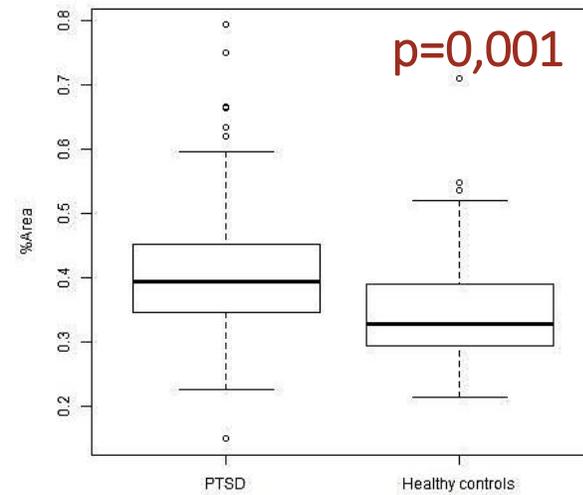
Triantenarni i tetraantenarni sijalizirani N-glikani povećani kod upalnih procesa (Gudelj et al. 2016) i hepatocelularnog karcionoma (Vanhooren et al. 2009)



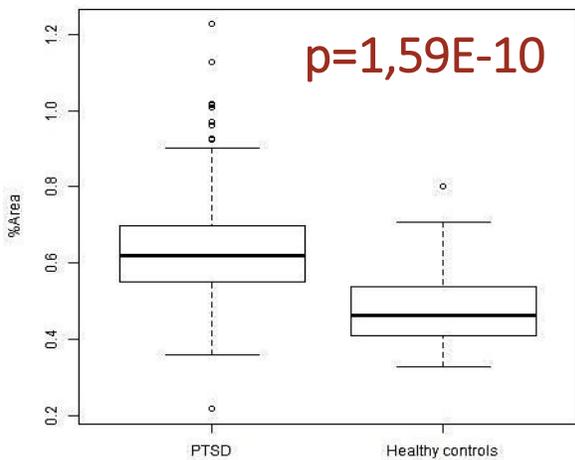
GP15 = A2BG2S1 - biantennary bigalactosylated and sialylated with bisecting GlcNAc



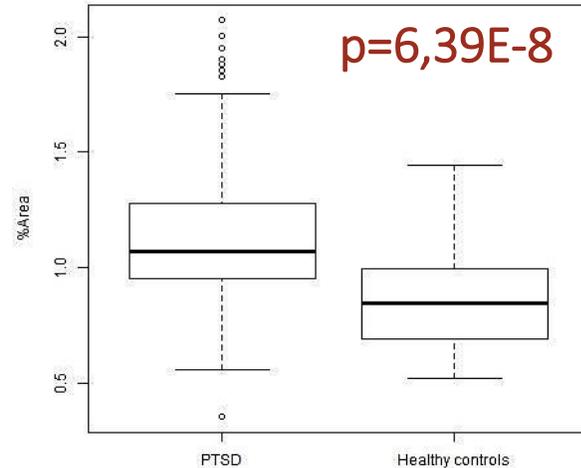
GP32 = A3F1G3S3 – triantenarny, trigalactosylated and trisialylated with antennary fucose



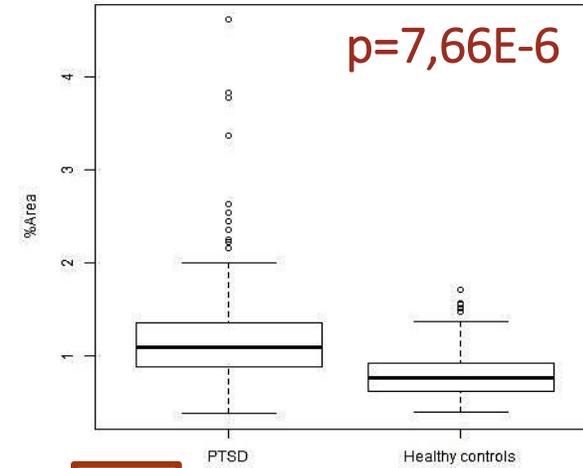
GP34 = A4G4S3 - tetraantenarny, tetragalactosylated and trisialylated



GP36 = A4S4 - tetraantenarny tetragalactosylated and tetrasialylated



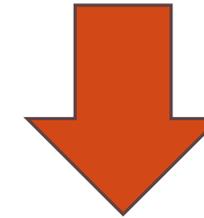
GP38 = A4G4S4 - tetraantenarny, tetragalactosylated and tetrasialylated



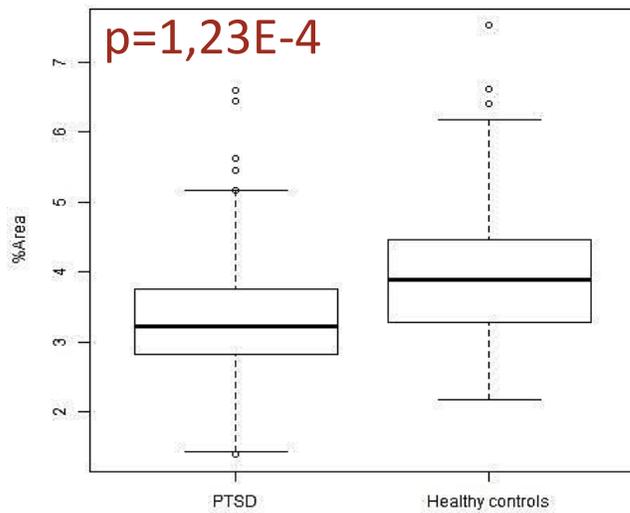
GP39 = A4F1G4S4 - tetraantenarny, tetragalactosylated and tetrasialylated with antennary fucose

Rezultati

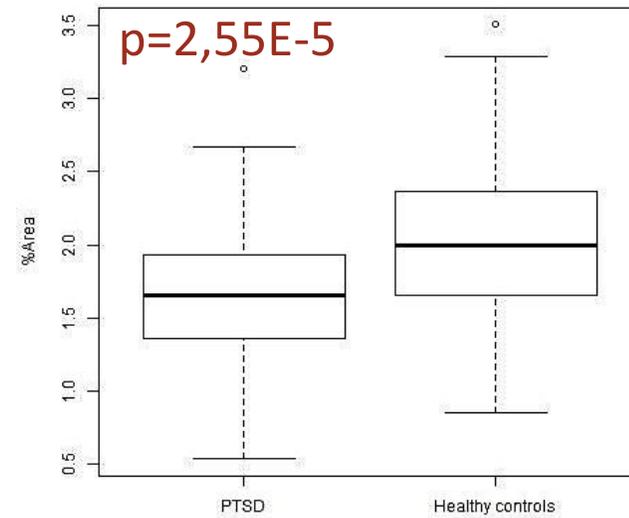
N-glikani u plazmi:



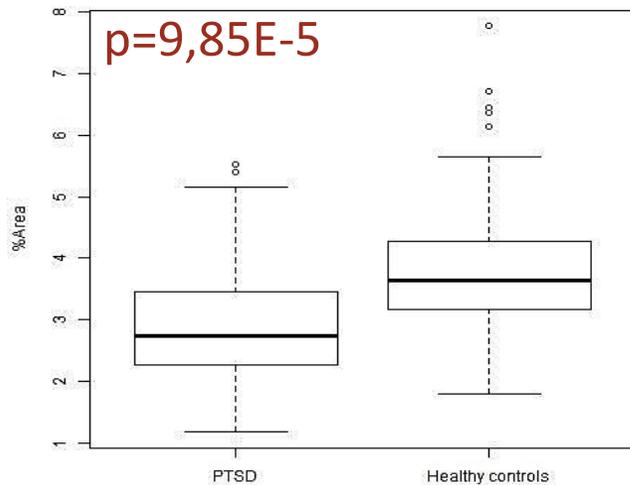
GP10 = FA2G2, vrijednost ovog N-glikana se drastično snižava s dobi



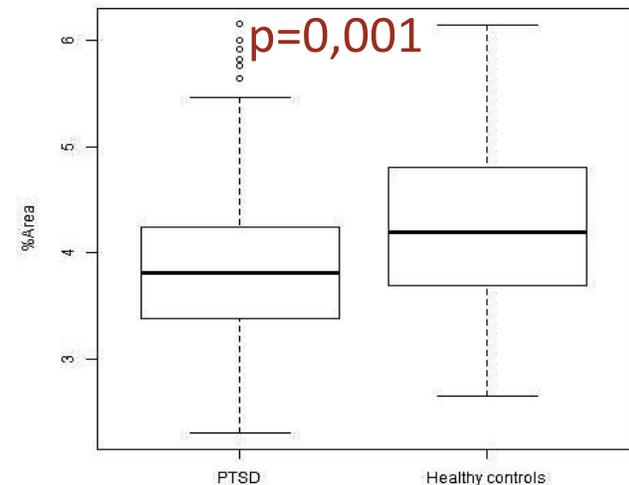
GP4 = FA2[6]BG1 -biantennary monogalactosylated with core fucose and bisecting GlcNAC



GP5 = FA2[3]BG1 -biantennary monogalactosylated with core fucose and bisecting GlcNAC



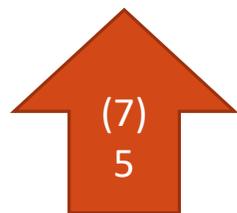
GP10 = FA2G2 -biantennary digalactosylated with core fucose



GP22 = FA2G2S2 -biantennary digalactosylated and disialyted with core fucose

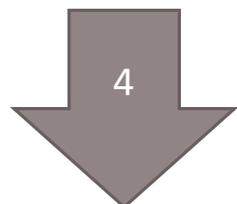
Rezultati

N-glikani vezani za IgG:

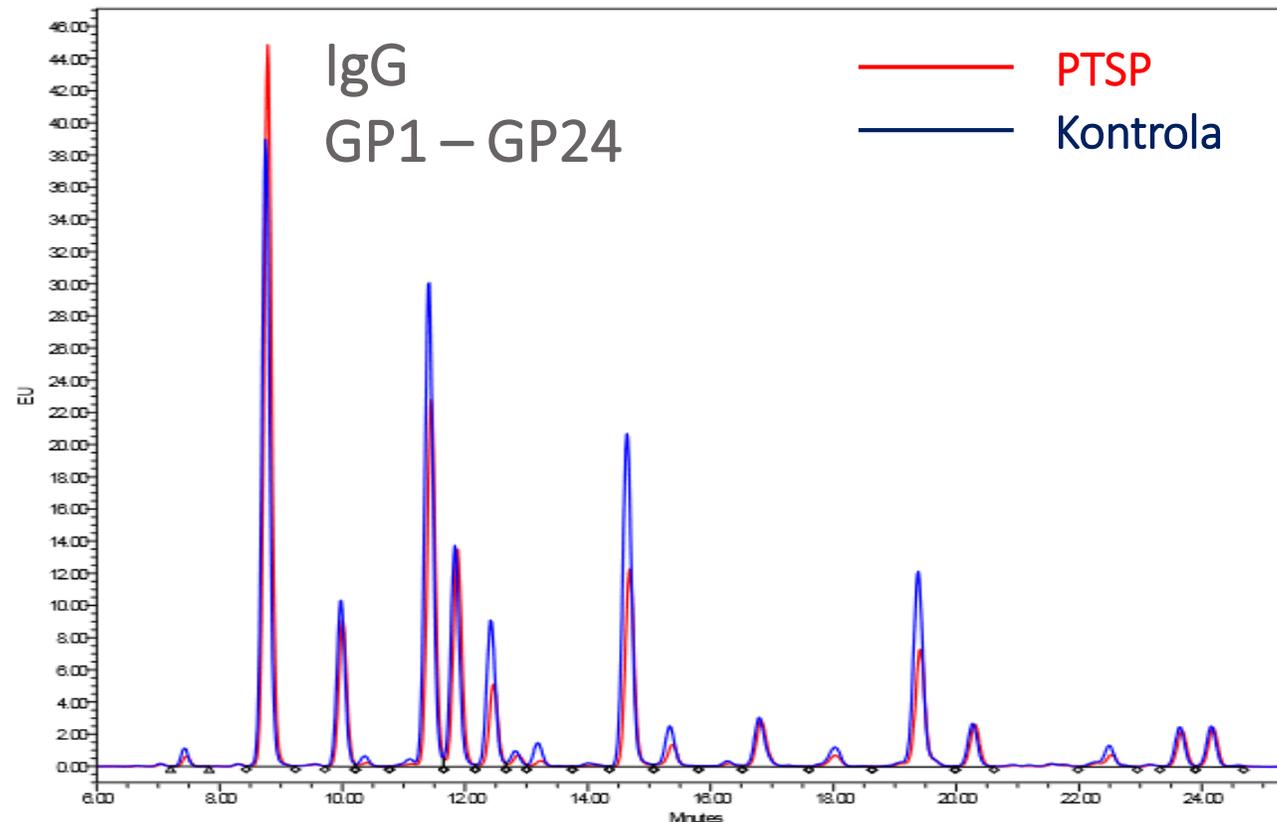


GP3, GP5, GP6, GP7, GP13,
GP17, GP22

PTSP



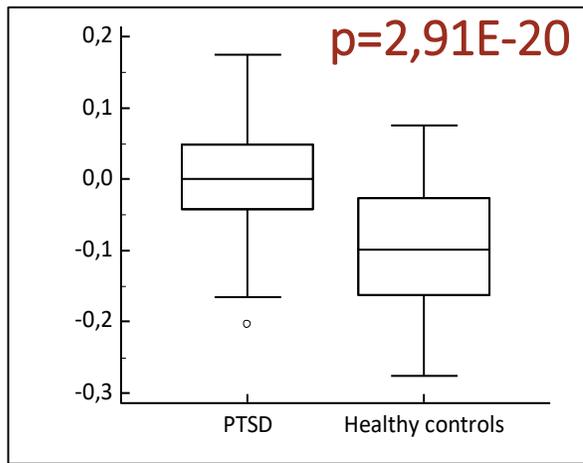
GP9, GP14, GP18, GP2



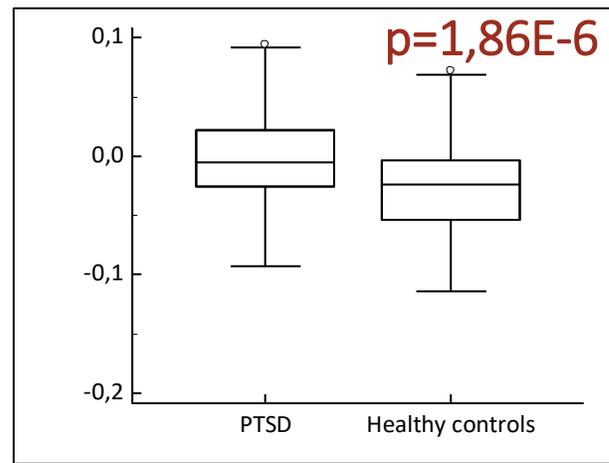
- snažan utjecaj dobi na razinu N-glikana → korekcija ($p=0,05$; korekcija na višestruko testiranje: $p=0,001^*$)

Rezultati

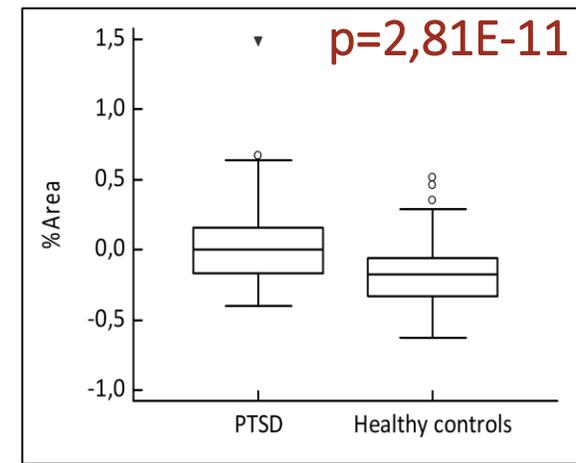
N-glikani vezani za IgG:



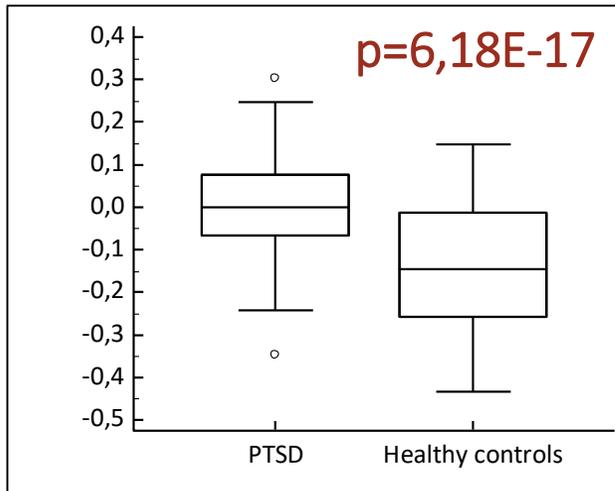
GP3 = A2B- Biantennary agalactosylated with bisecting GlcNAC



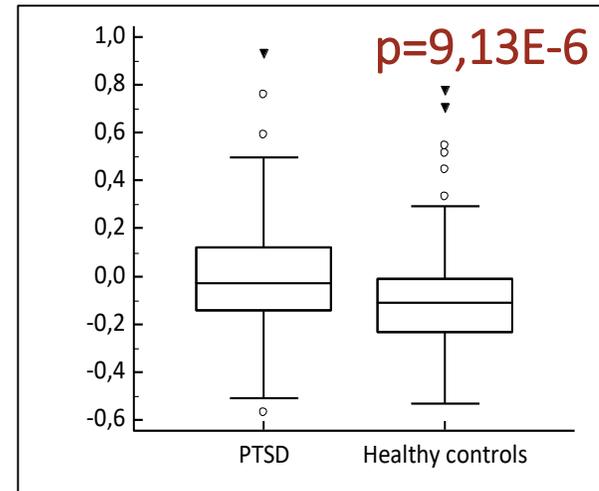
GP5 = M5 - High mannose



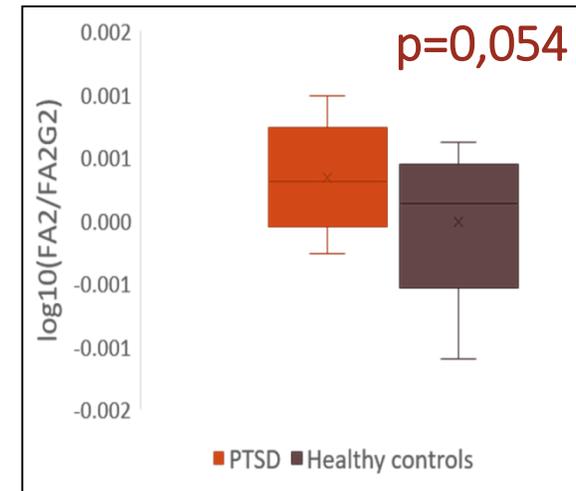
GP7 = A2G1 - Biantennary monogalactosylated



GP13 = A2BG1 - Biantennary digalactosylated with bisecting GlcNAC



GP17 = A2G2S1 - Biantennary digalactosylated and monosialylated

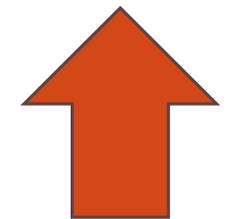


GlycoAge test

GlycoAge test

$$= \log_{10}(FA2/FA2G2)$$

$$= \log_{10}(GP4/GP14)$$



fiziološka dob
ispitanika s PTSP-om

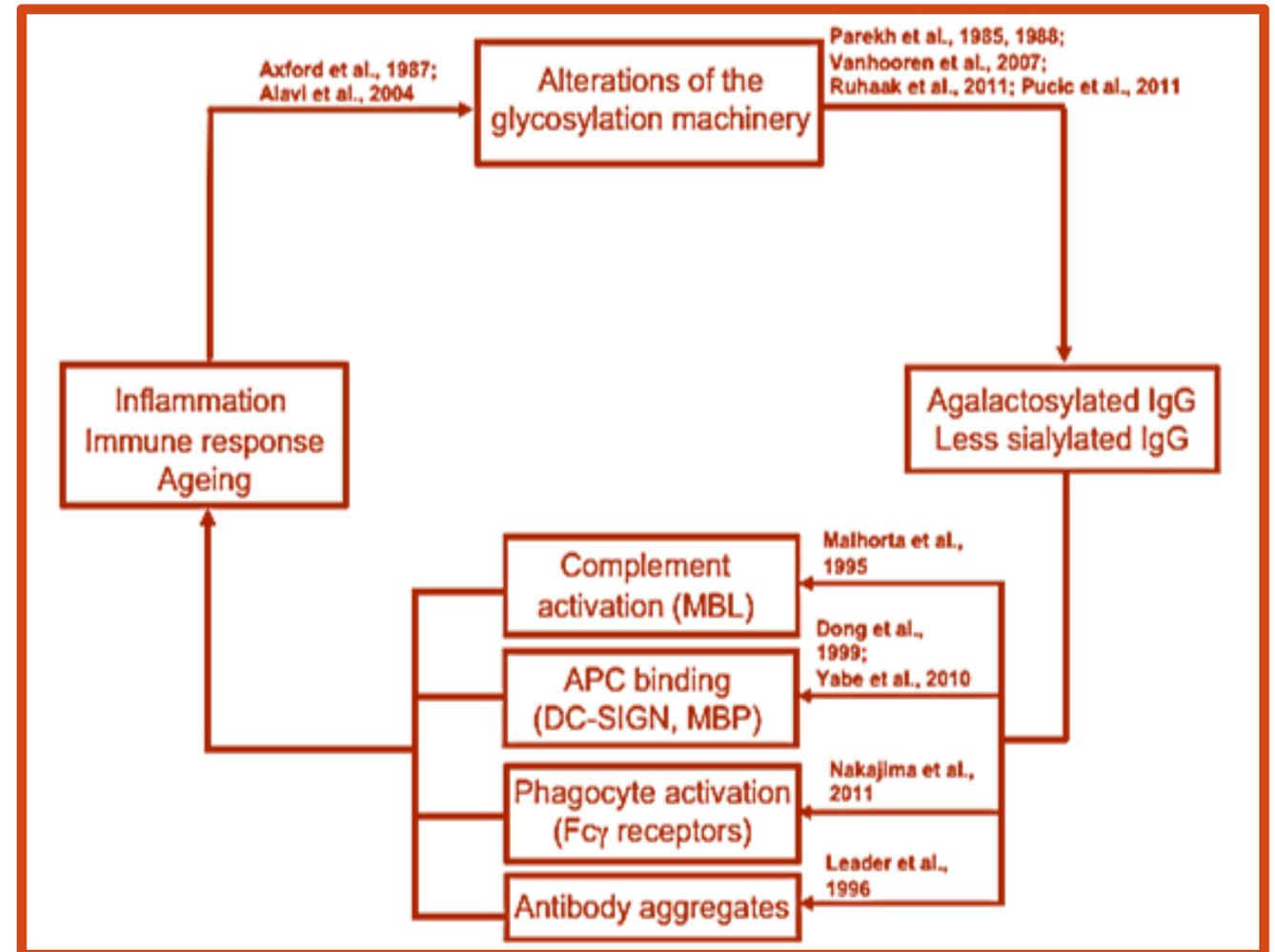
Zaključak

osobe oboljele od PTSP-a imaju statistički značajno:

- povišene razine N-glikana povezanih s upalnim procesima (Kaneko et al. 2006; Shibata-Koyama et al. 2009)
- povišene razine N-glikana povezanih s preuranjenim starenjem (Knezevic et al. 2009, Dall'Olio et al. 2012)

osobe oboljele od PTSP-a imaju:

- veću vrijednost GlycoAge testa (trend! ↑ grupe)



Daljnji ciljevi istraživanja

- ispitati razine N-glikana na preostalim uzorcima PTSP-a i kontrola
- istražiti povezanost razine N-glikana i pojedinih fenotipova PTSP-a (agitacija, psihoza...)
- u bolesnika s PTSP-om i kontrolnih ispitanika potvrditi polimorfizme (12) odabranih gena (6) (FUT8, HNF1A, FUT6/FUT3, SLC9A9, MGAT5 i B3GAT1) koji reguliraju glikozilaciju proteina, određenih GWAS studijama
- procijeniti utjecaj epigenetske regulacije (DNA metilacije) i genske ekspresije HNF1A u bolesnika s PTSP-om i kontrolnih ispitanika
- ispitati nove modele predviđanja stvarne dobi na temelju koncentracije pojedinih N-glikana u plazmi i N-glikana vezanih za IgG
- istražiti promjene N-glikana u animalnim modelima PTSP-a (prof. dr. sc. Dora Zelena, Mađarska akademija znanosti)
- neovisna replikacijska studija (prof. dr. sc. Tanja Jovanovic, Emory University, USA) na 200 ispitanika s PTSP-om (muškarci i žene, civilne žrtve traume-nasilje, silovanja, nesreće...) i 200 zdravih osoba uključenih u Grady Trauma Project (crna i bijela rasa, hispano populacija)

offset project CRO_A-00033

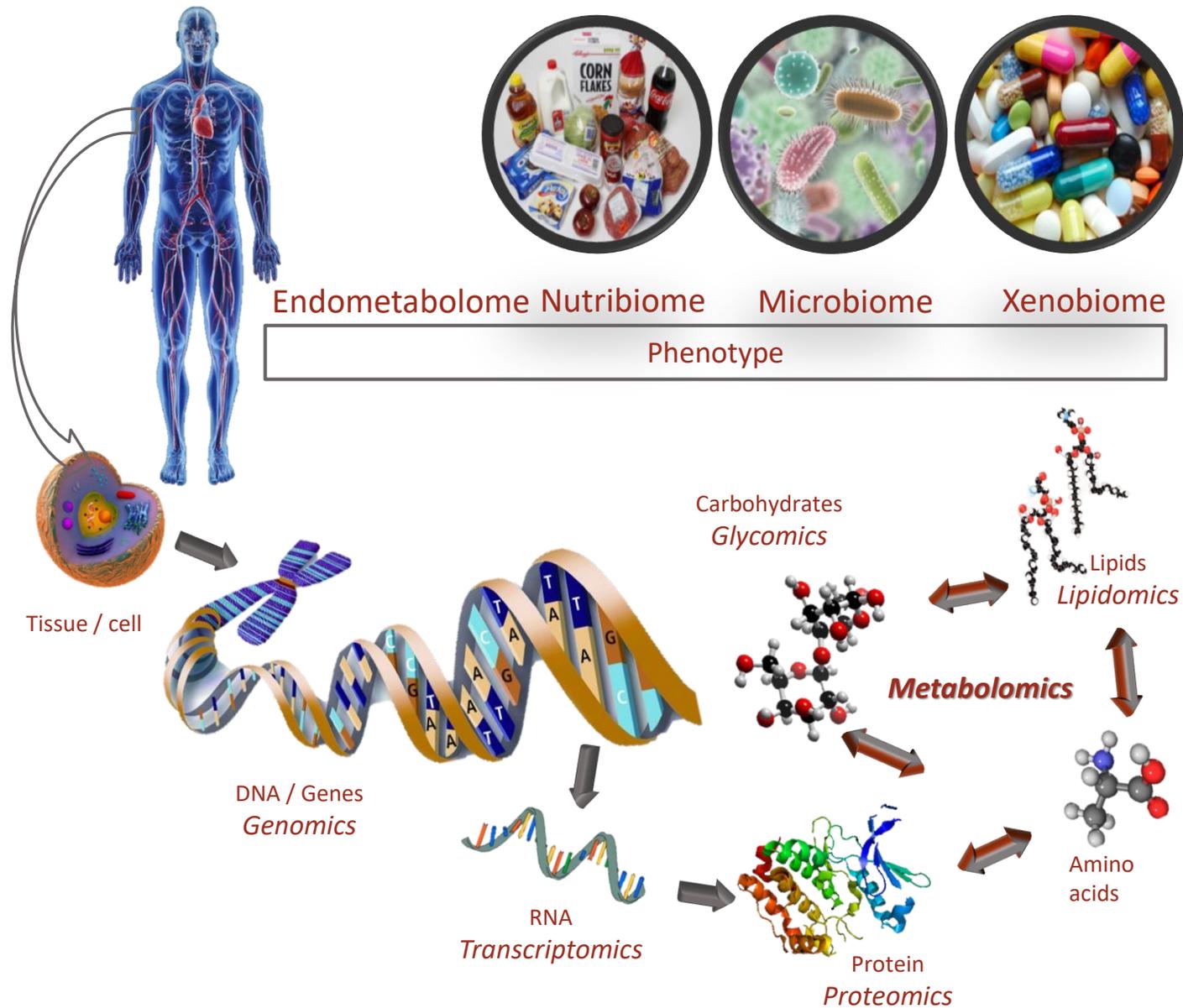
"Technology & Know-how Transfer in Metabolomics and Establishment of Latest Scientific Equipment in Zagreb",
financiran od tvrtke Patria, Finska
(voditelj: prof. dr. Neven Žarković)

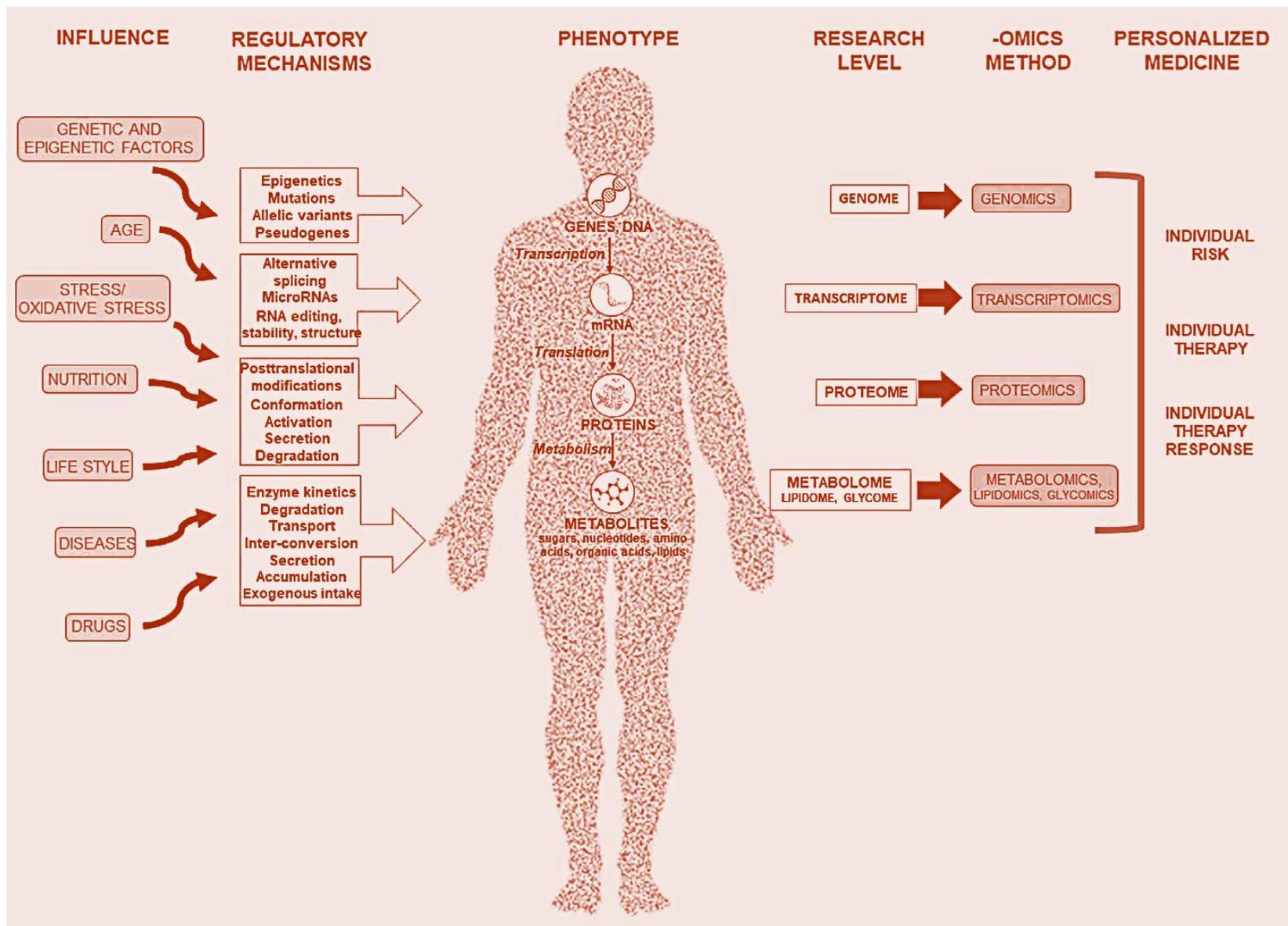
Cilj istraživanja

- ispitati razlike u metabolitima kod osoba s dijagnosticiranim PTSP-om (N=350) i zdravih kontrolnih ispitanika (N=350) primjenom neciljane višeplatformske analize metaboloma

Metabolomika

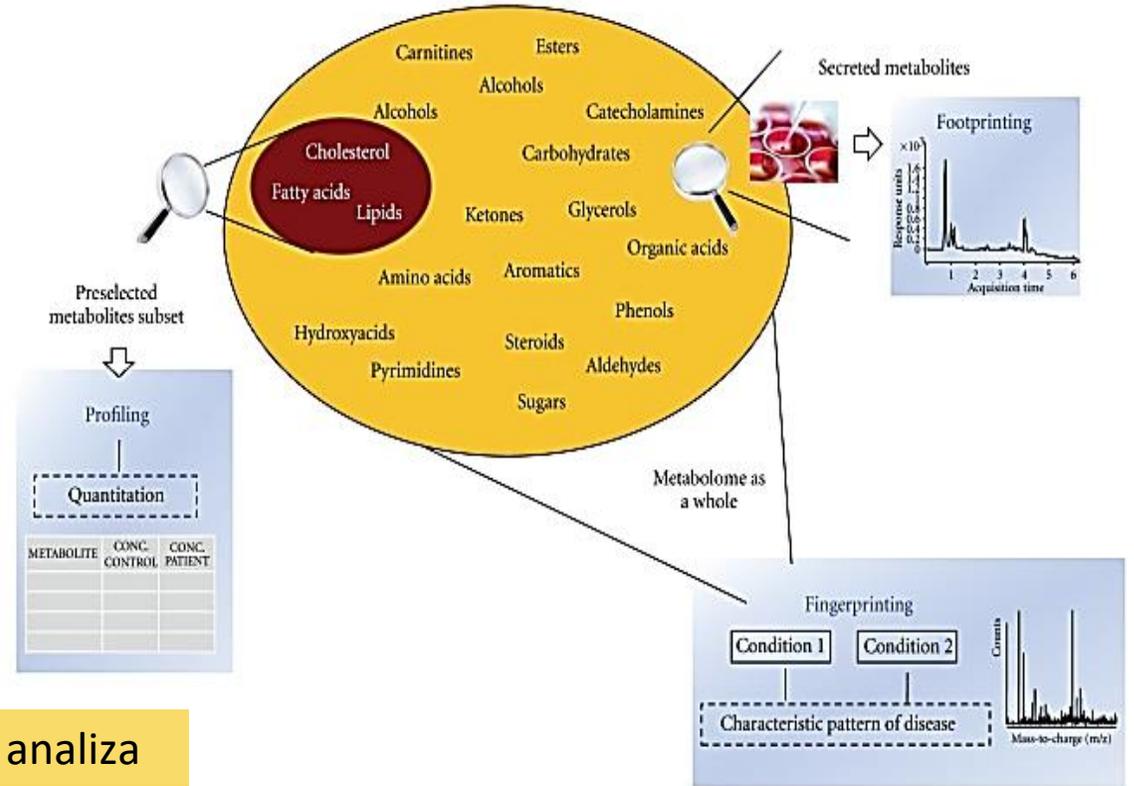
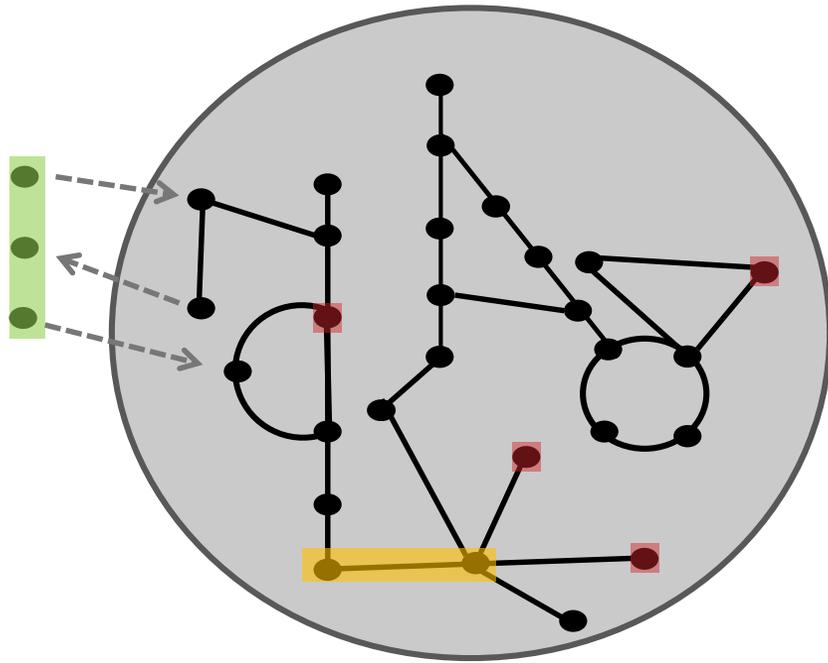
- proučava metabolom- niz spojeva niskih molekularnih težina (metabolita npr. nukleotidi, ugljikohidrati, aminokiseline, lipidi...) koji su rezultat ili su uključeni u metaboličke procese važne za normalno funkcioniranje stanice i cijelog organizma
- metabolički biomarkeri iz urina, krvi i sl. - važni u razjašnjavanju mehanizama bolesti, predviđanju odgovora na lijekove ili prehrambene intervencije ili toksičnih učinaka





- razine metabolita - vrlo osjetljive na genetske, epigenetske i okolišne čimbenike npr. dob, stres, bolest, liječenje, prehrambeni status...
- metabolomika - nadopunjuje "starija" - omics polja kao što su transkriptomika, proteomika i genomika
- personalizirani pristup u medicini

Pristupi u metabolomici: neciljana i ciljana analiza



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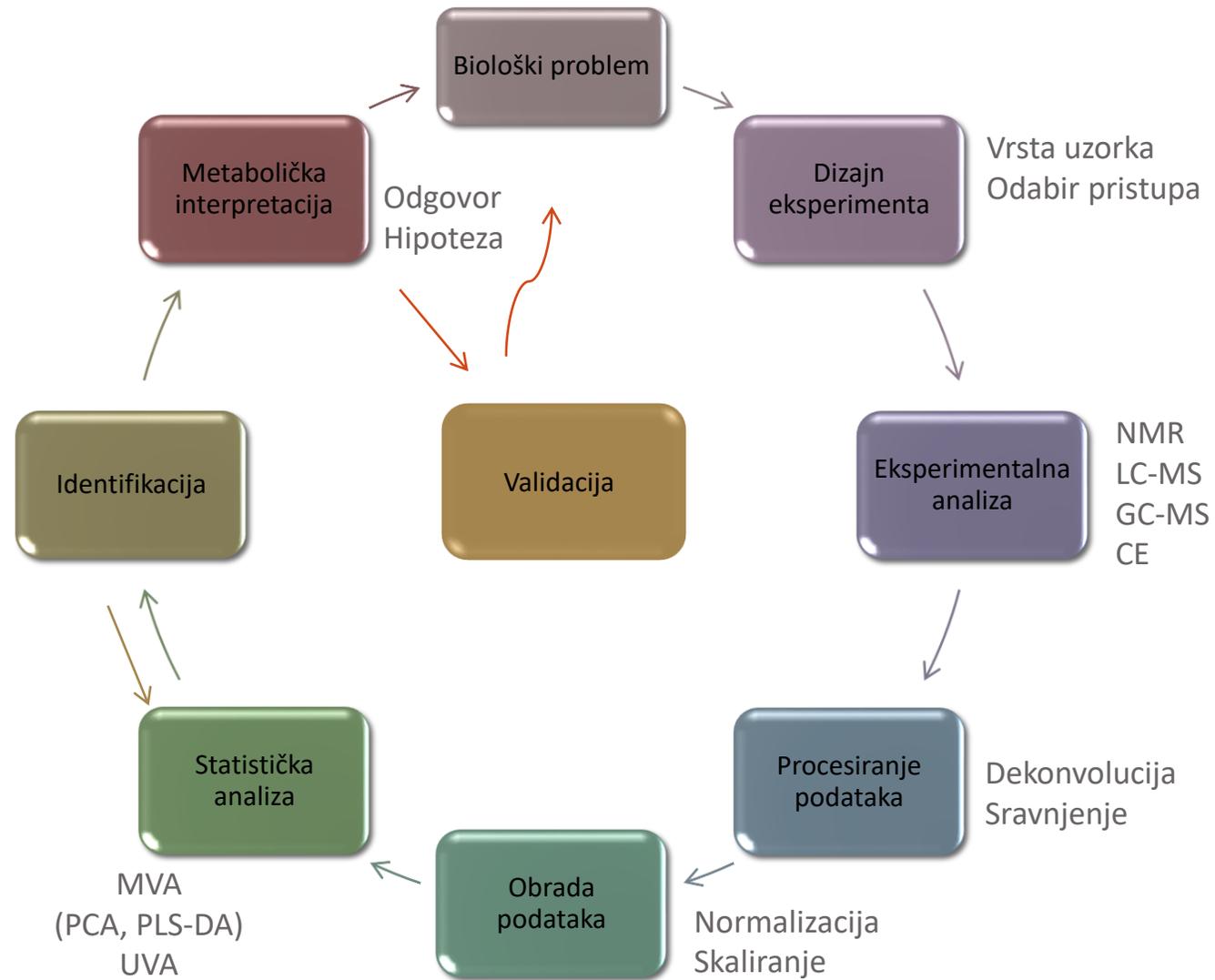
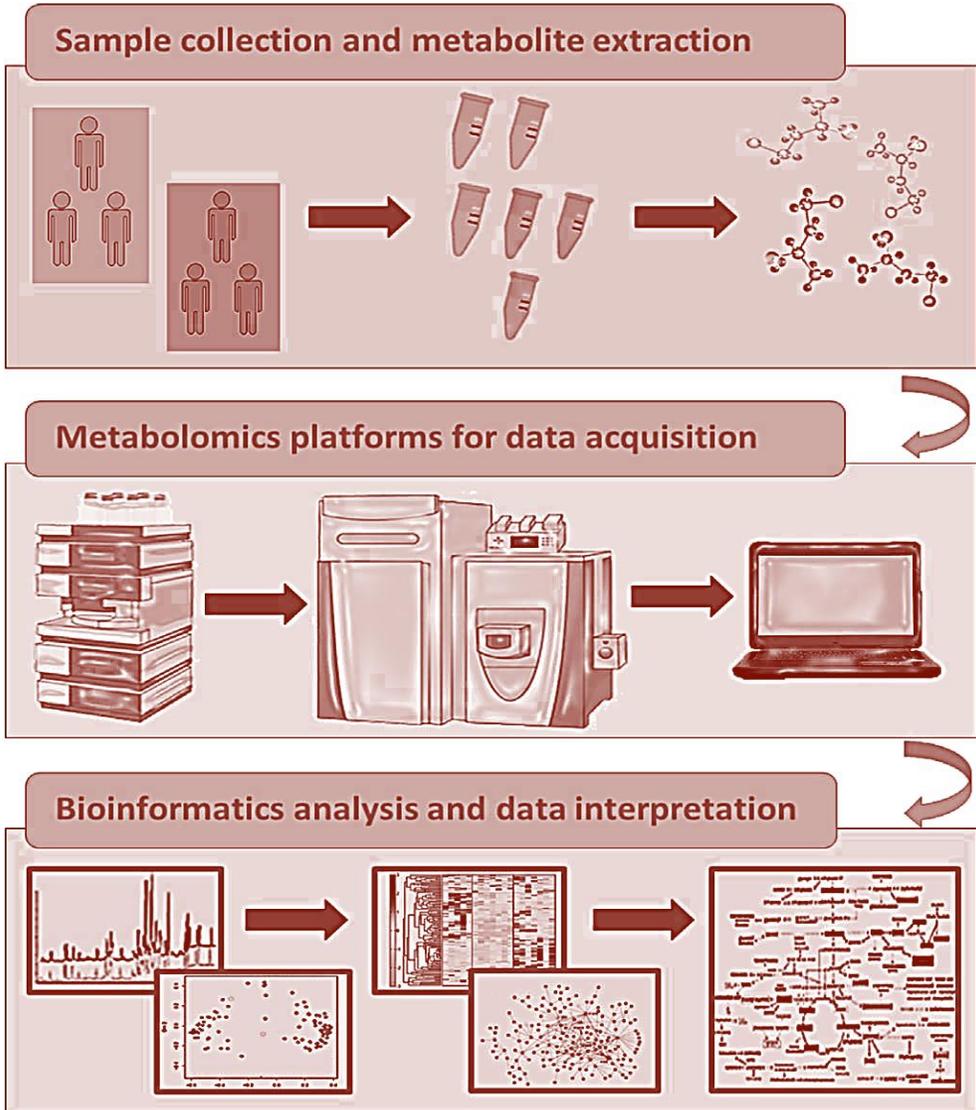
Profiliranje

Ciljana analiza

- Priprema uzoraka Standardi za kvantifikaciju Selektivnost +

+ Nepristranost Obrada podataka -

Tijek rada u metabolomici



Analitičke metode u metabolomici

	NMR	MS		
Princip detekcije	Magnetska svojstva atomskih jezgara (^1H , ^{13}C , ^{31}P)	Odnos mase i naboja ioniziranih čestica		
		LC	GC	CE
Priprema uzorka	+	+	-	+
Reproducibilnost/robustnost	+	-	+	-
Osjetljivost	-	+	+	-
Vrsta komponenti	Polarne i nepolarne	Širok spektar uporabe ovisno o tipu LC-a	Termostabilne i molekule koje isparavaju	Polarne nabijene molekule

Metabolomska istraživanja u psihijatrijskim poremećajima

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ELSEVIER

Review article

Short overview on metabolomic approach and redox changes in psychiatric disorders

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PSYCHIATRIC DISEASE	METABOLOMICS	
	Increased levels ↑	Decreased levels ↓
Depression	Lysophospholipids Monoglycerophospholipids Phosphatidylethanolamines Glutamate (serum) Aspartate (serum) Alanine Taurine Citrate Formate Glycine Isobutyrate Nicotinate α-KG Succinate Malonate Methylmalonate Succinyl-CoA γ-aminobutyric acid	Acyl carnitine Molecules Stearic amide Palmitic amide Lithocholic acid Deoxycholic acid Glycodesoxycholic acid Glycoursodeoxycholic acid Taurochenodeoxycholic acid Glucose Lactate Pyruvate Tryptophan Tyrosine 5-Hydroxyindoleacetic acid (CSF) Homovanillic acid (CSF) N-methylnicotinamide Isoleucine Valine Dopamine Ribulose 5-phosphate Malic acid Fumaric acid

PSYCHIATRIC DISEASE	METABOLOMICS	
	Increased levels ↑	Decreased levels ↓
Schizophrenia	Alanine Glycine Valine (urine) Glycine (urine) Lactate Lysophosphatidylcholines Ornithine 3-Indolebutyrate (microflora) Phenylalanine Tyrosine Proline Glutamic acid Pyruvic acid Saturated triglycerides	Glucose Phosphatidylcholine HDL LDL VLDL Unsaturated fatty acids Lipids Citrate α-KG Creatine Creatinine Acetoacetate 3-Hydroxybutyrate Arginine Glutamine Histidine Ketone bodies Hippurate (microflora) Trimethylamine-N-oxide (microflora)
PTSD	Glycerophosphoethanolamines (serum) Glycerophospholipids 7α,12α-dihydroxy-3-oxocholest-4-en-26-oic acid	Citric acid (mice) Isocitric acid (mice) Aconitic acid (mice) Succinic acid (mice) Oxalacetic acid (mice) N-acetylglucosamine-6-Phosphate, Palmitoylethanolamide Palmitic amide Guanosine Inosine Pantothenic acid 3α-hydroxy-5β-cholan-24-Oic acid Glycocholic acid

Common metabolites altered in schizophrenia, depression and PTSD.

METABOLITES	PSYCHIATRIC DISEASE		
	Schizophrenia	Depression	PTSD
Alanine	-	-	ND
α-KG	-	+	ND
Citrate	-	+	- (mice)
Glucose	-	-	ND
Glutamate	+	+	ND
Glycerophospholipids	+	ND	+
Glycine	+	+	ND
Lactate	+	-	ND
Palmitic amide	-	+	+
Pyruvate	+	-	ND
Succinate	ND	+	- (mice)
Tyrosine	+	-	ND
Valine	+	-	ND

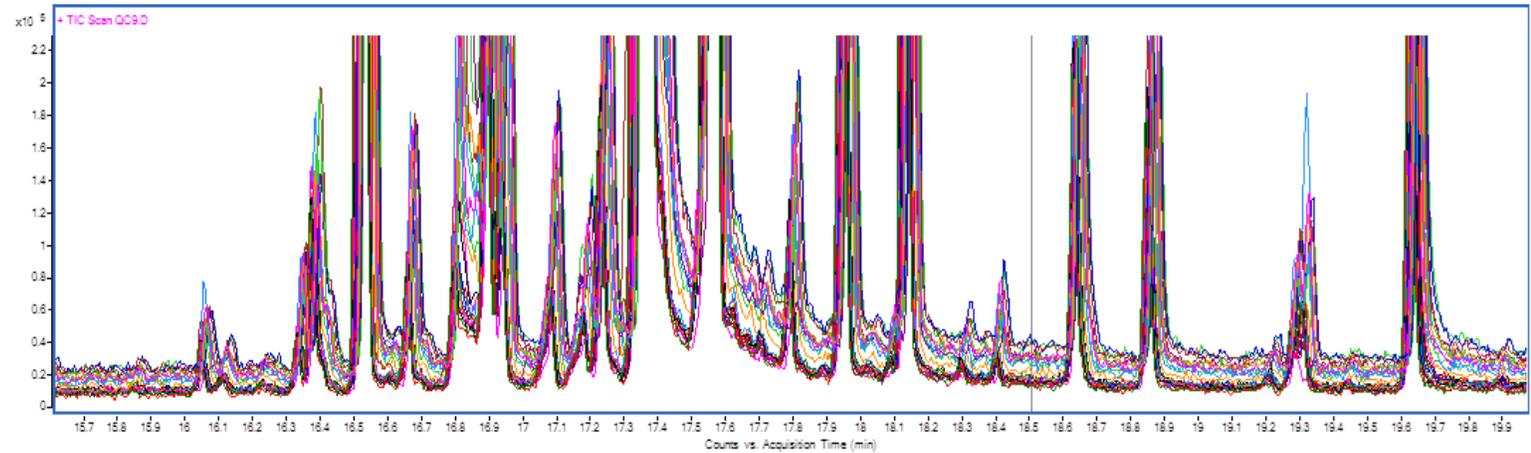
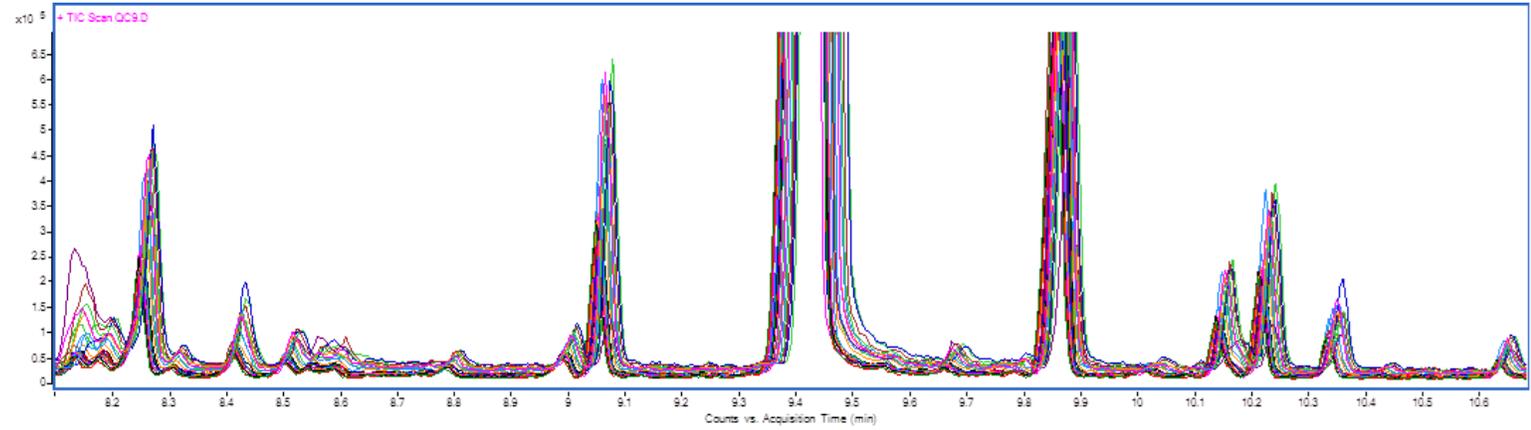
+ (increased levels); - (decreased levels); ND (not detected).

Rezultati

'Raw data'

➤ uzorci plazme 100 ispitanika - 50 ratnih veterana oboljelih od PTSP-a i 50 zdravih kontrolnih ispitanika

➤ neciljana višepatformska analiza metaboloma

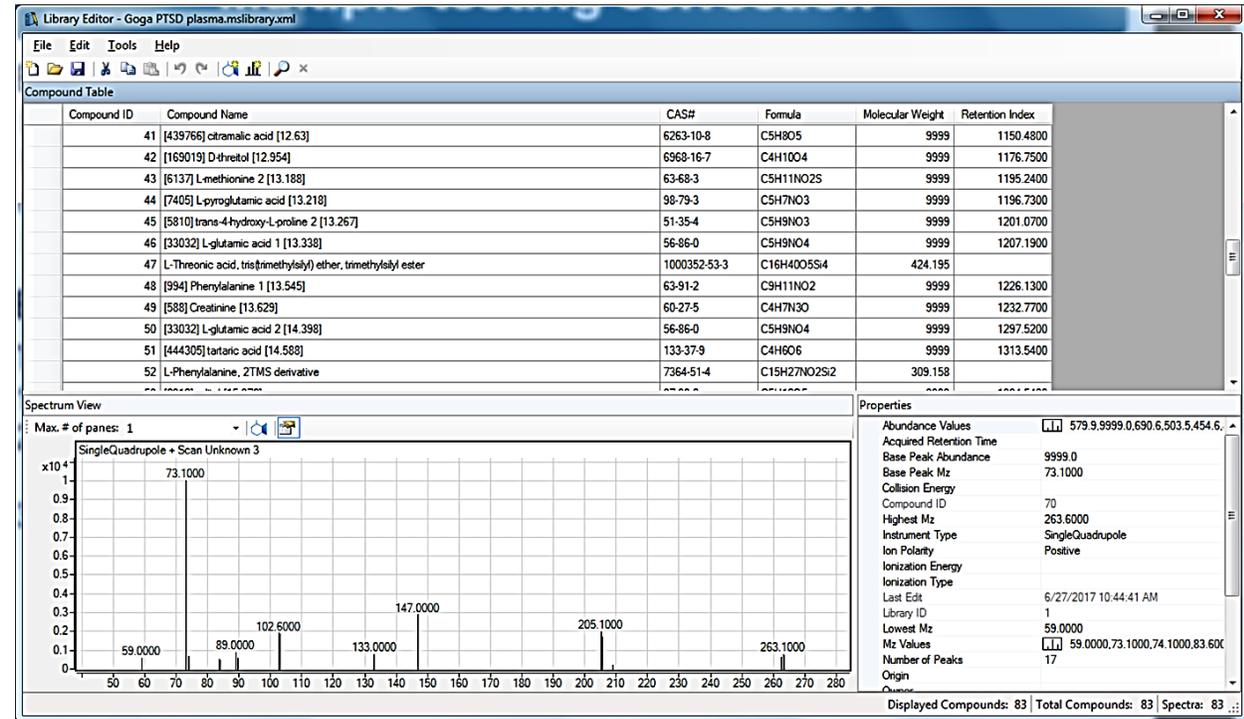
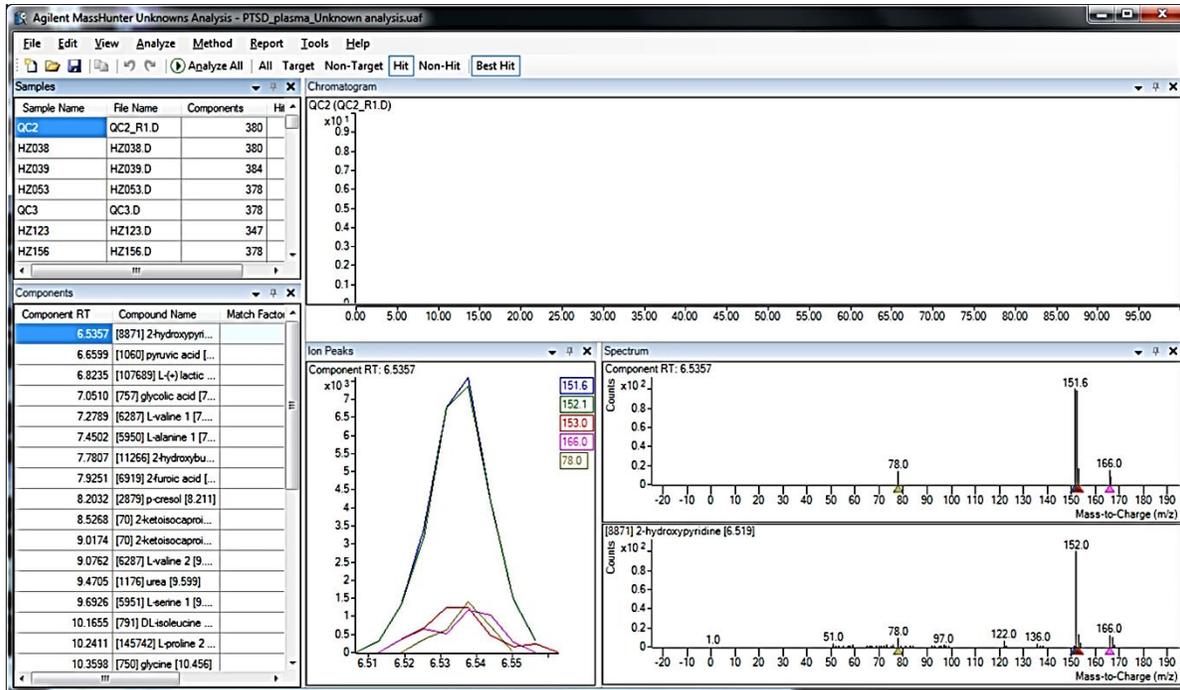


Rezultati

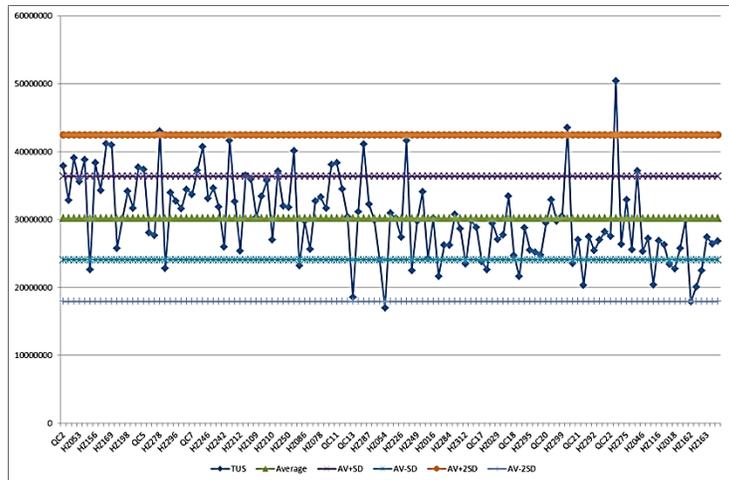
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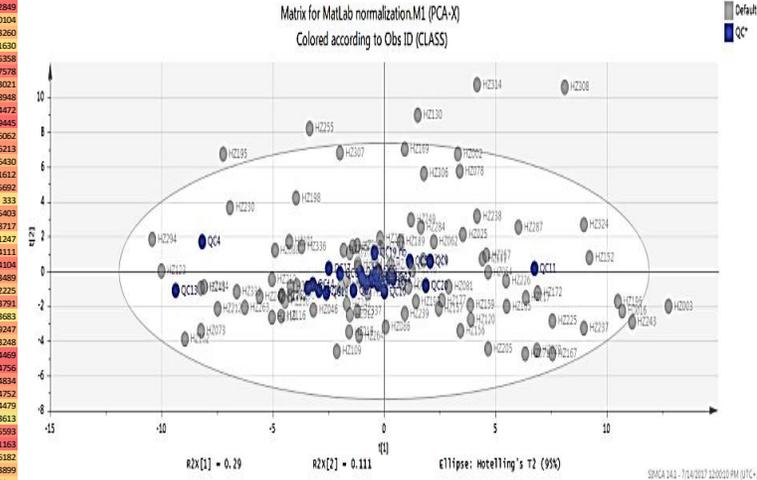


Obrada podataka

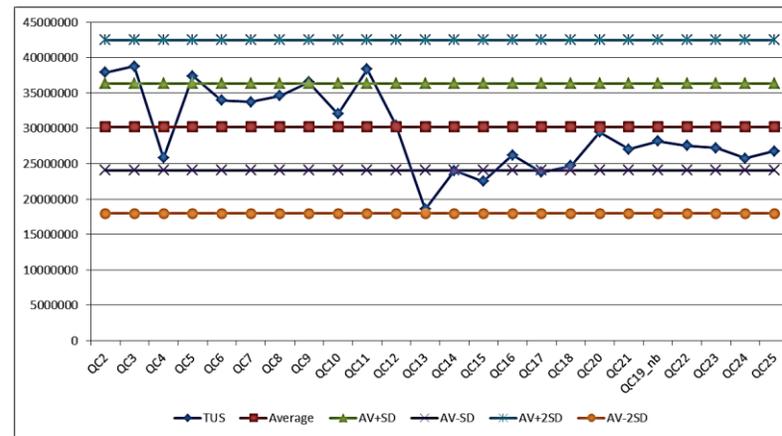
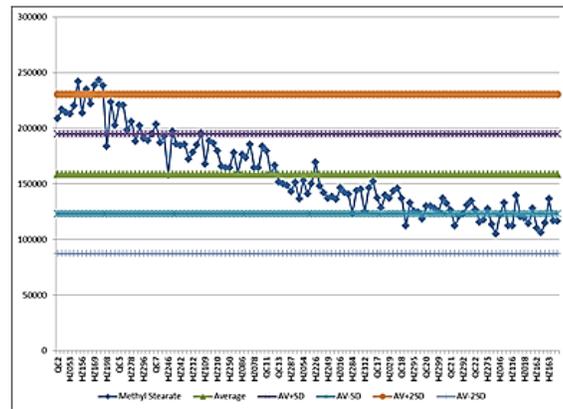


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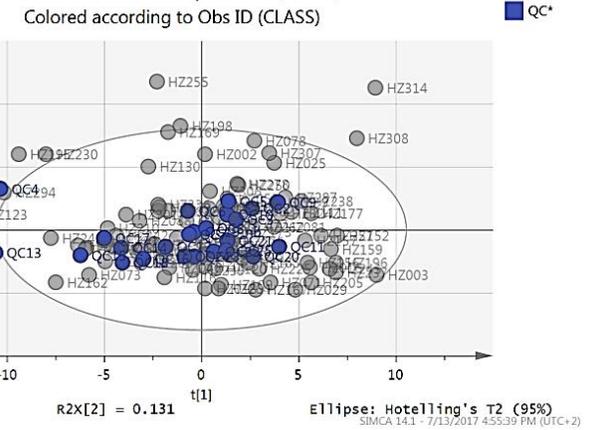
Normalizacija, skaliranje



Signal changes with injection. Only IS



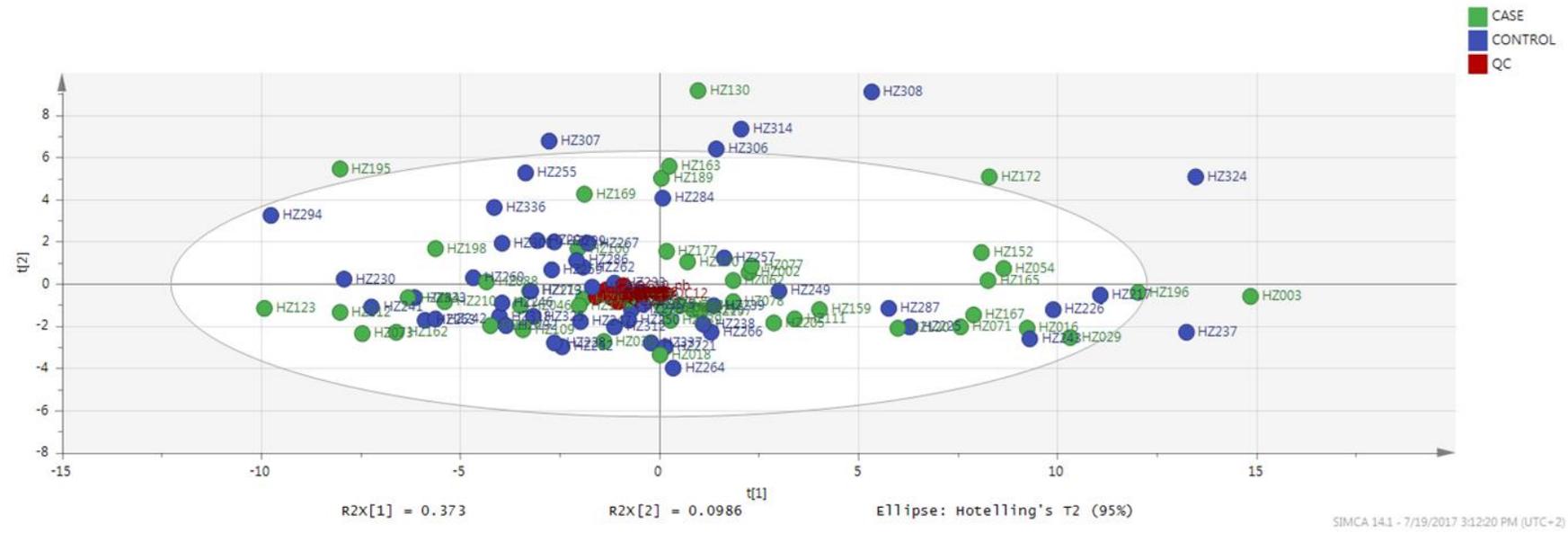
IS normalization all samples 2.M1 (PCA-X)



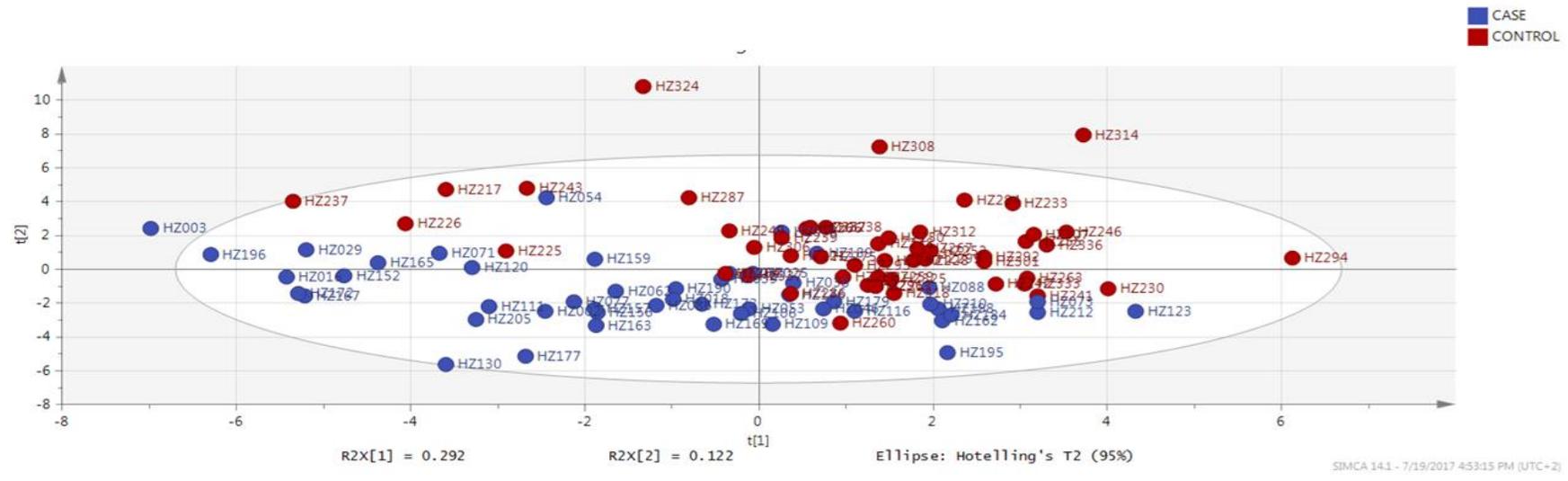
Statistička analiza

Multivarijantna statistička analiza (SIMCA 14.1)

PCA



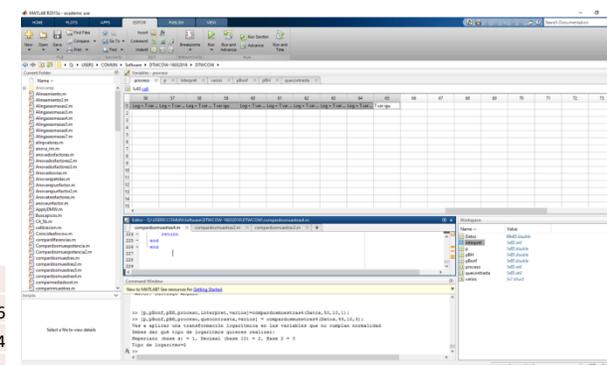
PLS-DA

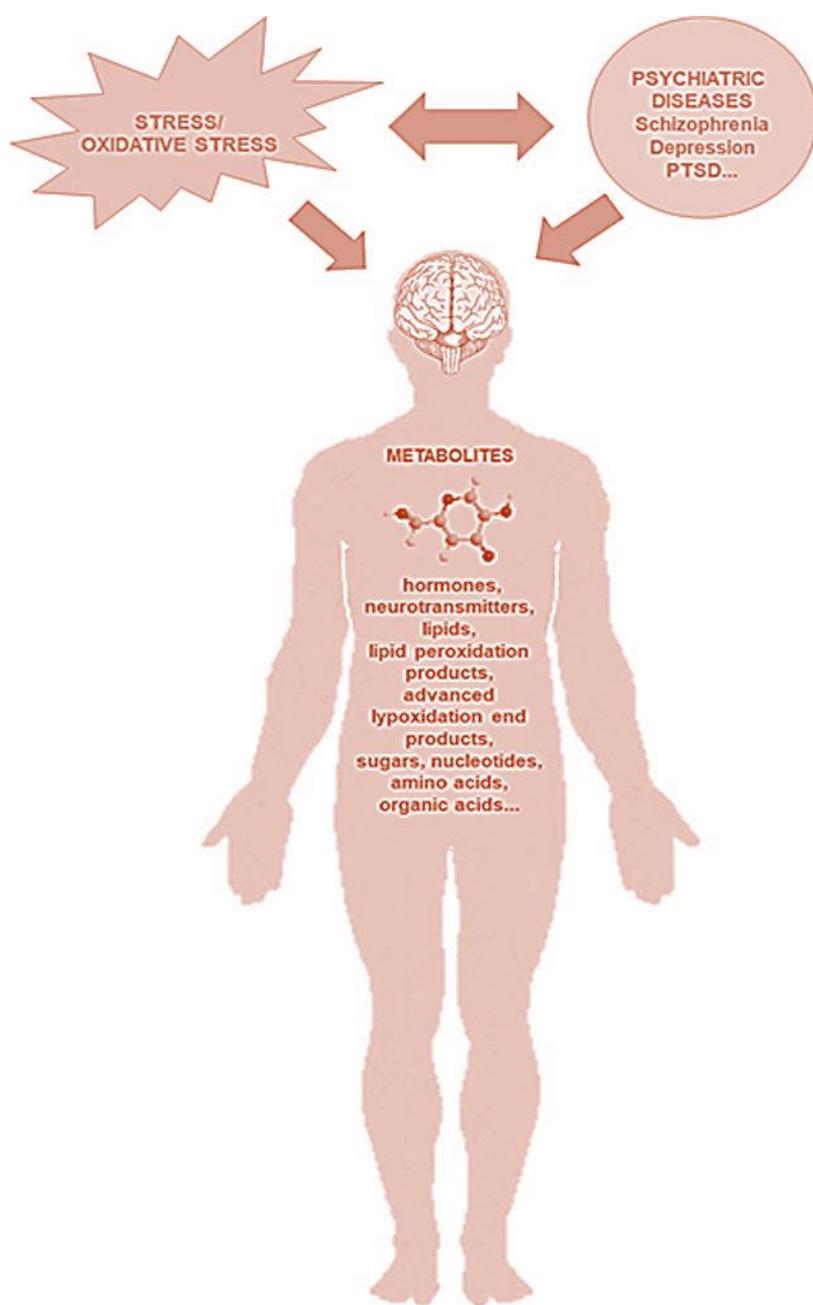


Univariјantna statistička analiza (MATLAB R2015a)

Compound	p value	p value after pBH
2-Deoxy-arabino-hexonic acid, 1,4-lactone	1.25 x 10⁻⁶	8.12 x 10⁻⁵
Aminomalonic acid	0.026192277	0.388454062
pyruvic acid	0.032976896	0.272873017
phosphoric acid	0.041030902	0.388454062
Methionine sum	0.057476969	0.388454062
Unknown sugar 1	0.063492937	0.388454062
Unknown sugar 2	0.068442229	0.388454062
citric acid	0.071976143	0.388454062
lactic acid	0.07418802	0.388454062
Glutamic acid sum	0.087389936	0.388454062
alanine 1	0.091604064	0.388454062
cholesterol	0.10112804	0.388454062
Glycine sum	0.103865585	0.388454062
2-ketoisocaproic acid sum	0.108403445	0.369878546
Tyrosine sum	0.110793438	0.388454062
Phenylalanine	0.118693867	0.388454062
Isoleucine sum	0.119859363	0.388454062
Pentose (D-lyxosylamine / lyxose)	0.121903834	0.388454062
myo-inositol	0.123995121	0.388454062
Proline sum	0.133468588	0.388454062
Leucine sum	0.135414432	0.388454062
palmitic acid	0.137452976	0.388454062
Phenylalanine sum	0.141902131	0.369878546
Pyranose sum	0.147377706	0.399147955
glycerol	0.162293708	0.421963641
Valine sum	0.206933683	0.498173681
oleic acid	0.218482358	0.507191189
glyceric acid	0.23215539	0.520348288
linoleic acid	0.273901801	0.593453903
stearic acid	0.306115525	0.627337325
threonine 2	0.308842991	0.627337325

Compound	p value	p value after pBH
Nonanoic acid	0.33243156	0.654789436
3-Hydroxybutyric acid	0.382868003	0.731953534
Serine sum	0.410894139	0.763089116
tartaric acid	0.446060843	0.783787944
urea	0.446156214	0.783787944
2-ketobutyric acid	0.469261482	0.785857131
Threonine sum	0.471514279	0.785857131
p-cresol	0.484681689	0.787607744
acetoacetate 2	0.498747586	0.790697393
2-hydroxybutyric acid	0.528852169	0.812178773
tryptophan 2	0.539227347	0.812178773
threitol	0.563890353	0.812178773
Unknown 1	0.57324981	0.812178773
pyroglutamic acid	0.57477267	0.812178773
Unknown 2	0.603222438	0.829581137
Creatinine	0.608008541	0.834168292
N-methylalanine	0.616213278	0.834168292
3-indolelactic acid 2	0.616364287	0.829581137
trans-4-hydroxy-L-proline 2	0.628834559	0.834168292
uric acid 1	0.642153093	0.834799022
xylitol	0.670371542	0.854395102
Unknown 4	0.705349038	0.881686298
glutamine 3	0.736285412	0.886269478
oxalic acid	0.755963561	0.893411481
citramalic acid	0.791087446	0.898863957
2-hydroxypyridine	0.8155264	0.898863957
Threonine acid	0.8158919	0.898863957
glycolic acid	0.866455272	0.9253313
Hydracrylic/Propanoic acid	0.868387835	0.9253313
gluconic acid 2	0.889298145	0.9323287
Furanose sum	0.908669948	0.937516613
2-Hydroxy-3-methylbutyric acid	0.979095359	0.98163448
2-furoic acid	0.98163448	0.98163448





Daljnji ciljevi istraživanja

- napraviti metabolomsku analizu na preostalim uzorcima PTSP-a i kontrola
- identificirati promijenjene metabolite iz plazme kao potencijalne biomarkere u PTSP-u
- utvrdite nove metaboličke putove uključene u patofiziologiju PTSP-a
- prijenos tehnologije i znanja u metabolomici u primjena najnovije znanstvene opreme u Zagrebu

Hvala na pažnji!

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