



GlikoGenPTSP

IP-2014-09-4289

## Promjene N-glikoma u PTSP-u

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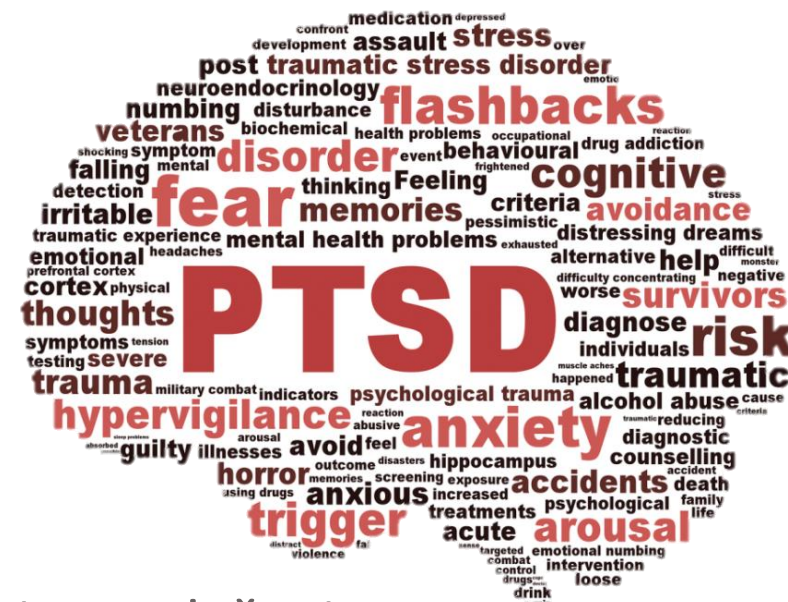
DR. SC. DUBRAVKA ŠVOB ŠTRAC, VIŠA ZNANSTVENA SURADNICA  
LABORATORIJ ZA MOLEKULARNU NEUROPSIHIJATRIJU  
INSTITUT RUĐER BOŠKOVIĆ

# Posttraumatski stresni poremećaj (PTSP)

- anksiozni (ili stresom uzrokovani) poremećaj koji nastaje nakon izlaganja ili svjedočenja teškom traumatskom događaju kod nekih, ali ne svih osoba

## Kriteriji DSM-V (APA, 2013):

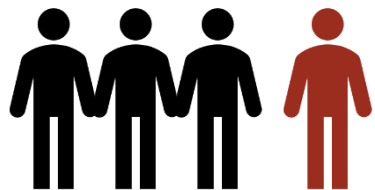
- postojanje traumatskog iskustva
- ponovno proživljavanje traume (eng. *reexperiencing*)
- izbjegavanje (eng. *avoidance*)
- pojačana pobuđenost (eng. *hyperarousal*)
- neosjetljivost (eng. *numbing*) i negativne promjene mišljenja i raspoloženja
- trajanje (najmanje 1 mjesec)
- znatan poremećaj u radnom, socijalnom ili drugom funkcioniranju



# 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**

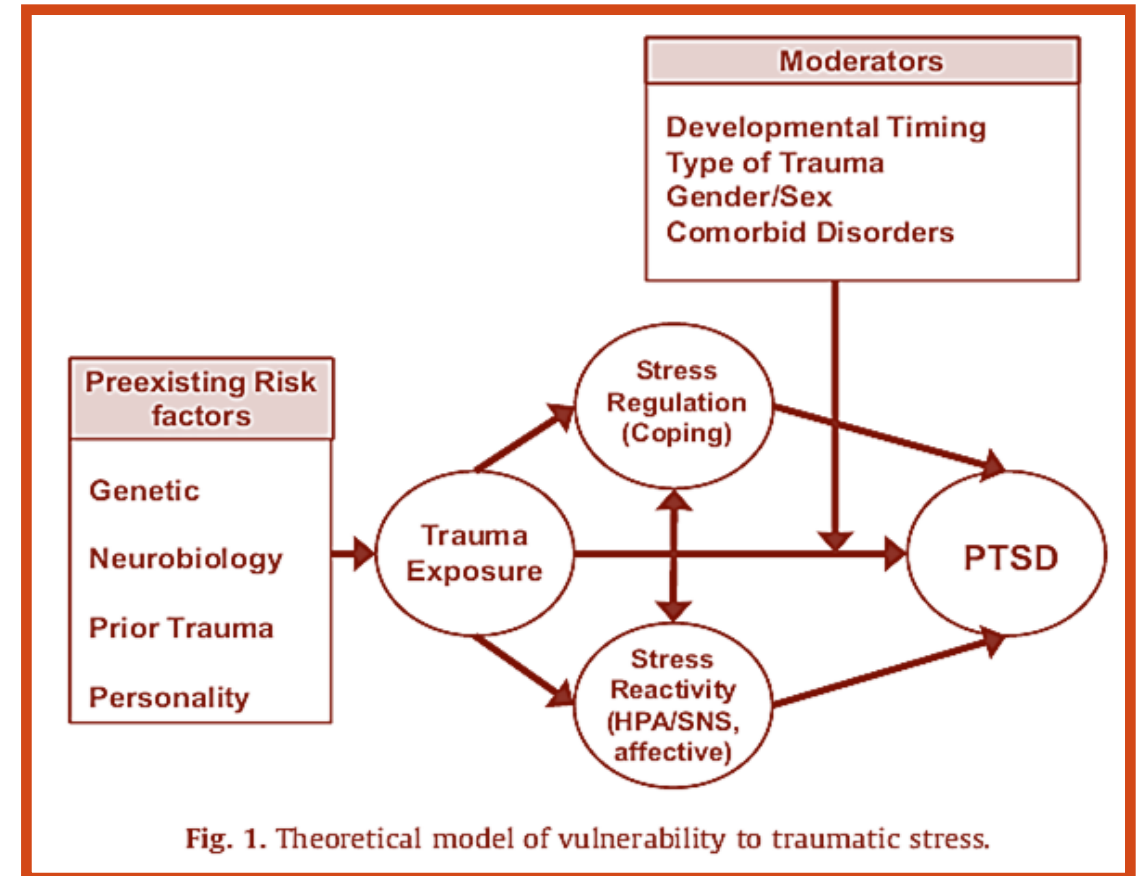
➤ kardiovaskularne bolesti, koronarna bolest srca, endokrine bolesti, metabolički sindrom, respiratorne bolesti, probavni poremećaji

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



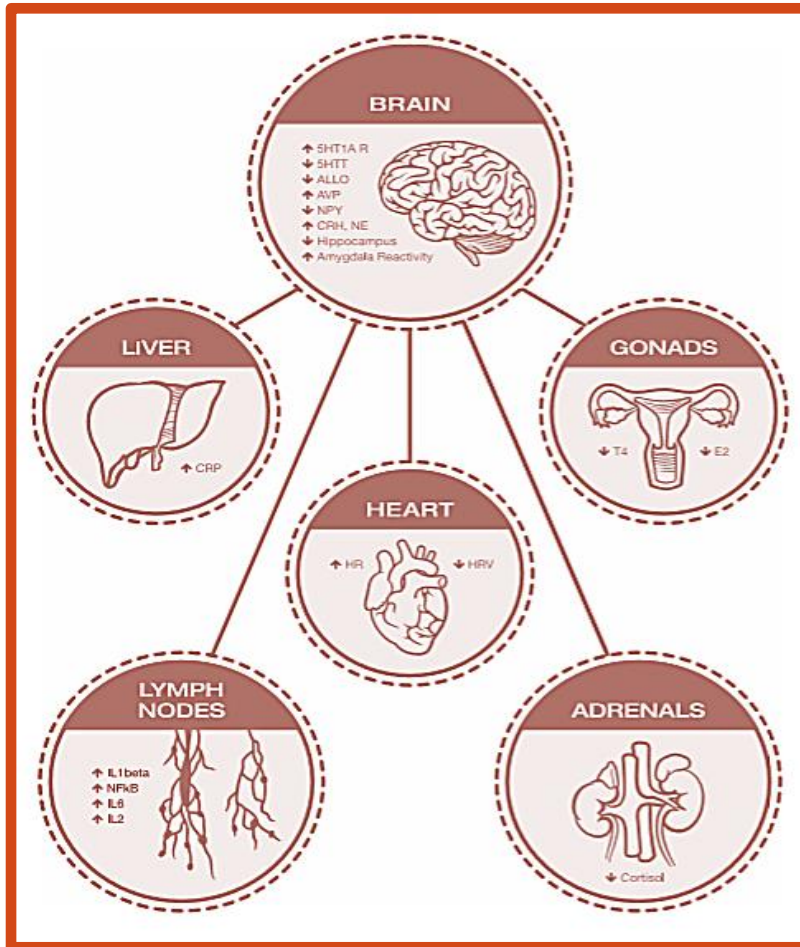
# Rizični čimbenici za razvoj PTSP-a

- 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



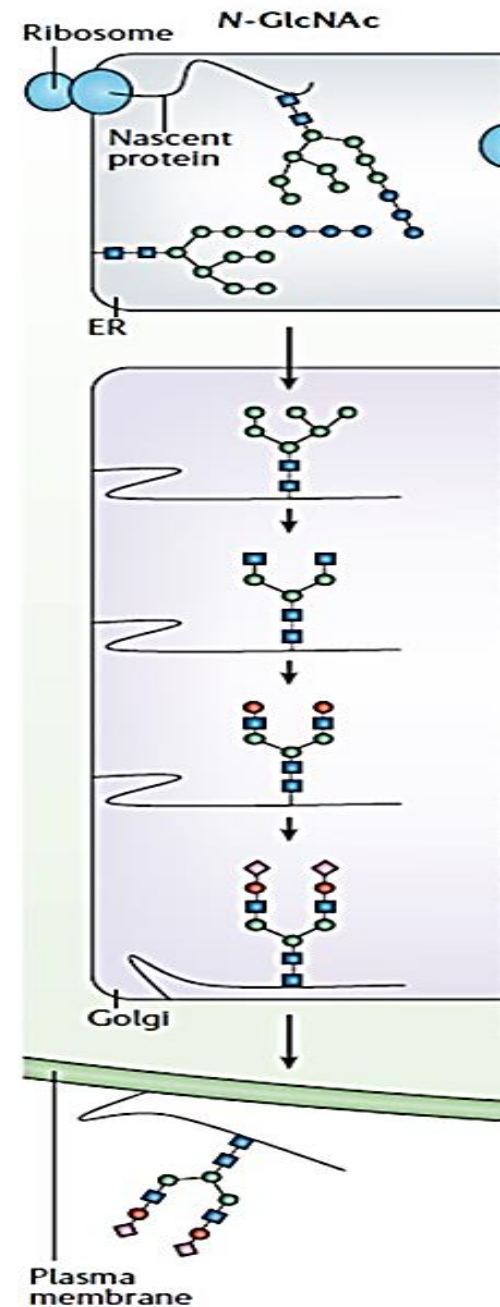
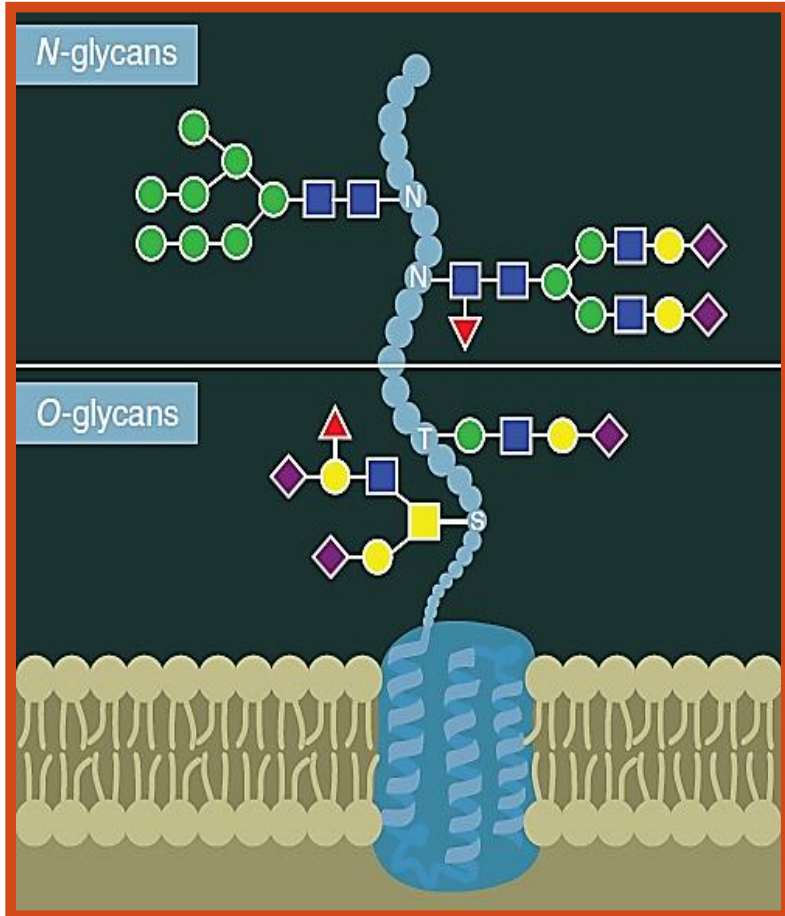
## Poremećena neuroendokrinologija i os HHN

- ↑ 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

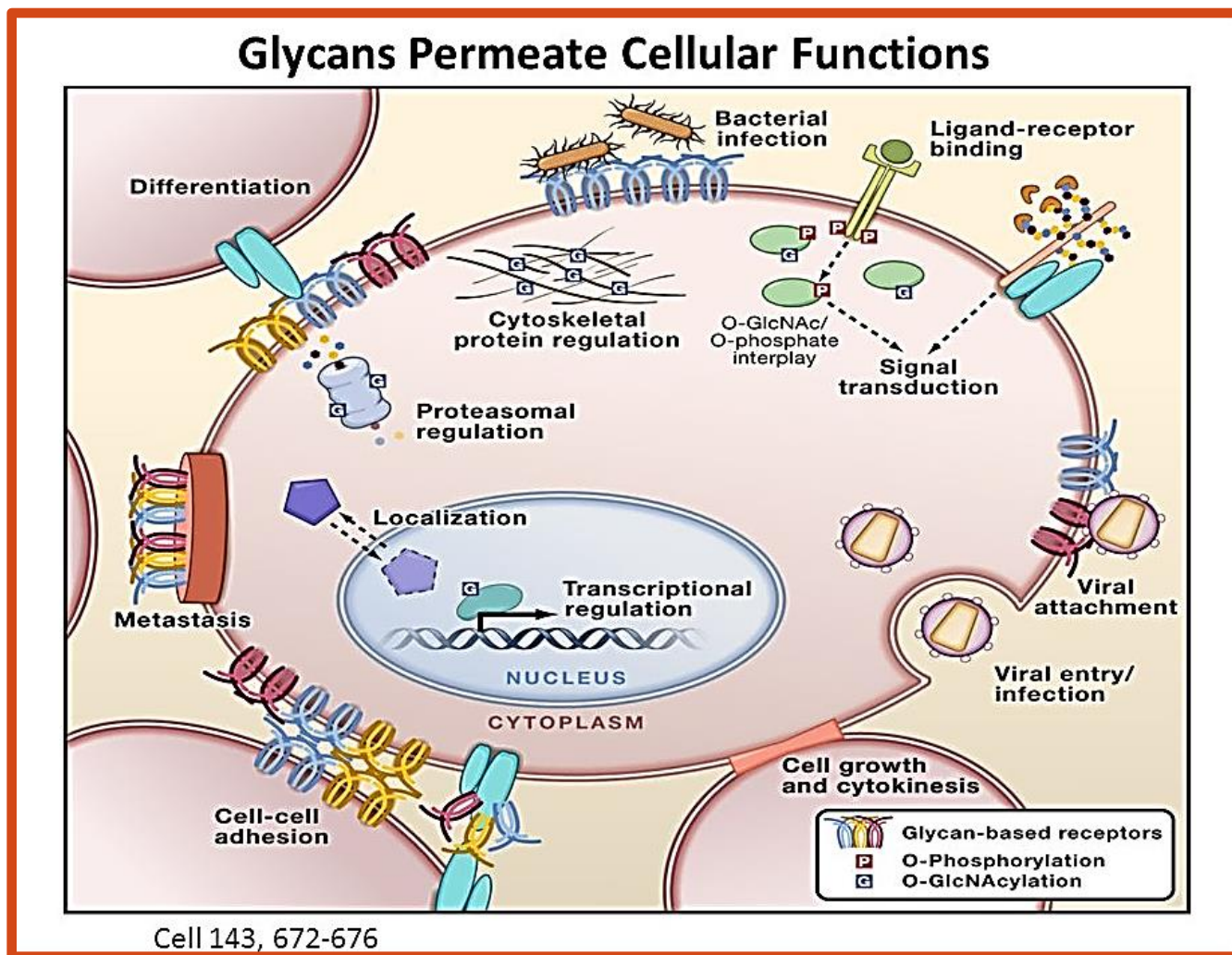


# Glikani

- 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



## 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<sup>a,1</sup>, Luisa Sturiale<sup>b,1</sup>, Angelo Palmigiano<sup>b</sup>, Mario Zappia<sup>c</sup>, Domenico Garozzo<sup>b,\*</sup>

*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<sup>‡,d</sup>, Ana Knežević<sup>§,d</sup>, Olga Gornik<sup>§</sup>, Maja Pučić<sup>¶</sup>, Wilmar Igl<sup>||</sup>, Hilde Peeters<sup>\*\*</sup>, An Crepel<sup>\*\*</sup>, Jean Steyaert<sup>\*\*</sup>, Mislav Novokmet<sup>¶</sup>, Irma Redžić<sup>§</sup>, Matea Nikolac<sup>‡</sup>, Vesna Novković Hercigonja<sup>‡‡</sup>, Katarina Dodig Ćurković<sup>§§</sup>, Mario Ćurković<sup>¶¶</sup>, Gordana Nedić<sup>‡</sup>, Dorotea Muck-Seler<sup>‡</sup>, Fran Borovečki<sup>|||</sup>, Igor Rudan<sup>ab</sup> and Gordan Lauc<sup>¶¶</sup>

## Alzheimer's Disease

Serum: decrease of digalactosylated core- $\alpha$ -1,6-fucosylated biantennary glycan. Increase of  $\alpha$ 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 $\beta$  glycopeptides [23]. Decrease of TTR brain-specific isoform [76]. Quantitative changes of apolipoprotein E, clusterin,  $\alpha$ -1- $\beta$ -glycoprotein and  $\alpha$ -1-AAT. Decreased glycosylation of one specific  $\alpha$ -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<sup>x</sup> 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  
© 2013 Macmillan Publishers Limited All rights reserved 2158-3188/13  
[www.nature.com/tp](http://www.nature.com/tp)



## ORIGINAL ARTICLE

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

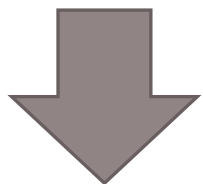
M Moreno-Villanueva<sup>1,7</sup>, J Morath<sup>2,7</sup>, V Vanhooren<sup>3,4</sup>, T Elbert<sup>2</sup>, S Kolassa<sup>5</sup>, C Libert<sup>3,4</sup>, A Bürkle<sup>1</sup> and I-T Kolassa<sup>2,6</sup>

- 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)

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### 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

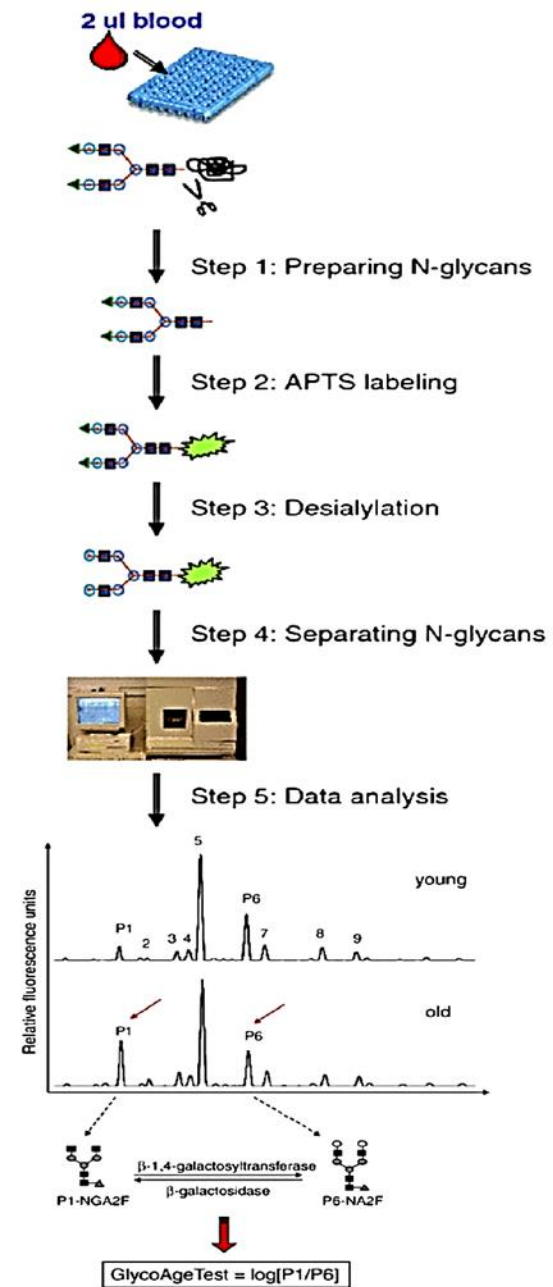
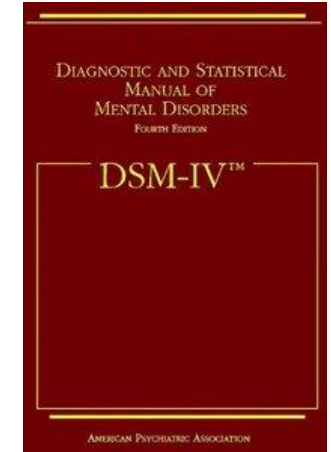
# Materijali i metode

## 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



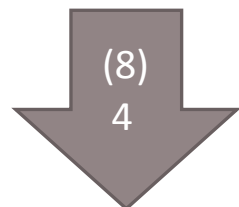
# Rezultati

## N-glikani u plazmi:

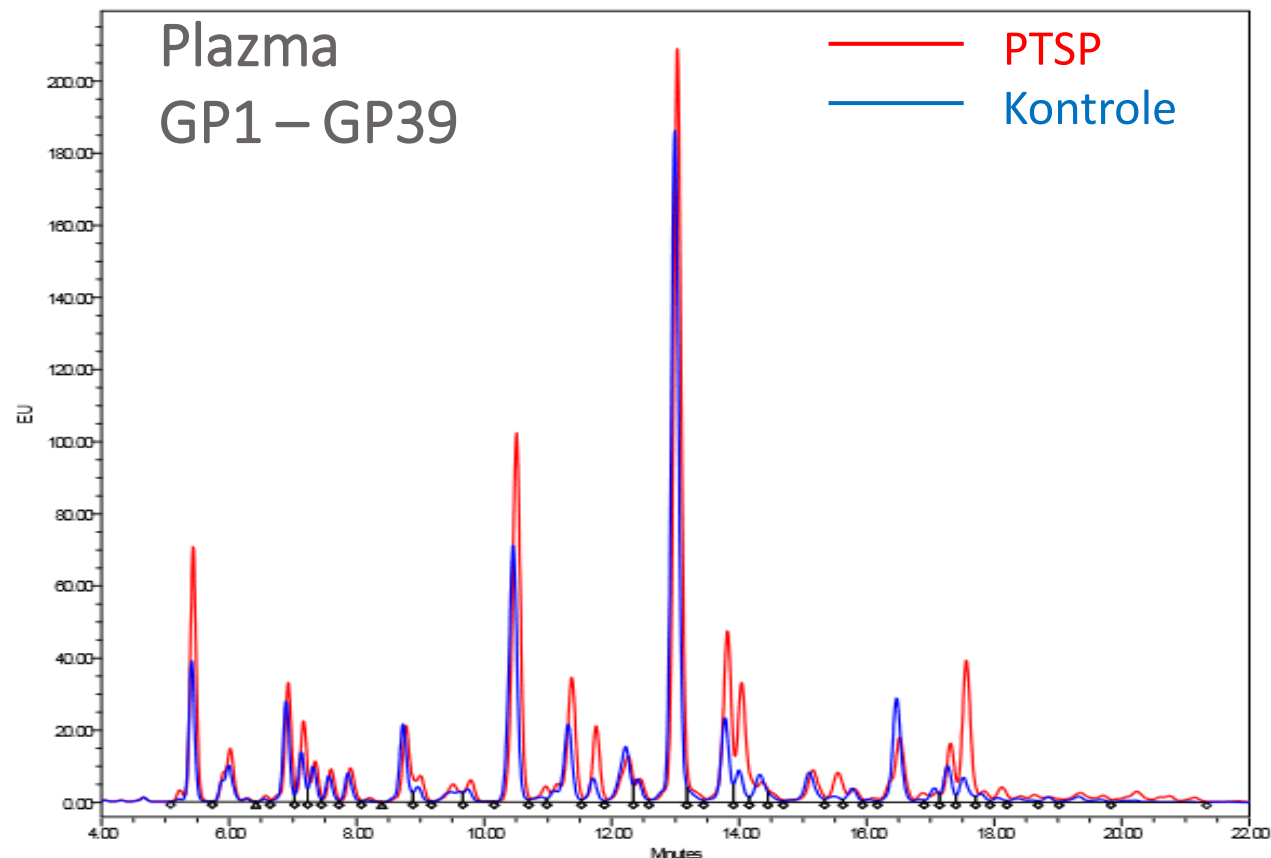


GP14, GP15, GP26, GP27, GP30,  
GP32, GP33, GP34, GP35, GP36,  
GP37, GP38, GP39

PTSP



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



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

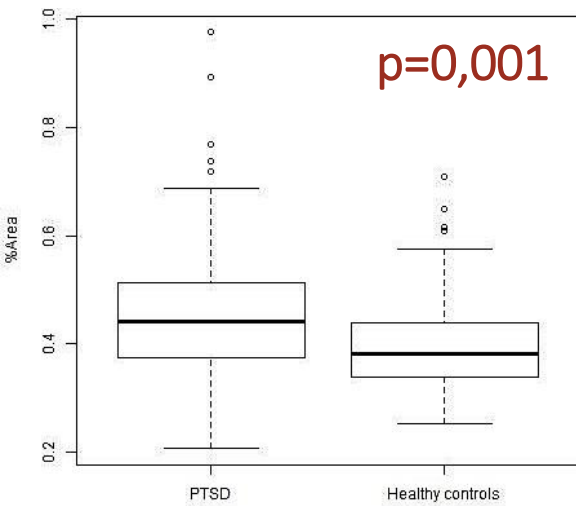


# Rezultati

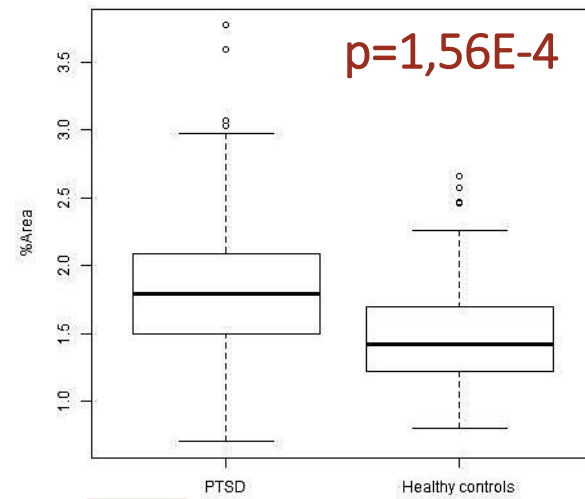
## N-glikani u plazmi:



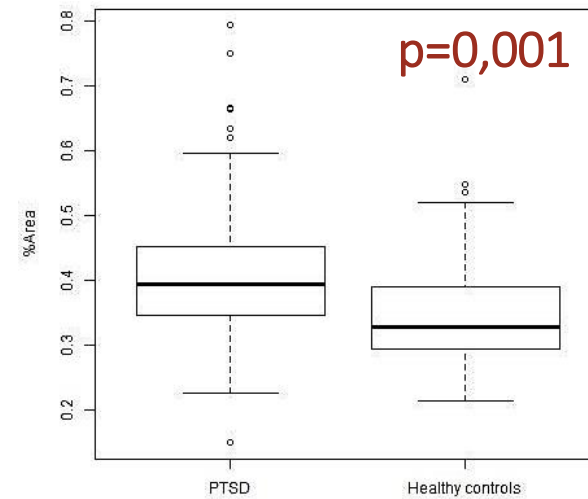
Triantenarni i tetraantenarni sijalizirani N-glikani povećani kod upalnih procesa (Gudelj et al. 2016) i hepatocelularnog karcinoma (Vanhooren et al. 2009)



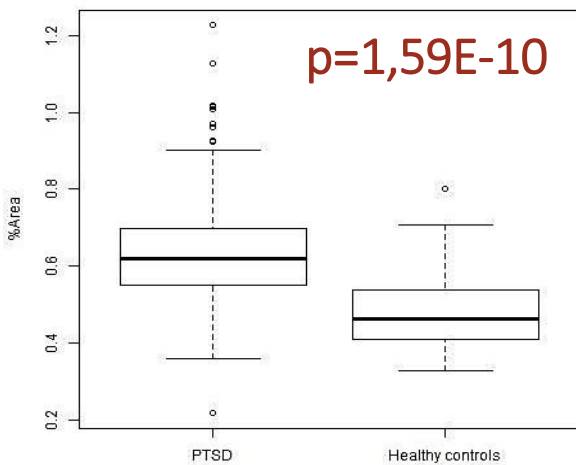
**GP15** = A2BG2S1 - biantennary bigalactosylated and sialylated with bisecting GlcNAc



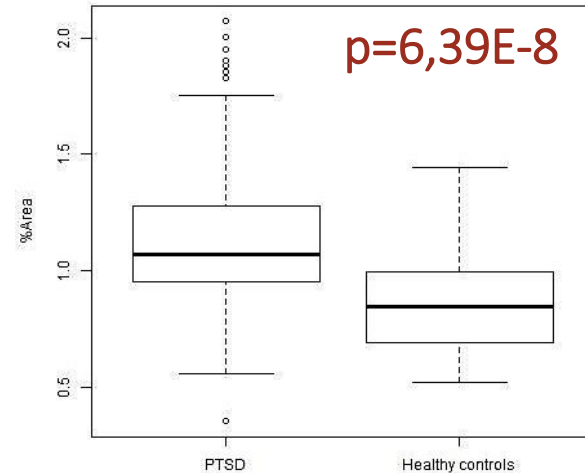
**GP32** = A3F1G3S3 – triantenarny, trigalactosylated and trisialylated with antennary fucose



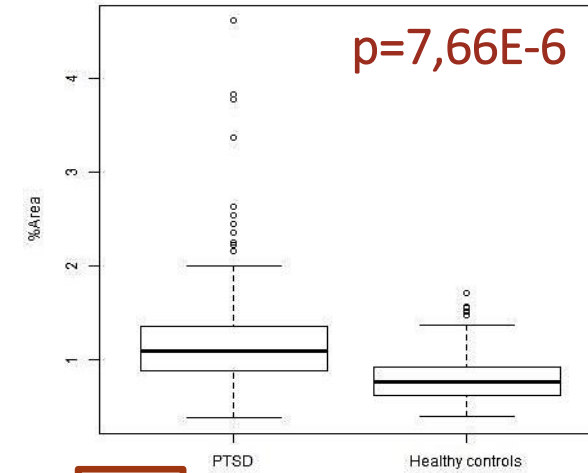
**GP34** = A4G4S3 - tetraantenarny, tetragalactosylated and trisialylated



**GP36** = A4S4 - tetraantenarny tetragalactosylated and tetrasialylated



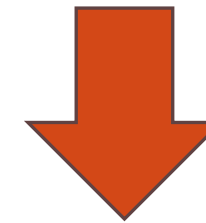
**GP38** = A4G4S4 - tetraantenarny, tetragalactosylated and tetrasialylated



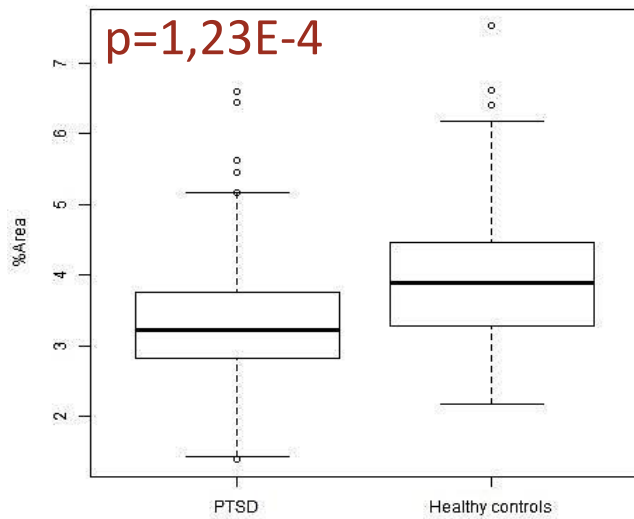
**GP39** = A4F1G4S4 - tetraantenarny, tetragalactosylated and tetrasialylated with antennary fucose

# Rezultati

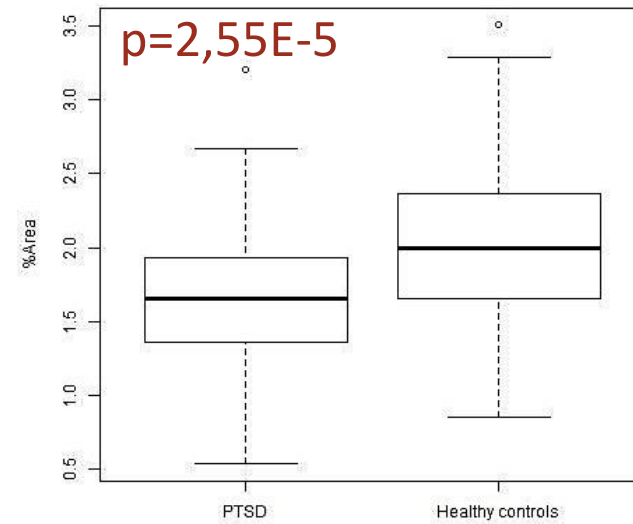
## N-glikani u plazmi:



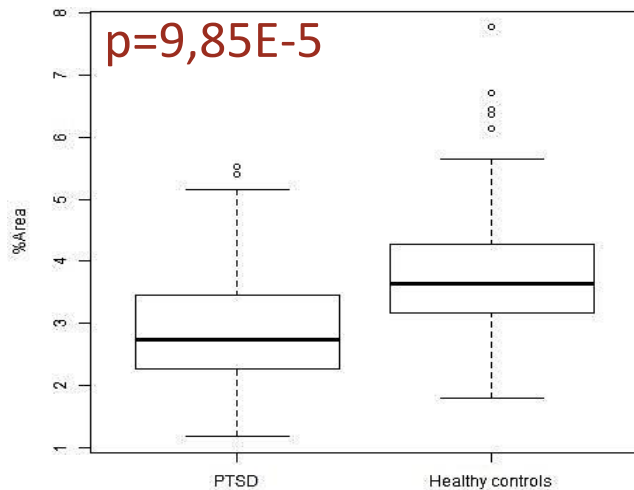
GP10 = FA2G2, vrijednost ovog N-glikana se drastično snižava s dobi



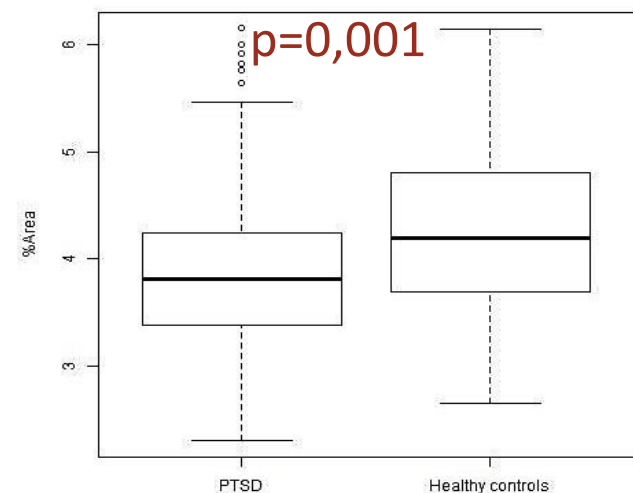
GP4 = FA2[6]BG1 -biantennary monogalactosylated with core fucose and bisecting GlcNAC



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

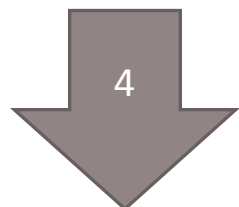
# Rezultati

## N-glikani vezani za IgG:

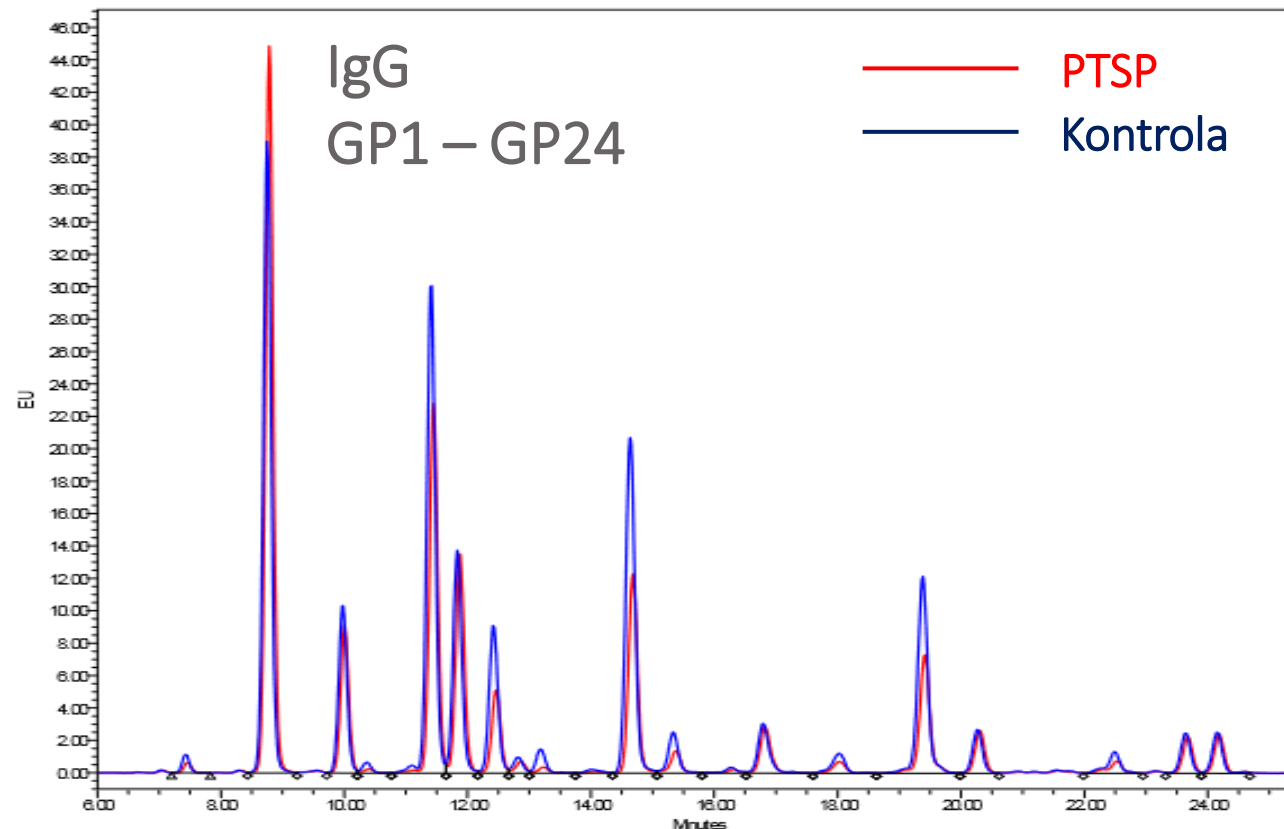


GP3, GP5, GP6, GP7, GP13,  
GP17, GP22

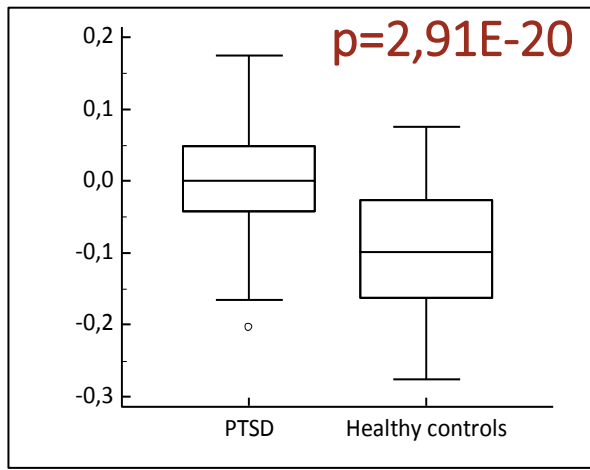
PTSP



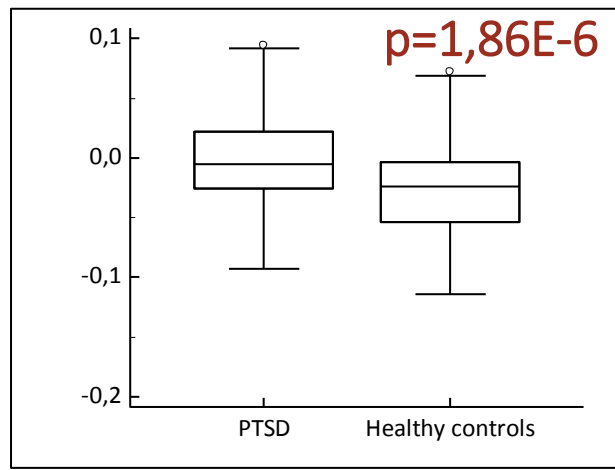
GP9, GP14, GP18, GP2



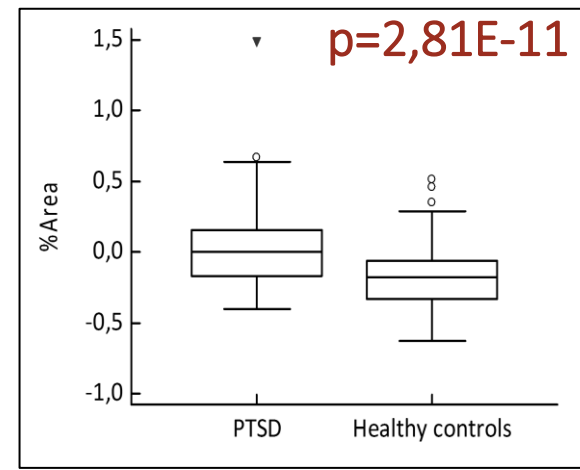
- snažan utjecaj dobi na razinu N-glikana → korekcija ( $p=0,05$ ; korekcija na višestruko testiranje:  $p=0,001^*$ )



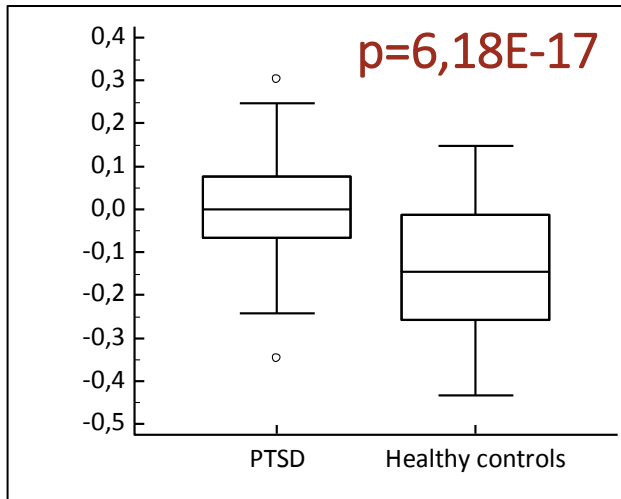
**GP3 = A2B- Biantennary agalactosylated with bisecting GlcNAC**



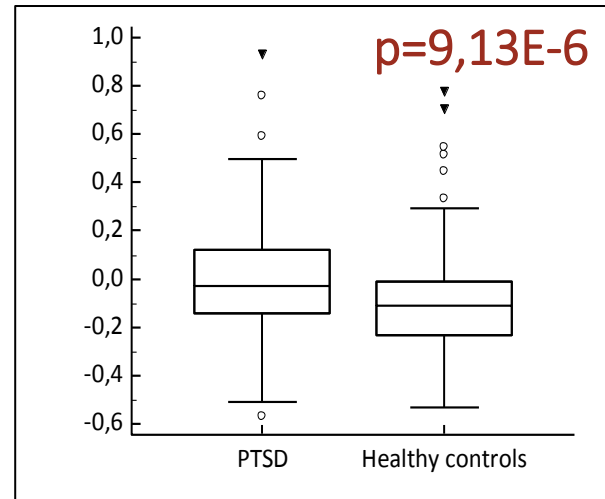
**GP5 = M5 - High mannose**



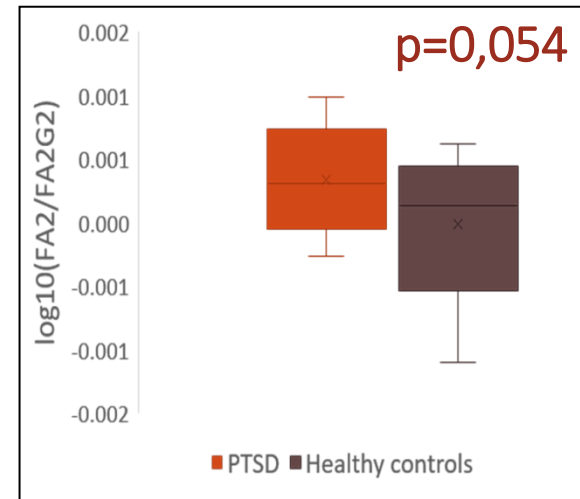
**GP7 = A2G1 - Biantennary monogalactosylated**



**GP13 = A2BG1 - Biantennary digalactosylated with bisecting GlcNAC**



**GP17 = A2G2S1 - Biantennary digalactosylated and monosialylated**



**GlycoAge test**

# Rezultati

N-glikani vezani za IgG:



GlycoAge test

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

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



fiziološka dob  
ispitanika s PTSP-om



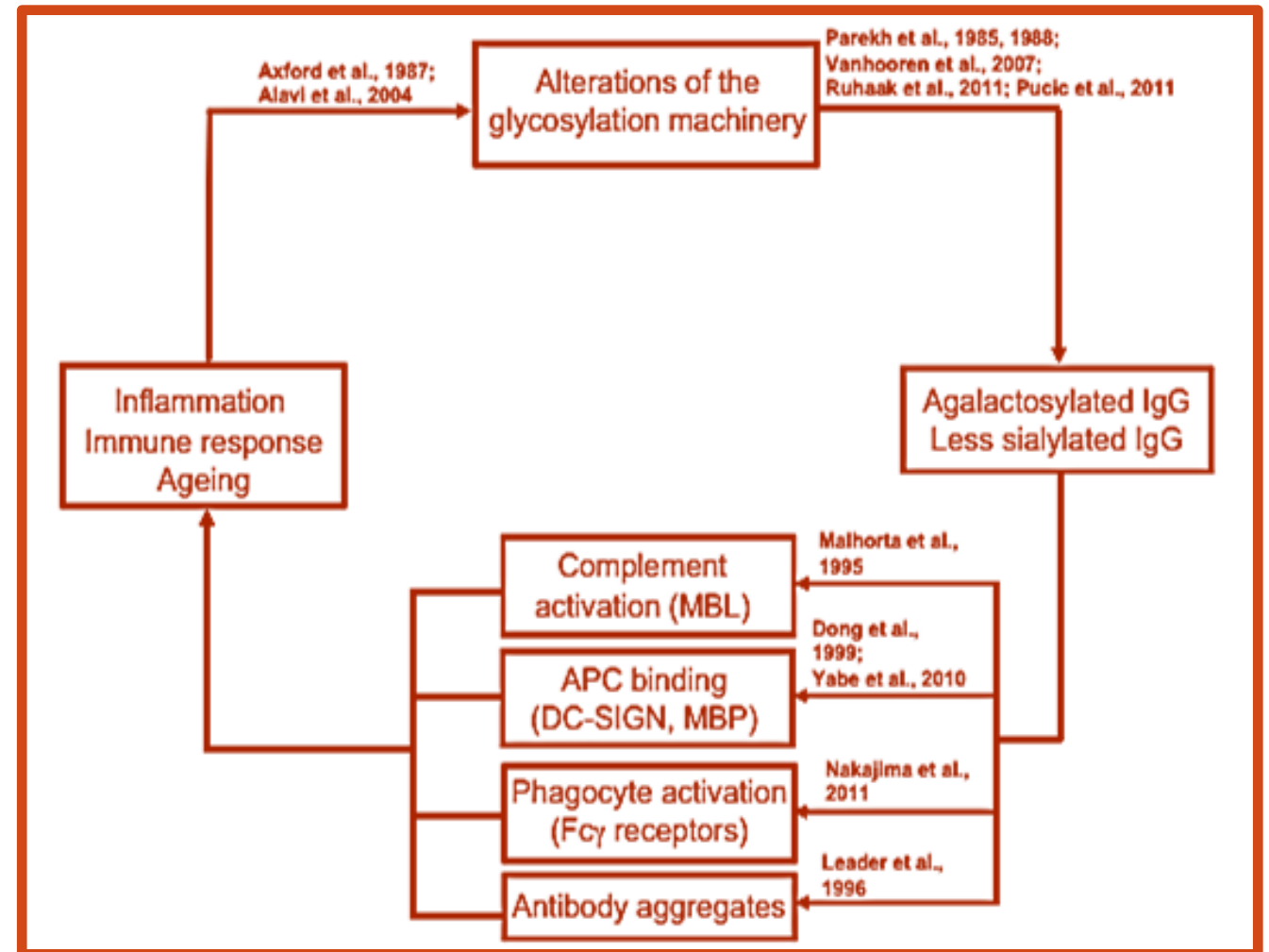
# Zaključak

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)

# Hvala na pažnji!



GlikoGenPTSP

Project No: IP-2014-09-4289

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Doc. dr. sc. Oliver Kozumplik, Klinika za psihijatriju Vrapče, Zagreb, Hrvatska

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