Metabolomic and glycomic findings in posttraumatic stress disorder

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Abstract

Posttraumatic stress disorder (PTSD) is a stressor-related disorder that develops in a subset of individuals exposed to a traumatic experience. Factors associated with vulnerability to PTSD are still not fully understood. PTSD is frequently comorbid with various psychiatric and somatic disorders, moderate response to treatment and remission rates. The term “theranostics” combines diagnosis, prognosis, and therapy and offers targeted therapy based on specific analyses. Theranostics, combined with novel techniques and approaches called “omics”, which integrate genomics, transcriptomic, proteomics and metabolomics, might improve knowledge about biological underpinning of PTSD, and offer novel therapeutic strategies. The focus of this review is on metabolomic and glycomic data in PTSD. Metabolomics evaluates changes in the metabolome of an organism by exploring the set of small molecules (metabolites), while glycomics studies the glycome, a complete repertoire of glycan structures with their functional roles in biological systems. Both metabolome and glycome reflect the physiological and pathological conditions in individuals. Only a few studies evaluated metabolic and glycomic changes in patients with PTSD. The metabolomics studies in PTSD patients uncovered different metabolites that might be associated with psychopathological alterations in PTSD. The glycomics study in PTSD patients determined nine N-glycan structures and found accelerated and premature aging in traumatized subjects and subjects with PTSD based on a GlycoAge index. Therefore, further larger studies and replications are needed. Better understanding of the biological basis of PTSD, including metabolomic and glycomic data, and their integration with other “omics” approaches, might identify new molecular targets and might provide improved therapeutic approaches.

Key words:

PTSD, metabolomics; glycomics; clinical data; animal models; theranostic biomarkers; patients

Abbreviations:

adenocorticotropic hormone (ACTH); atmospheric pressure chemical ionization (APCI); brain derived neurotropic factor (BDNF); capillary electrophoresis (CE); capillary electrophoresis-mass spectrometry (CE-MS); capillary gel electrophoresis with laser induced fluorescence detection (CGE-LIF); corticotropin releasing hormone (CRH); cerebrospinal fluid (CSF); C-reactive protein (CRP); docosahexaenoate (DHA); docosapentaenoate (DPA); eicosapentaenoate (EPA); electrospray ionization (ESI); gas chromatography (GC); gas chromatography-mass spectrometry (GC-MS); global arginine bioavailability ratio (GABR); hydrogen-nuclear magnetic resonance (HNMR); hydrophilic interaction liquid chromatography (HILIC); high performance liquid chromatography (HPLC); hypothalamic-pituitary-adrenal (HPA); immunoglobulin G (IgG); interleukin 1 beta (IL-1β); interleukin 6 (IL-6); interferon gamma (IFN-γ); laser desorption ionisation (LDI); liquid chromatography (LC); liquid chromatography-mass spectrometry (LC-MS); liquid chromatography electrospray mass spectrometry (LC-ESI-MS); liquid chromatography triple quadrupole mass spectrometry with multiple reaction monitoring (LC-QqQ-MS with MRM); mass spectrometry (MS); matrix-assisted lased desorption ionisation (MALDI); matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS); N-acetylaspartate (NAA); N-acetylglucosamine (GlcNAc); nano-liquid chromatography coupled to tandem mass spectrometry (nano-LC MS/MS); National institute of standard technologies (NIST) library; nicotinamide adenosine dinucleotide phosphate (NADP); nicotinamide adenosine dinucleotide phosphate oxidase 2 (NOX2); nitrogen monoxide (NO); nuclear magnetic resonance (NMR); one-dimensional NMR (1D-NMR); orthogonal partial least square-discriminant analysis (OPLS-DA); palmitoylethanolamide (PEA); partial least square-discriminant analysis (PLS-DA); principal component analysis (PCA); posttraumatic stress disorder (PTSD); reactive oxygen species (ROS); reverse-phase liquid chromatography (RPLC); time of flight MS (TOF-MS); tumor necrosis factor alpha (TNF-α); two-dimensional NMR (2D-NMR); ultra-performance liquid chromatography with fluorescence detection (UPLC-FLR), liquid chromatography electrospray mass spectrometry (LC-ESI-MS); uridine diphosphate N-acetylglucosamine (UDP-GlcNAc); weak anion-exchange (WAX)

1. Introduction

1.1. Posttraumatic stress disorder

Posttraumatic stress disorder (PTSD) is a trauma- and stressor-related disorder (APA, 2013) that requires direct or indirect exposure to a single traumatic event or prolonged exposure to stressful events. Such events may include witnessing or confrontation with a traumatic event that happened to a family member or a close friend, and even exposure to the consequences of traumatic events in some professions where exposure is common (Pai et al., 2017). Exposure to a traumatic experience involves serious injury, intense fear, actual or threatened death, horror and helplessness (Bisson, 2007; Yehuda, 2002). The majority of people are likely to be exposed to potentially traumatic events during their lifetime; however, rates of exposure differ among different populations (Kessler et al., 2017; Liu et al., 2017), and development of PTSD is significantly influenced by the type of the traumatic exposure. The traumatic experience most frequently leading to a high risk of PTSD is interpersonal violence (i.e. rape), while the unexpected death of a loved one is associated with a low risk of PTSD (Kessler et al., 2017). The proposed risk factors for PTSD are family and social status, female gender, severity, duration and number of traumatic incidents, childhood abuse and neglect, lack of family and social support, as well as a history of mental illness (Bisson, 2007; Karatzias et al., 2017; Nedic Erjavec et al., 2018; Zoladz and Diamond, 2013).

1.2. Resilience and vulnerability to develop PTSD

PTSD, as a multifactorial and polygenic disorder that shares genetic risk with other psychiatric disorders (Solovieff et al., 2014; Ryan et al., 2016; Duncan et al., 2018; Nievergelt et al., 2018), induces great suffering in patients and their families and carries enormous public health, social, and economic burden to society (Davidson, 2000). Traumatic experience has a major impact on an organism and its cellular, molecular, structural and biochemical systems (Girgenti et al., 2017; Michopoulos et al., 2015; Nedic Erjavec et al., 2018). However, not all individuals exposed to a traumatic event develop PTSD, and the extent to which individuals are vulnerable or resilient to PTSD depends on a variety of biological, genetic, and epigenetic factors (Domschke, 2012; Dulka et al., 2017; Duncan et al., 2018; Ryan et al., 2016; Schmidt et al., 2011), and the interaction between them. Resilience is a process of positive adaptation to particular environmental stress, tragedy, trauma, or adversity, developed through complex interaction of genetic, epigenetic, environmental and neurochemical factors (Li et al., 2017). Resilience or vulnerability to develop PTSD after traumatic exposure differs significantly among exposed individuals (Michopoulos et al., 2015), and therefore the prevalence of PTSD is considerably lower than exposure to traumas (Bisson et al., 2005). It has been estimated that the lifetime prevalence of PTSD ranges from 1.3% to 8.8% (Atwoli et al., 2015). However, combat and war-related traumas are

associated with much higher prevalence of PTSD, ranging from 10.1-30.9% in U.S. veterans (Kang et al., 2003; Mellon et al., 2018), and estimated to be 18% in Croatian veterans (Priebe et al., 2010).

PTSD is rarely an isolated disorder and commonly co-occurs with other psychiatric disorders (Flory, 2015), but also with different somatic disorders (Britvic et al., 2015; Mellon et al., 2018). PTSD interferes with many metabolic pathways causing biological alterations (Michopoulos et al., 2015; Wolf et al., 2016a; 2016b; Mellon et al., 2018). Comorbid somatic disorders include cardiovascular, dermatological, musculoskeletal, pulmonary diseases (Britvic et al., 2015), immune dysfunction and metabolic syndrome (Mellon et al., 2018; Wolf et al., 2016a). Subjects with PTSD have higher prevalence of obesity (Kozaric Kovacic et al., 2009), increased risk for neurodegenerative diseases, cognitive decline and premature aging (Miller et al., 2014; Wolf et al., 2016a; 2016b). More specifically, frequent occurrence of metabolic syndrome in combat-related PTSD is associated with decreased cortical thickness and consequently cognitive decline induced by temporal and frontal neurodegeneration (Wolf et al., 2016a; 2016b). In addition, PTSD is often characterized with psychotic features (Hamner, 2011; Pivac and Kozaric-Kovacic, 2006; Compean and Hamner, this issue), that worsen the clinical presentation of PTSD. Novel assessment in International Classification of Diseases, 11th revision, proposes the distinction between PTSD and complex PTSD (Karatzias et al., 2017). In complex PTSD, besides core PTSD symptoms, disturbances in self-organization should be present, describing the pervasive psychological disturbances that can follow traumatic exposure (Hyland et al., 2018). Complex PTSD is often severe and highly debilitating (Karatzias et al., 2017). Patients with complex PTSD display significantly higher levels of dissociation, depression, anxiety, borderline personality disorder symptoms, suicidal ideation and self-harm (Hyland et al., 2018). In addition, early life trauma, as well as multiple and interpersonal traumas, are more frequently associated with complex PTSD (Karatzias et al., 2017).

1.3. Treatment of PTSD

The treatment of PTSD includes psychological interventions (APA, 2017) and pharmacotherapy (Goodnight et al., this issue). Spontaneous remission rates from PTSD in the absence of treatment vary considerably (8-89%), however average remission rates of 44% have been reported (Morina et al., 2014). Recommendations, such as clinical practice guidelines for the treatment of PTSD in adults, given by the American Psychological Association (2017) are cognitive behavioral therapy, cognitive processing therapy, cognitive therapy, and prolonged exposure therapy as the first line psychotherapy, followed by the use of brief eclectic psychotherapy, eye movement desensitization and reprocessing, as well as narrative exposure therapy. Pharmacotherapy of PTSD depends on the treatment goals, such as reduction of comorbidity as well as improvement of stress resistance, whereas the response to

certain pharmacotherapy is due to individual genetic, epigenetic and experiential characteristics (Kao, 2015). The most commonly used pharmacotherapy is treatment with selective serotonin reuptake inhibitors, such as sertraline, fluoxetine, and paroxetine, which decrease hyper-arousal, fear response, and numbing (Kao, 2015; Ravindran and Stein, 2009). In addition to these medications, selective serotonin and norepinephrine reuptake inhibitors, tricyclic antidepressants, monoamine oxidase inhibitors, noradrenergic agents, benzodiazepines and atypical antipsychotics are used in treatment of PTSD. These drugs reduce numbing, hyper-arousal, anxiety and depressive symptoms, sleep disturbances, irritability, re-experiencing and psychotic symptoms (Ravindran and Stein, 2009). However, regarding pharmacotherapy, only fluoxetine, paroxetine, sertraline, and venlafaxine are recommended for treatment of PTSD (APA, 2017).

1.4. Biological findings in PTSD

The focus of this review is on metabolomic and glycomic findings in PTSD. Although this paper is not intended to be an exhaustive review of the biology of PTSD (as this topics will be covered in other reviews in this issue), patients with PTSD show alterations in different biological systems (higher sensitivity of sympathetic nervous system, dysregulated activity of hypothalamic-pituitary-adrenal (HPA) and thyroid axis, altered function of the neurotransmitters (norepinephrine, serotonin, dopamine) and brain derived neurotropic factor (BDNF), increased and premature aging processes shown in shortened telomeres, increased DNA damage, mitochondrial dysfunction and altered values of the “GlycoAge test” (Fragkaki et al., 2016; Gautum et al., 2015; Kao, 2015; Karabatsiakis et al., 2015; Kovacic Petrovic et al., 2016; Mellon et al., 2018; Moreno-Villanueva et al., 2013; Mouthaan et al., 2014; Mustapic et al., 2007; Nedic Erjavec et al., 2018; Pivac et al., 2006; 2007; 2012; Ryan et al., 2016; Sautter et al., 2003; van Zunden wt al., 2015; Yehuda, 2002; 2006; Yehuda and Seckl, 2011). In addition, PTSD is frequently associated with disturbed metabolic pathways (Mellon et al., 2018). Furthermore, inflammation is a frequent finding in PTSD, since C-reactive protein (CRP) and pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), and interleukin 6 (IL-6) are increased in PTSD (Mellon et al., 2018). Inflammatory markers have even been proposed to be used as biomarkers of PTSD (Baker et al., 2012; Michopoulos et al., 2015). While these data point to PTSD as a systemic disease, it remains to be evaluated whether immune activation precedes development of PTSD after traumatic exposure or is a consequence of PTSD (Mellon et al., 2018).

Besides altered biological function, genetic data from family association, twin, and genome-wide association studies suggest that heritability of PTSD ranges from 30-40% (Almli et al., 2014; Duncan et al., 2018). As in other complex mental disorders, a number of genes with small effect sizes contributes to the cumulative risk for PTSD (covered by Ressler et al., this issue). There are different findings

regarding the association (or a lack of association) between the candidate genes, coding for the proteins involved in different biological systems, and risk for PTSD (Almli et al., 2014; Digangi et al., 2013; Solovieff et al., 2014; Ryan et al., 2016). However, most of the data confirm hypothesized biological underpinnings of PTSD, showing risk gene variants in the serotonergic, dopaminergic, and GABA systems, immune system, HPA axis, neuropeptide Y, BDNF, apolipoprotein E, and other genes related to response to stress, fear, startle, cognition and anxiety (Nedic Erjavec et al., 2018; Ryan et al., 2016). Complex gene and environment interactions further complicate the understanding of the PTSD etiology, and it has been suggested that vulnerability to develop PTSD after traumatic experience results from numerous, independent gene x environmental interactions (Ryan et al., 2016). Candidate genes investigated in genetic or epigenetic studies of PTSD included genes for dopamine receptors type 2 and 4, dopamine transporter, catechol-o-methyl transferase, dopamine-beta-hydroxylase, ankyrin repeat and kinase domain containing 1, for serotonin receptor type 2A and serotonin transporter, for CRH receptor type 1, glucocorticoid receptor and FK506 binding protein 5, for BDNF, apolipoprotein E, pituitary adenylate cyclase-activating polypeptide type I receptor, cannabinoid receptor, C-reactive protein, mannosidase alpha class 2C member 1, opioid receptor-like 1, protein kinase C alpha and spindle and kinetochore-associated complex subunit 2, and many others (Ryan et al., 2016). Genome wide association studies (GWAS) offered a great number of novel loci and novel genes, but these variants are rarely replicated, or do not survive corrections for the genome-wide significance (Duncan et al., 2018).

Epigenetic changes can influence gene expression (Domschke, 2012; Ryan et al., 2016; Kim et al., 2017; Uddin et al., 2011), and therefore epigenetic research in PTSD (covered by Morrison et al., this issue) might provide an explanation how the environment affects or interacts with genes, making some individuals more vulnerable or resilient to develop PTSD after trauma exposure. Studies show that early childhood traumatic experience influences DNA methylation patters and people with PTSD show a unique DNA methylation signatures (Klengel et al., 2013). Therefore, improved knowledge on the genetic and epigenetic changes in PTSD might help in the early prediction of vulnerable individuals, offering possible targeted preventative interventions (Ryan et al., 2016). It has been proposed that PTSD is the result of the physiological adaptations affected by early life experiences, to allow survival after traumatic events (Yehuda and Seckl, 2011).

There is great heterogeneity in the biological (genetic and epigenetic) underpinning of PTSD as well as in the molecular architecture associated with vulnerability to development of PTSD after traumatic exposure. Due to this heterogeneity, there are few validated biomarkers for PTSD, and some proposed biomarkers (Zhang et al., 2011) still lack specificity and sensitivity. Biomarkers are non-invasive and

objective measures of patient diagnosis, prognosis, and treatment (Nikolac Perkovic et al., 2017). Some genetic or epigenetic marks might be used as early biomarkers to predict vulnerable individuals (Ryan et al., 2016). Theranostics is a discipline that combines diagnosis, prognosis, and therapy and offers targeted therapy based on specific analyses. Novel high throughput techniques and approaches called "omics" include information of the entire genome data (genomics), transcription products (transcriptomics), protein products (proteomics) and metabolic products (metabolomics) of an organism (Figure 1). "Omics" approach and high-throughput technologies enable the analyses of relationships, roles and mechanisms of action of various molecules, and provide better understanding of the normal physiological but also pathological processes and offer a new approach to explore various disorders and diseases.

Therefore, a theranostic approach, combined with "omics" approaches, might improve the knowledge about the biological underpinning of PTSD, and offer novel diagnostic, prognostic, and therapeutic biomarkers and ultimately novel therapeutic strategies. There is a high priority for clearly defined and validated biomarkers, based on the "omics" approach. With these novel approaches, metabolomics and glycomics might also increase understanding of the biological alterations in PTSD. Studies using metabolomics and glycomics should more deeply investigate complex neurometabolic alterations in PTSD. These novel biomarkers should be used to discriminate between individuals who are vulnerable or resilient to development of PTSD, provide insights into the disease pathogenesis, and could be potentially used for development of targeted treatments and interventions. If identified early, vulnerable individuals exposed to trauma might receive appropriate psychological or pharmacological interventions prior the full development of PTSD (Ryan et al., 2016).

2. Metabolomics

2.1. Metabolomic approaches

Metabolomics is a fast-developing scientific discipline and one of the analytical "omics" technologies with the most comprehensive approach (Figure 1), (Alonso et al., 2015; Kaddurah-Daouk and Krishnan, 2009; Quinones and Kaddurah-Daouk, 2009; Wood, 2014). It seeks to study changes of an organism in a set of small molecules (metabolites) that are the final or intermediate products of biochemical processes driven by genetic regulation (Gonalez-Pena et al., 2015; Kaddurah-Daouk and Krishnan, 2009; Mastrangelo et al., 2016; Quinones and Kaddurah-Daouk, 2009). Additionally, the metabolome, as a complex, large, and dynamic set of metabolites, can be affected by various exogenous and endogenous factors. Alternations in the metabolome might occur due to the dysfunction in metabolic pathways, or could represent adaptation mechanisms to some internal changes (physiological or pathological) or to external, environmental factors such as stress, nutrition, or drug administration

(Danielsson et al., 2012; German et al., 2008; Knee et al.,2013; Nedic Erjavec et al., 2018; Pontes et al., 2017).

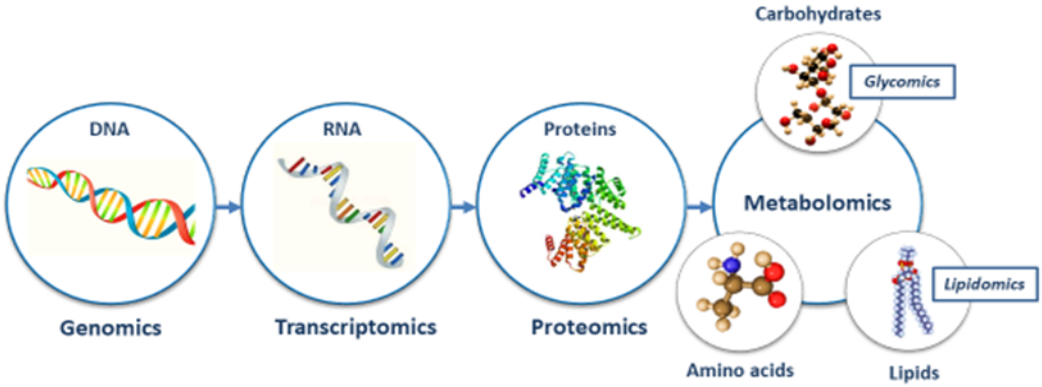

Figure 1. "Omics" technologies: genomics, transcriptomics, proteomics, metabolomics

Several strategies, such as metabonomics, metabolomics, metabolic fingerprinting, metabolite profiling and target analysis are used. While metabolite profiling refers to quantification of previously identified metabolites, metabolomics is an unbiased approach. Metabolic fingerprinting provides classification, metabolite target analysis refers to quantification and qualitative analysis of specific metabolites, and metabonomics enables evaluation of altered metabolites due to treatment or disease (Dunn and Ellis, 2005). Metabolomics has a wide application in various scientific areas, such as zoology, botany, environment, pharmacology, nutrition, microbiology, toxicology, diagnostic, biomedicine and in many others (Alonso et al., 2015; Beckonert et al., 2007; Dunn et al., 2013; Pontes et al., 2017; Wu et al., 2008). In biomedicine, metabolomics plays an important role in identification and development of potential biomarkers for medical treatments and various diseases (Alonso et al., 2015; Danielsson et al., 2012). Analytical strategies applied in metabolomics can be classified into untargeted, semi-targeted and targeted analyses. Usually studies start with an untargeted, non-hypothesis driven approach, which later helps to generate hypothesis for further targeted steps (Kaal and Janssen, 2008; Naz et al., 2014; Patti, 2011). In contrast to untargeted metabolomics, identities of metabolites in semi-targeted and targeted metabolomics are known before data collection (Dunn et al., 2013; Patti, 2011). Therefore, targeted metabolomics provides absolute, while untargeted metabolomics reveals relative values (Wood, 2014). The workflow in a metabolomics study is summarized in Figure 2. Clear identification of the biological question will lead to the proper sample selection. Any type of biological matrix (biofluids, tissue, or cells) can be used in metabolomics with an adequate sample pretreatment (Gonzalez-Pena et al., 2016; Mastrangelo et al., 2016; Naz et al., 2013). The biofluids most frequently analyzed in metabolomics are saliva, serum, plasma, CSF, and urine (Cruickshank-Quinn et al., 2017;

Nedic Erjavec et al., 2018; Wood, 2014), whereas other biological materials include human cell lines, tissue biopsies, brain dialysates and blood cells (Wood, 2014).

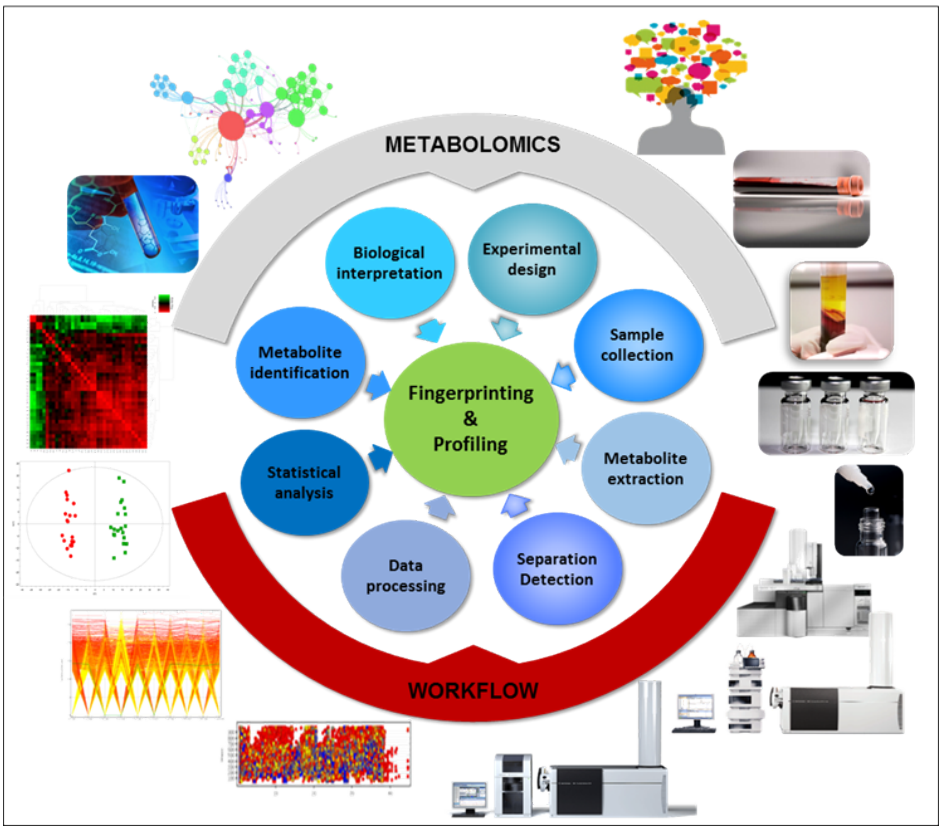

Figure 2. Metabolomics workflow

Sample preparation always starts with deproteinization and depends on the type of the separation technique applied. The selection of the analytical technique or set of techniques is based on the availability of the instrumentation, amount (volume) of sample, time and financial resources, as well as to the study target and sample type. After getting the global profile of the samples, profiles are overlaid, univariate and multivariate statistics are applied, and the metabolites that are statistically different are identified. Finally, the metabolites altered by the condition or disease should be included in biochemical pathways and their changes explained based on previous knowledge. When the potential biomarker is proposed as a diagnostic marker, it should be validated in a relevant cohort and with an ad hoc technique (Gonzalez-Pena et al., 2016; Mastrangelo et al., 2016; Naz et al., 2013). The study of metabolome is very complex from an analytical point of view, due to the variability of physico-chemical properties and ranges of concentrations that metabolites exhibit. Polarities can include small ionic compounds, such as oxalate, or non-polar lipids as triglycerides and concentration ranges between molar and picomolar. For that reason, one single analytical technique does not suffice to obtain all the information (Gonzalez-Pena et al., 2016; Mastrangelo et al., 2016; Naz et al., 2013). Two

groups of techniques have been classically used: 1) Hydrogen-Nuclear Magnetic Resonance (HNMR), which is robust but with low sensitivity 2) Mass spectrometry (MS), with high sensitivity but low robustness, which is usually coupled to a separation technique such as liquid chromatography (LC), gas chromatography (GC), and/or capillary electrophoresis (CE). In addition to concentration and quantification, one-dimensional NMR (1D-NMR), which is commonly used, as well as two-dimensional NMR (2D-NMR), provide information about chemical structure (Alonso et al., 2015).

2.2. Metabolomic findings in patients with PTSD

Only a few studies have examined the metabolome in PTSD and compared these metabolite data to control subjects (reviewed in Mellon et al., 2018; and Nedic Erjavec et al., 2018). Some studies reported possible associations between PTSD and gut microbiome, visceral adipose tissue and sympatho-medullary-adrenal activity, but more research is necessary for better understanding of these findings (Hemmings et al., 2017; Mellon et al., 2018; Pace and Heim, 2011). Lower N-acetylaspartate/creatine ratio was found in the anterior cingulate region of 11 children and adolescents with PTSD who were maltreated, compared with 11 healthy matched subjects (De Bellis et al., 2000), but a small sample size, the use of a single voxel proton magnetic resonance spectroscopy, tissue heterogeneity within the voxel, and a lack of absolute quantification of metabolite measurements limited the significance of this finding. In the first study evaluating metabolites in adult patients with PTSD, which included 20 PTSD participants and 18 healthy controls, 20 metabolites were identified as potentially associated with psychopathological alterations in PTSD (Karabatsiakis et al., 2015). These metabolites, based on their structure and function, can be divided into six categories: monosaccharides, nucleosides, fatty acid metabolites, glycerophospholipids, bile acids, and antioxidants (Karabatsiakis et al., 2015). However, both the multivariate and the univariate analysis in the study Karabatsiakis et al. (2015) revealed palmitoylethanolamide (PEA) and glycerophosphoethanolamine PE(17:1(9Z)/18:0) as the strongest candidates for involvement in PTSD. Clinical studies revealed the importance of endocannabinoid and glycerophospholipid pathways in PTSD patients, while altered metabolites in PTSD were associated with carbohydrate, lipid and amino acid metabolism, especially with glucose metabolism, i.e. citric acid cycle (Karabatsiakis et al., 2015; Mellon et al., 2018). In addition, plasma saturated and unsaturated fatty acids were altered in PTSD patients. Fatty acids such as linolenate, linoleate, docosahexaenoate (DHA), eicosapentaenoate (EPA) and docosapentaenoate (DPA), which have an important role in neuroprotection, were decreased in the plasma of subjects with PTSD (Mellon et al., 2018). Lower levels of DHA, vaccenic acid and eicosatrienoic acid, but higher levels of erucic acid were found in erythrocytes of 49 patients with civilian trauma-related PTSD when compared to 46 control subjects (de Vries et al., 2016). However, after adjusting for sociodemographic and dietary factors, only changes in DHA levels (i.e. lower levels in PTSD patients vs. controls) and

erucic acid levels (i.e. higher levels in PTSD patients vs. controls) were significant (de Vries et al., 2016). The metabolite differences between PTSD patients and control subjects can result from mitochondrial dysfunction, alterations in diet, microbial antigens or metabolism, inflammation and energy metabolism (Mellon et al., 2018). Among antioxidants, pantothenic acid (vitamin B5), which plays an important role in protecting against oxidative stress, was down-regulated in PTSD (Karabatsiakis et al., 2015). Oxidative stress, defined as a metabolic imbalance between anti- and pro-oxidants generating excessive amounts of reactive oxygen species (ROS) (Cipak et al., 2008), is suggested to be bidirectionally associated with PTSD (Miller and Sadeh, 2014). In the case of lipid peroxidation, reactive aldehydes are generated, which are known to act as "second messengers of free radicals" for long periods and are effective on humoral, systemic levels (Gveric-Ahmetasevic et al., 2009; Sredoja-Tisma et al., 2009). 4-hydroxynonenal and related aldehydes are acting as bioactive markers of oxidative stress involved in the pathology of major human diseases, including metabolic and neurodegenerative disorders (Borovic Sunjic et al., 2005; Weber et al., 2013; Zarkovic et al., 2006; 2017). Their associations with PTSD are currently studied by metabolomics complemented by specific immunochemical methods (Fedorova and Zarkovic, 2017; Nedic Erjavec et al., 2018). In line with the assumption that oxidative stress and pro-inflammatory signaling pathways are affected or initiated by stress or trauma (Miller and Sadeh, 2014) are also findings of lipid peroxidation involvement in generalized anxiety disorder and of decreased antioxidant capacities in panic disorder (Bulut et al., 2013; Ozdemir et al., 2012). In addition to these metabolites, nitrogen monoxide (NO) plays different physiologically significant roles, including regulation of stress response, acting both as reactive oxygen and nitrogen species. Abnormalities in metabolism of NO are observed both in PTSD and in acute stress disorder (Bersani et al., 2016; Yeh et al., 2002). As NO is associated with inflammation processes and PTSD, global arginine bioavailability ratio (GABR) (GABR = arginine/citrullin + ornithine) has been determined, which represents the capacity for NO synthesis (Bersani et al., 2016). The study reported that PTSD subjects had increased levels of TNF-α, CRP, IL-6, and ornithine, as well as decreased levels of arginine, while citrullin did not differ between PTSD and control subjects; i.e. GABR value was decreased in PTSD patients compared to values in healthy controls (Bersani et al., 2016). However, comorbid depression and antidepressants could affect these GABR findings in PTSD subjects (Bersani et al., 2016). Moreover, choline levels were unchanged, whereas N-acetylaspartate (NAA) and creatine levels were decreased, in hippocampus of PTSD patients compared to levels in healthy controls (Schuff et al., 2001). A lower ratio of NAA and creatine was also detected in adolescents and children with PTSD (De Bellis et al., 2000). However, alcohol abuse and metabolic and oxidative impairments might be partially responsible for the observed reductions (De Bellis et al., 2000; Schuff et al., 2001).

A small number of studies evaluated differences in particular metabolites in subjects with PTSD and healthy control subjects, and these findings suggest that some metabolomics markers, as well as some markers of oxidative stress, could be associated with particular mechanisms of PTSD. One can speculate that observed differences in metabolites between PTSD patients and healthy controls could also be the result of the suboptimal adaptation to changed environmental circumstances, such as trauma exposure. In order to suggest more concrete conclusions about the direct association between metabolites and mechanisms included in the etiology of PTSD, more studies should be conducted, confirmed, and replicated in larger groups of subjects with PTSD.

2.3. Metabolomic findings in animal models of PTSD

In addition to human subjects, animal models with PTSD-like features are also used in metabolomic studies. Although animal models cannot completely replicate the processes, genetic regulation, and characteristic symptoms in humans, they can be a valuable tool to understand altered metabolic pathways and brain circuits, and could be used to develop potential biomarkers and pharmaceuticals (Kao, 2015). Development of PTSD-like symptoms in animal models can be modelled with various stressors, such as early life stress, physical, psychogenic, and psychosocial stress (Kao, 2015). In animal models of PTSD induced by a single prolonged stress, behaviors related to anxiety and elevated fear learning were accompanied by elevated oxidative stress and neuroinflammation, expressed as increased levels of malondialdehyde, IL-6, hippocampal nicotinamide adenosine dinucleotide phosphate oxidase 2 (NOX2), and 4-hydroxynonenal, as well as decreased density of hippocampal parvalbumin interneurons (Liu et al., 2016). In a predator stress paradigm, another putative animal model of PTSD, increased concentrations of ROS and pro-inflammatory cytokines were found (Wilson et al., 2013). C57BL/6 strain mice repeatedly exposed to a trained aggressor mouse of a different strain were used as an animal model with PTSD features, by applying a modified, resident-intruder, social defeat paradigm. Subject mice first showed effects relevant to acute stress disorder, such as gaining body weight and developing inflammation, and after 1.5 and 6 weeks of stress showed behaviors similar to PTSD symptoms (Gautam et al., 2015). The study demonstrated that a long stressful period of 5 days' duration caused alterations in 40 metabolites measured in plasma after 24 hours and 14 metabolites measured after 1.5 weeks. Similarly, after 10 days long stressful period, 20 metabolites in plasma were altered at 4 weeks and 37 metabolites were changed at 24 hours (Gautam et al., 2015). The finding that more mice subjected to a 10-day stress protocol had less alterations in metabolites, suggests that mice adapt to stressors and their metabolite levels normalize over time. In addition, gut microbiota derived metabolites (hippurate, phenylpropionylglycine and 3-phenylpropionate) were increased after chronic and acute stress protocols in aggressor-exposed mice (Gautam et al., 2015). However, metabolites in plasma, including lipids, carbohydrates and amino acids, differed between

acute and chronic stress protocols. Carbohydrate metabolite levels were elevated after acute protocols but reduced following withdrawal from stress. In addition to carbohydrates, groups subjected to 5- and 10-day stress protocols showed differences in amino acid levels, whereas lipid metabolites were increased at all-time points (Gautam et al., 2015). Acute alternations observed in this animal model of PTSD included inflammation, altered plasma metabolite levels and tissue damage, while chronic changes included altered metabolite levels, activation of lipid metabolism, hyperlipidemia and changed gene expression (Gautam et al., 2015). Moreover, mitochondrial dysfunction, upregulation of 35 mitochondrial genes and altered fatty acid metabolism were observed in mice exposed to stress (Mellon et al., 2018; Zhang et al., 2015). Metabolomics of microdialysates from mouse brains revealed an association between basal metabolites in the prefrontal cortex and fear associated with foot shock stress (Kao et al., 2015). Metabolites, such as sarcosine, nicotinate, kynurenic and xanthurenic acid were associated with behavioral changes in this mouse model of PTSD (Kao et al., 2015). Twenty-eight days after the foot shock, prolonged fluoxetine treatment reduced PTSD-like behaviors and alterations in nucleus accumbens and anterior cingulate cortex of mice, by affecting metabolic pathways involved in energy metabolism (Kao et al., 2016). Additionally, a trend in citric acid cycle downregulation was observed, while metabolites like succinic, isocitric, citric, aconitic and oxalacetic acids were decreased in nucleus accumbens of shocked mice. In anterior cingulate cortex of foot-shocked mice, metabolites such as succinic, oxolutaric and oxalacetic acid were increased, whereas aconitic, isocitric, citric and pyruvic acids were decreased (Kao et al., 2016). Another metabolomic study in mice and hamsters was performed in order to determine possible metabolite differences between animals resistant and susceptible to development of PTSD-like behaviors (Dulka et al., 2017). Amino acids such as cystine, fumarate, and methionine were elevated in the nucleus accumbens of resistant compared to susceptible mice, while in hamsters, fumarate levels were increased in dominant compared to subordinate animals. On the other hand, susceptible mice had elevated GABA levels in hippocampus, whereas dominant hamsters had elevated tyrosine levels in ventromedial prefrontal cortex and lower serotonin levels in basolateral/central amygdala. Elevated levels of ATP products were also observed in ventromedial prefrontal cortex of resistant mice (Dulka et al., 2017). In a rat model of chronic unpredictable mild stress, susceptible and resilient rats exposed to stress had different metabolite levels and significantly decreased body weight compared to control rats (Li et al., 2017). The reduction in sucrose preference was observed in susceptible rats, but not among resilient rats when compared to control animals. Adenosine, creatinine, stigmasterol, serotonin, oleic acid, β and γ-tocopherol, 4,5-dimethyl-2,6 dihydroxypyrimidine, N-acetyl-glucosamine and myo-inositol were increased, while metabolites like glycine, malic and dehydroascorbic acid, ornithine, L-lysine and L-glutamic, were decreased in resilient compared to control rats (Li et al., 2017). Components of citric acid cycle, such as pyruvate, malic, citric, fumaric and lactic acid were decreased, whereas cysteine, xanthine, aspartic

acid and lanosterol were elevated in resilient rats (Li et al., 2017). However, only malic acid presented a resilient- specific metabolite. In line with decreased levels of amino acid ornithine in resilient group of rats (Li et al., 2017), increased ornithine levels were found in plasma of PTSD-like subjects (Bersani et al., 2016), suggesting an important role of ornithine and arginine, as a components of urea cycle, in resilience to PTSD.

In summary, the presented findings reveal significant stress-induced changes in metabolome in animal models. According to the results of several above mentioned studies, the affected metabolic pathways include different processes such as (neuro-) inflammation, auto-immune reactions, oxidative stress, and energy metabolism. These results further underscore the complex etiology of PTSD, as well as the fact that PTSD is a neuropsychiatric disorder with systemic effects. Of course, further studies are needed to clarify the biological underpinnings of PTSD.

3. Glycomics

3.1. Glycomic approach

Glycans are oligosaccharide chains covalently attached to biomacromolecules such as proteins and lipids which can significantly alter their physiochemical properties and consequently their biological role (Varki A, 1993). Glycomics is an “omics” approach technology that determines all glycans released from glycoproteins (Miura and Endo, 2016) and represents the systematic study of the glycome, a complete repertoire of glycan structures with their functional roles in biological systems (i.e. in cells, tissues or organisms), which can be observed under specific conditions such as time, location and environment. Each organism and even each cell type has its own distinct glycome, determined by both genetic and environmental factors, although its size is yet unknown. The great diversity and dynamics of the glycome during development as well as in various conditions and disorders, suggests that glycans should be studied in whole by using glycomics (Rudd et al., 2017). In complex organisms, glycans play an important role in virtually all processes that involve more than a single cell (National Research Council, 2012). The glycan parts of (glyco)proteins are integral elements of the final molecular structure and together with amino acids in the polypeptide backbone form a single molecular entity that performs biological functions. The majority of membrane and secreted proteins are post-translationally modified by covalent addition of glycans with very high site occupancy, thus changes in glycosylation of cell membrane receptors in response to external or internal stimuli, can drastically change their affinity to target molecules or cells (Zielinska et al., 2010) and in this manner participate in cell signaling, migration, angiogenesis and development (Ohtsubo and Marth, 2006). However, glycans generally do not turn physiologic processes on and off, but rather modify the behavior of the cell by responding to internal or external stimuli. This is particularly seen in immunological system

where glycans play a role in leukocyte migration (Mitoma et al., 2007), antibody-dependent cytotoxicity (ADCC) (Masuda et al., 2007) and pathogen-host interactions (Ilver et al., 1998). For example, glycans attached to immunoglobulin G (IgG) have very profound effects on protein structure (Subedi and Barb, 2015) and alternative glycosylation (attachment of different glycans) affects binding of IgG to all Fc receptors and is in this way analogous to variation in protein sequence due to genetic variations (Subedi and Barb, 2016). Glycans contribute to both structure and function of glycoproteins, but, contrary to the polypeptide part that is directly encoded in the genome, glycan parts of glycoproteins do not have a direct genetic template (Kristic et al., 2014). Instead, final glycan structures are determined through dynamic interactions between hundreds of proteins and small molecules that form the complex pathway of glycan biosynthesis (Shen et al., 2017). Namely, glycans are shaped by fine-tuning of genetic variations, epigenetic and gene expression regulation, post-translational modifications, and activity of corresponding proteins (Gornik et al., 2012). The majority of glycosylation processes occurs in the endoplasmic reticulum and Golgi apparatus and involves many different enzymes such as glycosyltransferases, glycosidases and transporters, transcriptional factors, and other proteins (Moremen et al., 2012). The major types of glycans present in mammals, N-glycans, O-glycans, glycosphingolipids and glycosaminoglycans, are very heterogeneous as they differ in size, charge, occurrence, and complexity (Moremen et al., 2012). Enormous variability of glycan determinants results from different number, order, and type of sugar monomers, diverse in their numeric configuration, position, and branching at protein multiple sites (Cummings, 2009). In addition, protein glycosylation is tissue-specific and therefore glycoproteins in distant tissues, although sharing the same protein sequence, might differ in glycan profile (Almeida and Kolarich, 2016). Free glycans or glycans that are part of glycoproteins or glycolipids bind to glycan receptors - lectins. In animals, major classes of lectins based on their amino acid sequences and biochemical properties, are C-type lectins such as selectins, collectins and endocytic lectins, and S-type lectins such as galectins (Ghazarian et al., 2011). Lectin–glycan interactions are crucial for various physiological as well as pathological processes (Kletter et al., 2013). The most common protein glycosylation is N-glycosylation, and its absence is embryonically lethal (Marek et al., 1999), whereas mutations that obstruct proper glycosylation cause debilitating diseases (Freeze, 2006). Variations in genes have been found to be associated with changes in glycosylation (Hennet, 2012; Huffman et al., 2011; Lauc et al., 2010), whereas epigenetic regulation of some genes was also found to affect protein glycosylation (Zoldos et al., 2012). After the initial discovery of altered IgG glycosylation in rheumatoid arthritis (Parekh et al., 1985), hundreds of studies demonstrated altered glycosylation in different diseases. Inter-individual differences in glycosylation are important and associated with predisposition and course of numerous diseases, such as congenital, immunological and infectious disorders, cardiovascular and neurodegenerative diseases, cancer, and diabetes. (Lauc et al., 2016). However, the functional relevance of differences in glycosylation found in

disease compared with healthy states and mechanisms underlying these differences are still insufficiently understood (Lauc et al., 2016). As glycans integrate genetic and environmental factors, they are more closely associated with complex diseases than sequence variations in the genome (Zoldos et al., 2013). Glycosylation reflects the biological state of an organism and hence represents a potential biomarker for disease susceptibility and its course, as well as to treatment response (Lauc et al., 2016). Therefore, no biological analysis at systems level is complete without investigating the glycome in addition to the genome, transcriptome, proteome and metabolome. However, due to glycan complexity and methodological issues, glycomics has been falling behind genomics and proteomics, and in comparison to proteins and genes, fewer techniques have been available for glycan analysis (Sato, 2016).

Recently, various techniques have been developed for studying the glycome at different levels (Hart and Copeland, 2010). A number of high-resolution and sensitive techniques are available today for glycan research, such as CE, high performance liquid chromatography (HPLC), MS and lectin microarrays (Hart and Copeland, 2010). In addition, a variety of high-throughput approaches for glycan analysis are currently in use including ultra-performance liquid chromatography with fluorescence detection (UPLC-FLR), liquid chromatography electrospray mass spectrometry (LC-ESI-MS), capillary gel electrophoresis with laser induced fluorescence detection (CGE-LIF), matrix assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), nano-liquid chromatography coupled to tandem mass spectrometry (nano-LC MS/MS) and liquid chromatography triple quadrupole mass spectrometry with multiple reaction monitoring (LC-QqQ-MS with MRM) (Trbojevic-Akmacic et al., 2016). Glycans can be obtained from cell lysates, homogenized tissue, enriched membranes, as well as from different body fluids such as serum/plasma, urine, saliva, tears, milk, semen or amniotic fluid, but most analytical methods provide relative rather than absolute quantification of glycans in a sample (Etxebarria and Reichardt, 2016). In general, glycans are first enzymatically or chemically released from glycoproteins, then separated using different stationary phases and finally detected (Figure 3). Labelling or chemical modification of released glycans is performed in order to optimize HPLC and CE, and improve MS detection employing linkage-specific glycosidases and/or the use of glycan standards.

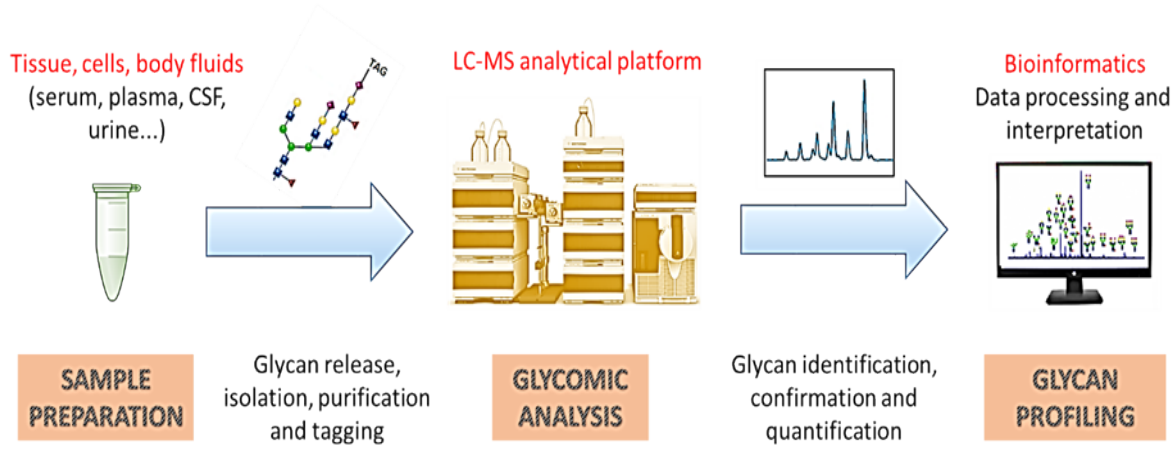

Figure 3. Glycomics workflow

A combination of approaches is usually applied, investigating the individual glycan repertoire as well as performing global glycan analysis (Rudd et al., 2017). Basic classes of glycomic analyses are glycoprofiling, glycan class characterization, and full structural analysis, each providing specific information. Glycoprofiling is one-dimensional separation used to obtain a signature of the glycome profile by applying methods such as HPLC, CE and MS. Glycan class characterization uses technologies such as weak anion-exchange (WAX) HPLC and MS to separate glycan mixtures into different glycan types and to provide their relative quantification. Detailed (full) structural analysis determinates monosaccharide sequence and modifications, anomericity, and linkage of the glycans in a glycome, using HILIC separation and MS. All of these glycomic approaches produce high quantities of data that need to be analyzed. Although progress in bioinformatics and databases for major analytical platforms used in glycan research has recently been accelerating, it is still at the beginning compared to genomics and proteomics (Aoki-Kinoshita, 2008). Therefore, the development of bioinformatic analysis tools such as algorithms to support the characterization of glycan structures for high-throughput applications, as well as generation of databases containing well-structured glyco-related data, will foster the integration of glycobiology into all fields of biomedical science.

3.2. Glycomic findings in human subjects with PTSD

While significant changes of N-glycome were found in several psychiatric and neurodegenerative disorders, such as schizophrenia (Bauer et al., 2010; Stanta et al., 2010), major depressive disorder (Park et al., 2018), attention-deficit hyperactivity disorder (Pivac et al., 2011), dementia (Vanhooren et al., 2010) and Alzheimer's disease (Lundström et al., 2014), studies of N-glycosylation in PTSD are scarce. One small study (Moreno-Villanueva et al., 2013) involving 13 individuals with PTSD, 9 trauma-exposed (high-stress) subjects and 10 subjects exposed to low-stress, measured nine plasma N-glycan structures in association with traumatic load and stress exposure. It has been previously shown that a

GlycoAge Test, the logarithmic ratio of two N-glycan structures, agalactosylated core-a-1,6-fucosylated biantennary N-glycan (FA2), whose concentration grows with aging, and bigalactosylated core-a-1,6-fucosylated biantennary (FA2G2), whose concentration decreases in older age, can be used as a predictor of biological age (Pucic et al., 2011; Vanhooren et al., 2007; 2010). In this study, subjects with PTSD and higher traumatic load had a significantly higher GlycoAge index compared to low stress group and this effect correlated with the amount of stress an individual experienced (Moreno-Villanueva et al., 2013). The presented results suggested accelerated aging in PTSD subjects in comparison to age-matched healthy individuals (Moreno-Villanueva et al., 2013), supporting findings using GlycoAgeTest in patients with dementia or Cockayne syndrome, involving neurodegeneration and premature ageing (Vanhooren et al., 2010). In patients with PTSD (Moreno-Villanueva et al., 2013), decreased bigalacto core-1,6-fucosylated bisecting biantennary glycans (FA2B2G2) and increased agalacto core-1,6-fucosylated bisecting biantennary glycans (FA2B) were detected. FA2B2G2 glycans, which are reduced in PTSD, are also lower in patients with hepatocellular cancer (Liu et al., 2007) and in patients within 24 hours of having major abdominal surgery (Gudelj et al., 2016). On the other hand, increased biantennary glycans (FA2B) in PTSD are usually found to be elevated in older age. As older age and PTSD are frequently associated with inflammation, these findings suggest that subjects with PTSD have higher markers of inflammation and older age, although tri- and tetra-antennary sialyted, and not biantennary glycans, are found to be increased in various inflammatory process (Gudelj et al., 2016). The discrepancies might be explained with the lower resolution of N-glycan determination and/or the small number of subjects included in the above PTSD study (Moreno-Villanueva et al., 2013).

Both acute and chronic stress increase the risk of immune system dysregulation, and this is particularly seen in individuals suffering from PTSD (Boscarino, 2004; Glaesmer et al., 2011; Mellon et al., 2018; Pacella et al, 2013), and PTSD patients are more prone to development of autoimmune (O'Donovan et al., 2015) and inflammatory diseases (Boscarno, 2004; Lindqvist et al., 2014; Wang et al., 2017). Some studies have also shown that antennary fucosylation with increased number of antennas and sialic acid residues, as well as smaller numbers of added galactoses on IgG bound N-glycans, which is characteristic of acute and chronic inflammation, and observed in patients with rheumatoid arthritis (Arnold et al., 2008; Reiding et al., 2017). Tri- and tetra-antennary sialyted plasma glycans are usually increased in inflammatory processes (Gudelj et al., 2016), whereas a lack of core fucose on IgG drastically increases antibody-dependent cellular cytotoxicity (Masuda et al., 2007). Therefore, strong connections between PTSD and inflammatory processes are evident; however, their underlying mechanisms and associations with changes in N-glycome are still uncertain. Higher number of subjects with PTSD and more precise determination of glycans are necessary to provide more definitive conclusions on whether and how the glycosylation patterns mediate PTSD symptomatology. The

possible biological mechanisms involved in PTSD include cytokine disbalance (Wang et al., 2017) and changes in proportion of naïve, cytotoxic and memory T-lymphocytes (Sommershof et al., 2009), which follow the pattern of T-cells distribution in older age individuals (Shen et al., 1999). It has been shown that individuals with PTSD have elevated proinflammatory cytokines such as IL-1, IL-6 and IFN- (Wang et al., 2017), as well as significantly higher ”pro-inflammatory score” (Lindqvist et al., 2014). Elevated peripheral inflammatory cytokines could trigger neuroinflammation, by crossing the blood-brain barrier and causing the inhibition of neurogenesis and death of dopaminergic neurons (de Pablos et al., 2014), consequently leading to characteristic PTSD symptoms (Muhie et al., 2017). Other potential contributors to PTSD symptomatology are metabolic syndrome, insulin resistance (Heni et al., 2015) and mitochondrial damage (Naviaux, 2014; Picca et al., 2017; Zhang et al., 2016), which are all commonly present in subjects with PTSD. Namely, patients with PTSD more often develop metabolic diseases, such as diabetes and metabolic syndrome (Blessing et al., 2017; Michopoulos et al., 2016). On the other hand, changes in glycosylation patterns were also reported in people with increased body mass index (BMI) (Nikolac-Perkovic et al., 2014) and diabetes (Lemmers et al., 2017).

There are only a few reports of the observed differences in glycosylation profile in sera of highly stressed individuals who experienced imprisonment in war camps (Barisic et al., 1996) and professional soldiers (Flögel et al., 1996; Lauc et al., 1998), but these subjects did not have a diagnosis of PTSD. In these studies, the N-glycosylation profile was determined by Western blot using five digoxigenin-labelled lectins isolated from plants, each with specificity for different glycosidic bonds. Significantly higher concentrations of 57 kDa glycoprotein (subsequently named stressin) in sera of war prisoners (Barisic et al., 1996) and soldiers (Flögel et al., 1996) was found in comparison to control subjects. The authors concluded that concentrations of this glycoprotein could be positively correlated with stress intensity. Additional analysis (Lauc et al., 1998) showed that N-oligosaccharides, mostly sialic acid, contributed to more than 40% of the total mass of this highly glycosylated protein, in agreement with more recent experiments showing higher sialylation of proteins in inflammatory processes (Gudelj et al., 2016).

These findings of altered particular glycans in acutely stressed, traumatized individuals and subjects with disorders of the central nervous system (Barone et al., 2012) and PTSD (Moreno-Villanueva et al., 2013) suggest that stress-related disorders might be affected or mediated by changes in crucial N-glycan patterns on immunoglobulins and immune cells receptors. However, these results should be validated and replicated using larger samples.

3.3. Glycomic findings in animal models of stress

There is no information on glycosylation pattern in animal models of PTSD, however there are few studies on stressed animals. Although in these studies, the animals were exposed to some traumatic events or acute and chronic stress conditions, no behaviors corresponding to PTSD symptoms in humans were measured. The first studies of glycosylation in animal models of stress were conducted by Tsukada et al. (1989) and Kitajima et al. (1990), who investigated changes in glycosylation patterns of gastric mucosa in rats. They observed that rats exposed to traumatic events had a significantly lower percentage of N-acetylgalactosamine incorporation in gastric proteins than control animals (Tsukada et al., 1989), as well as changes in binding of several lectins to gastric mucosa proteins such as peanut agglutinin (PNA) lectin, specifically binding galactose (Kitajima et al., 1990). More recent experiments studied glycosylation patterns in Atlantic salmon (Salmo salar L.) exposed to long-term stress conditions (Liu et al., 2008). This study used MS analysis to monitor intact glycan serum O-acetylation of glycoprotein bound sialic acid (Liu et al., 2008), the most common modification of sialic acid (Klein and Roussel, 1998). O-acetylated sialic acids could partly regulate virus binding, cell signaling and intercellular interaction, cancer progression, immune system regulation, lectin recognition, as well as gene expression (Corfield et al., 1999; Ghosh et al., 2005; Schauer et al., 2000; Shen et al., 2004; Severi et al., 2007; Sjoberg et al., 1994). Previous studies also identified di-O-acetylation of sialic acid as a major modification of N-glycans in fish serum (Ylonen et al., 2001; 2002). Liu et al. (2008) observed that a major glycoform in salmon sera was monoacetylated sialic acid, which decreased after two weeks' exposure to a stressor, while di-O-acetylated sialic acid significantly increased in comparison to the stress-free fish. After 4 weeks, levels of mono- and di-O-acetylated sialic acids returned to the baseline levels, indicating a possible role of this sialic acid derivative in maintenance of organism homeostasis, adaptation to environmental stress and monitoring of biological response to short- and long-term stress (Liu et al., 2008). However, to our knowledge, there are still no studies showing N-glycosylation patterns in animal models of PTSD, which might elucidate major glycan differences between acutely and chronically stressed or between traumatized vs. non-traumatized animals.

4. Conclusion

To the best of our knowledge, only a few studies evaluated metabolomic and glycomic profiling in patients with PTSD (Table 1). Since these studies included a small number of patients (De Bellis et al., 2000; de Vries et al., 2016; Karabatsiakis et al., 2015; Moreno-Villanueva et al., 2013), further studies with larger groups of patients with PTSD, and replications are needed to confirm or to reject the proposed conclusions. Stress exposure can considerably compromise the immune system and accelerate cellular aging, which can be seen as alterations in the N-glycan profile and in the metabolome of animals or humans. The findings from the literature indicate possible roles of altered

metabolome and changes in N-glycosylation as biomarkers or mediators of acute stress and PTSD, as well as potential targets enabling personalized medicine for PTSD or other diseases. The advances in "omics" technologies, especially in metabolomics and glycomics, combined with other "omics" approaches, might be used to improve the understanding of the molecular, cellular, and circuit basis of biological underpinning of PTSD (Nievergelt et al., 2018). "Omics" based biomarkers will play a major role in identification, characterization, and understanding of PTSD, offering new knowledge that would reveal impairments in metabolic and glycan pathways, metabolite levels, glycoproteins, biochemical processes and their complexity (Lauc et al., 2016; Miura and Endo, 2016; Patti et al., 2012; Zhang et al., 2012). The potential of glycan biomarkers lays in the fact that glycans and glycoproteins reflect the physiological and pathological conditions of individual patients (Lauc et al., 2016; Miura and Endo, 2016). PTSD, like other neuropsychiatric disorders, is a complex polygenic disease and therefore multiple factors are involved in its progression and development. Previous studies have shown the importance of animal models with PTSD-like features in revealing altered metabolites and biochemical processes, which might offer insights into pathophysiology of PTSD and propose novel potential biomarkers (Dulka et al., 2017; Gautam et al., 2015; Kao et al., 2016), although glycomic studies on animal models of PTSD are missing to date. However, in addition to animal studies, clinical studies on human participants are necessary, because animal models cannot completely replicate some specific symptoms, emotions, and processes characteristic of humans exposed to traumatic events and their experience of the trauma. Therefore, both animal models and clinical metabolomic and glycomic studies and their integration with other "omics" technologies, like lipidomics, genomics, epigenomics, transcriptomics and proteomics will improve knowledge about PTSD and might provide insights into biological origin of PTSD, identify molecular targets responsible for the onset of PTSD after exposure to trauma, offer novel theranostic biomarkers and ultimately might provide novel therapeutic approaches (Girgenti et al., 2017; Lauc et al., 2016; Nedic Erjavec et al., 2018; Ryan et al., 2016; Wolf et al., 2016; Zhang et al., 2012). Here we have reviewed a few studies showing metabolomics and glycomics data in subjects with PTSD: at present, we cannot confirm whether these metabolic/glycomic changes are risk factors for PTSD or consequences of PTSD (Mellon et al., 2018). The presented data suggest that metabolomics and glycomics findings might be primarily used as diagnostic and prognostic markers. However, based on the integration of all novel "omics" approaches, further targeted mechanistic studies might provide insight into the possible therapeutic targets, and therefore metabolomic/glycomic studies in PTSD might offer theranostic applications.

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Table 1. Overview of significant findings in the field of metabolomics and glycomics in PTSD

Paper	Subjects	Sample type	Method	Main findings
Kao et al., 2015	FS-induced mouse model of PTSD (exposed and non-exposed group; N_{total}=NA)	Microdialysate fractions of mPFC extracellular fluid	LC-MS	Central metabolites included in citrate cycle, glyoxylate and dicarboxylate metabolisms correlated with the FS-related early changes in arousal. Xanthurenic acid, glucose-1-phosphate, sarcosine and spermidine levels correlated with the FS-related longer-term PTSD-symptoms
Kao et al., 2016	FS-induced mouse model of PTSD (exposed and non-exposed group; N_{total}=15)	Brain tissue punches	LC-MS	Downregulation of the citric acid cycle in NAc and ACC in shocked mice.
Gautam et al., 2015	Mouse model exposed to a trained aggressor mouse and developing behavioral features of PTSD (8 groups: control/treatment, 5/10 weeks of treatment, sampling 24 hours/1.5 weeks/4 weeks after treatment; N_{total} = 40-48)	Blood plasma	GC-MS LC-MS	Gut-derived metabolites (3-phenylpropionate, phenylpropionylglycine, hippurate) altered acute and chronically. Metabolites of gut microbiota (2-4-hydroxyphenyl propionate, indole lactate, phenyl lactate) elevated in treated mice. Carbohydrates, amino acids and lipids levels correlated with stressor duration and the length of stress-withdrawal period.
Karabatsi-akis et al., 2015	PTSD patients and healthy control subjects (N_{total}=38)	Blood serum	LC-MS	13 metabolites (4 glycerophospholipids, 2 fatty acid metabolites, 2 nucleosides, 2 bile acids and derivates, 1 monosaccharide, 1 anti-oxidant) significantly altered in PTSD patients; 12 metabolites correlated with PTSD symptoms determined according to CAPS scores.
De Bellis et al., 2000	Children and adolescents with PTSD and healthy control subjects (N_{total}=22)	Anterior cingulate	Single voxel proton MRS	N-acetylaspartate to creatine ratio was lower in the maltreated children with PTSD.
de Vries et al., 2016	Patients with PTSD and healthy control subjects (N_{total}=92)	Erythrocytes	Capillary GC	DHA was significantly lower in PTSD patients after adjusting for sociodemographic and dietary factors.
Moreno-Villanueva et al., 2013	Patients with PTSD, trauma-exposed patients and control subjects (N_{total}=32)	Blood plasma	DSA-FACE	Higher Glycoage test score in PTSD and trauma-exposed patients. Decreased FA2B2G2 in patients with PTSD. Increased FA2B in patients with PTSD.

NA-not available; LC-liquid chromatography; GC-gas chromatography; MS-mass spectrometry; FS-foot shock; NAc-nucleus accumbens; ACC-anterior cingulate cortex; MRS-magnetic resonance spectroscopy; DHA- docosahexaenoic acid; DSA-FACE- DNA sequencer-assisted flurophore-assisted carbohydrate electrophoresis; FA2B2G2-bigalacto core-1,6-fucosylated bisecting biantennary glycan; FA2B-agalacto core-1,6-fucosylated bisecting biantennary glycan; CAPS- clinician administrated PTSD scale