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III Parksepp, M., **Leppik, L.**, Koch, K., Uppin, K., Kangro, R., Haring, L., Vasar, E., & Zilmer, M. (2020). Metabolomics approach revealed robust changes in amino acid and biogenic amine signatures in patients with schizophrenia in the early course of the disease. *Scientific Reports*, 10(1), 13983. https://doi.org/10.1038/s41598-020-71014-w

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Paper I: the author contributed to data collection, carried out the literature search and critically revised the manuscript.

Paper II: the author contributed to data collection, carried out the literature search and critically revised the manuscript.

Paper III: the author carried out the literature search and reviewed the manuscript.

ABBREVIATIONS

AA(s)	amino acid(s)
Ac-Orn	acetylornithine
Ala	alanine
alpha-AAA	alpha-aminoadipic acid
AP(s)	antipsychotic(s)
Arg	arginine
Asn	asparagine
Asp	aspartate
ATP	adenosine triphosphate
BA(s)	biogenic amine(s)
BMI	body mass index
BPRS	Brief Psychiatric Rating Scale
Cys	cysteine
CS	control subjects
DA	dopamine
DOPA	dihydroxyphenylalanine
DSM(-5)	Diagnostic and Statistical Manual of Mental Disorders (5-th Edition)
FEP	first-episode psychosis
GABA	gamma-aminobutyric acid
GLM	general linear models
Gln	glutamine
Glu	glutamate
Gly	glycine
GPL(s)	glycerophospholipid(s)
HDL	high-density lipoprotein
His	histidine
ICD(-10/-11)	International classification of diseases for mortality and morbidity statistics (10-th/11-th Revision)
Ile	isoleucine
Kyn	kynurenine
KYNA	kynurenic acid
Leu	leucine
LLOQ	lower limit of quantification
LOD	level of detection
Lys	lysine
LysoPC	lysophosphatidylcholine
Met	methionine
Met-So	methionine-sulfoxide
NAD	nicotinamide adenine dinucleotide
NICE	National Institute for Health and Care Excellence
NMDA	N-methyl-D-aspartate

Orn	ornithine
PC	phosphatidylcholine
PE	phosphatidylethanolamine
Phe	phenylalanine
PL(s)	phospholipid(s)
Pro	proline
PS	phosphatidylserine
PUFA(s)	polyunsaturated fatty acid(s)
Ser	serine
SCZ	schizophrenia
SL(s)	sphingolipid(s)
SM(s)	sphingomyelin(s)
SSD(s)	schizophrenia spectrum disorder(s)
Thr	threonine
Trp	tryptophan
Tyr	tyrosine
Val	valine
WHO	World Health Organization

1. INTRODUCTION

Schizophrenia spectrum disorders (SSDs) directly affect 20 million people worldwide (James et al., 2018). These often debilitating psychiatric conditions are a great burden not only to the sufferers and their close ones personally but to the society as well, mainly through healthcare costs and lost years of productivity (Huajie Jin & Mosweu, 2017). The life expectancy of schizophrenia (SCZ) patients is on average 14.5 years shorter than in general population (Hjorthøj et al., 2017). Although the rate of suicide in individuals with SSD is high (about 10%) and the risk of death by accident increased (Hellemose et al., 2018; Sher & Kahn, 2019), the majority of premature deaths in SSD patients' population is caused by more natural causes (Laursen, 2019). The latter include somatic co-morbidities and their suboptimal treatment, substandard lifestyle, probable accelerated aging related genetic factors and adverse effects of medication (Laursen, 2019).

Reasons for a below standard lifestyle among SSD patients are versatile. They can originate primarily from the symptoms of the illness like motivational disturbances but also secondarily due to socio-economic problems and stigmatisation. Common lifestyle risk factors for premature mortality in people with severe mental disorders include smoking, lower dietary quality and little physical activity (Jakobsen et al., 2018). Aforementioned factors in turn increase the risk of dyslipidemia, hypertension, type 2 diabetes mellitus and overall cardiovascular risk (Jakobsen et al., 2018). Additionally, people with co-occurrent SSD are generally less likely to receive guideline treatment for their somatic illnesses, making the unfavourable outcomes even more probable (Laursen, 2019).

Furthermore, cardiovascular risk in SSD patients is increased by yet another factor general population is unaffected by. Namely, the second-generation antipsychotic (AP) medications have shown to have such remarkable metabolic adverse effects, that impose limitations on their use (Correll et al., 2017; Hirsch et al., 2017; Pillinger et al., 2020). First-generation APs, on the other hand, may evoke "typical" side effects – catalepsy and other disabling movement disorders through dysregulation of the involuntary motor system (Nucifora et al., 2017; Gründer et al., 2009). Therefore, one of the biggest challenges in treating SSDs is the constant need to re-evaluate the balance between the medications' benefits and potential harmfulness. Nevertheless, although AP medication is associated with side-effects that may shorten the life expectancy of patients, the overall impact of AP treatment on mortality has been shown to be greatly beneficial compared to medication non-use (Taipale et al., 2018).

Derived from both primary and secondary effects of SSD on persons' physiology, the need to routinely assess patients' physical health, including cardiovascular and metabolic indicators, is evident and highly recommended (National Institute for Health and Care Excellence [NICE], 2014). Monitoring metabolic indicators, however, may not only provide necessary information to support improving patient care directly but also shed light upon the course of the disease itself.

Understanding the pathophysiological mechanisms of psychotic diseases and identifying their biomarkers have the potential to enable both great diagnostical and therapeutical advancements desperately needed (Tayeb et al., 2019). However, in the clinical context, significant limitations apply to measuring biomolecule concentrations, foremost due to its potential invasiveness. Therefore, as a widely accessible and relatively harmless method, the measurements can be done from peripheral blood samples. To understand the metabolic processes accompanying SSDs in more detail, the metabolomic approach can be used. Combination of these methods has been used in research successfully, revealing alterations in the metabolomic profiles of SSD patients (Cao et al., 2019; Comes et al., 2018; D. Wang et al., 2019).

However, the data concerning specific shifts of metabolite levels are still contradictive and insufficient to make comprehensive conclusions due to the small number of studies. Furthermore, SSDs usually have extended duration and different stages of the illness have been shown to have different biochemical characteristics (Boll et al., 2017; Galińska-Skok et al., 2019). Studies taking the duration of the illness into consideration are even more scarce. Our research contributes to filling the gap in the knowledge by describing the shifts in metabolomic profiles of specific lipids, amino acids (AAs) and biogenic amines (BAs) at certain time points of the psychotic illness. In our longitudinal study SSD patients are followed since the outbreak of SSD up to five years into the treatment and the disease's progression. We describe numerous robust changes in metabolite levels that accompany psychotic disorders and their treatment and discuss the potential underlying mechanisms of these alterations to achieve a more thorough insight to the pathophysiology of SSDs.

2. REVIEW OF LITERATURE

2.1. Concept of psychosis

Psychosis, as a functionally disruptive syndrome that may occur in different medical conditions, is an important and often essential target of evaluation and treatment in neurological and psychiatric practice (Arciniegas, 2015). The definition of psychosis has been a matter of dispute and over time different approaches have been taken to confine this state. Both wide-ranging indefinite descriptions and very narrow depictions concentrating on few essential symptoms have been used throughout researching psychosis. American Psychiatric Association and the World Health Organization (WHO) define psychosis narrowly by requiring the presence of hallucinations without insight and/or delusions (American Psychiatric Association, 2013; WHO, 1992). In Kaplan and Sadock´s Synopsis of Psychiatry psychosis is defined as a "mental disorder in which the thoughts, affective response, ability to recognize reality, and ability to communicate and relate to others are sufficiently impaired to interfere grossly with the capacity to deal with reality". Impaired reality testing, hallucinations, delusions and illusions are subsequently listed as its classical characteristics (Sadock et al., 2015).

2.2. Classification of psychotic disorders

Psychotic disorders differ from one another greatly by the manifestation of the psychotic symptoms and associated characteristics, thereby are identified as spectrum disorders (Arciniegas, 2015; Guloksuz & Os, 2018). To emphasize this, descriptive diagnostic guideline the Diagnostic and Statistical Manual of Mental Disorders fifth edition (DSM-5), primarily used in the USA, uses division "SCZ Spectrum and Other Psychotic Disorders" in their classification of psychotic disorders (American Psychiatric Association, 2013). The International Classification of Diseases 11th Revision (ICD-11) Classification of Mental, Behavioral and Neurodevelopmental Disorders classify psychotic disorders as "SCZ or other primary psychotic disorders" (WHO, 2018) and its 10th revision (ICD-10) as "SCZ, schizotypal and delusional disorders" (WHO, 1992). Nevertheless, when it comes to diagnosing first-episode psychoses, the DSM and ICD manuals do not differ from each other significantly and have both high prospective diagnostic stability (Fusar-Poli et al., 2016).

2.3. Symptoms of psychotic disorders

Psychotic symptoms can be divided into three categories – positive symptoms, negative symptoms and cognitive symptoms. Classified as positive, are the most characteristic psychotic symptoms such as thought disorders, delusions and

hallucinations (Stępnicki et al., 2018). Negative symptoms include apathic signs like motivational impairment, social withdrawal, anhedonia and signs of reduced expression like flattened affect and alogia (Klaus et al., 2018). Cognitive symptoms comprise a range of memory, attention, reasoning and executive function impairments that are commonly associated with chronic SSDs but do appear already at the early stage of the illness and can be detectable even before the outbreak of the first psychotic episode (Sheffield et al., 2018). As said before, combinations of these phenomena are variable, thereby enabling a wide range of clinical manifestations.

2.4. Course of schizophrenia

Although the progress of SCZ may vary broadly, there are common disease progress patterns. The prodromal phase is the time period marking the occurrence of mild fluctuating psychotic symptoms often accompanied by other nonspecific psychosocial symptoms preceding the characteristic manifestation of psychosis (George et al., 2017). The acute phase starts with first-episode psychosis (FEP) when symptoms exceed the threshold for a psychosis diagnosis for the first time (Power, 2017). Even though more than a half of the FEP patients achieve remission (Lally et al., 2017; Zipursky et al., 2020), majority of them will suffer from residual symptoms afterwards (Khan et al., 2017). If the remission of the first psychotic episode is achieved, the next stage of the illness is characterized by alternating remissions and acute psychotic episodes that may lead to persistent drug-resistant psychotic symptoms (Power, 2017). However, in the minority of the cases, the syndrome may be treatment-resistant since the first episode or, in the best scenario, recovery is achieved without further recurrent episodes. The long-term outcome of SSDs depends on multiple medical and social factors and is highly variable (Volavka & Vevera, 2018). The challenge for clinical practice lies in distinguishing between patients who need long-term AP maintenance treatment from the ones who do not (Zipursky et al., 2020; Volavka & Vevera, 2018).

2.5. Etiology of schizophrenia

The genetics of SCZ is eminently complex. Both rare but highly penetrant, and common but individually modestly significant genetic variants comprise the disease risk of this polygenic disorder (Henriksen et al., 2017). The neurodevelopmental hypothesis integrates genetic, environmental and medical risk factors with aberrant neural development (Murray et al., 2017) and includes, for example, alterations in genes involved in dopamine (DA) synthesis, low socioeconomic status and birth complications, respectively (Stilo & Murray, 2019). As an explanation for the complexity of the disorder the idea has been proposed that SCZ is not a unitary illness but rather a common symptomatic endpoint of a great variety of brain dysfunctions (Guloksuz & Os, 2018; Sethi et al., 2017). Integrating

data from different fields of neurobiology, several recurring neurochemical patterns have emerged. The interplay of dopaminergic, glutamatergic and inflammatory dysregulation has been associated with surfacing psychotic disorders (Kahn & Sommer, 2015).

Mounting evidence reveals new aspects of etiology and pathology of SSDs continuously. Nevertheless, the three main theories about the etiology of SCZ – the genetic theory, the neurodevelopmental theory and the neurobiological theory – stay inconclusive (Kim, 2016). Neurobiological approach, as it can complement the existing knowledge on treatment targets and improve predicting the outcome by describing the underlying pathophysiological mechanisms of the illness, is the foundation of this dissertation.

2.6. Pathophysiology of schizophrenia

2.6.1. Membrane phospholipids hypothesis of schizophrenia

The membrane phospholipids hypothesis of SCZ suggests that the manifestation of psychotic symptoms is a consequence of altered lipid-composition of the cell membrane that exerts on the level of membrane receptors' function. To be more precise, it has been considered that the overactivity of the phospholipases A_2 may induce change in membranes' phospholipids (PLs) that result in modified N-methyl-D-aspartate (NMDA) and DA receptor function (Horrobin, 1998; Horrobin et al., 1994).

PLs' metabolism has a crucial role in neuronal and synaptic growth and remodelling, hence, the defects in this system may result in neurodevelopmental impairments related to SCZ (Sethi et al., 2017). Studies imply that the increased calcium-independent phospholipase A_2 activity in the brain may lead to hypo-dopaminergia and neurodegeneration through accelerating the breakdown of membrane PLs (Schaeffer et al., 2012).

Fortunately, the increased activity of phospholipases A_2 alone is not sufficient to erupt SCZ. Combined with an additional increase in PLs hydrolysis and a decrease in their synthesis due to the lessened integration of polyunsaturated fatty acids (PUFAs) into PLs, the disbalance in PLs metabolism can reach the verge of evoking SCZ symptoms (S. Sethi et al., 2017).

2.6.2. Dopaminergic model of schizophrenia

The DA hypothesis has had the central role in explaining the pathophysiology of SCZ for decades. As the knowledge about the DAs' functions and interactions in the brain has advanced, so has the DA theory. In the '70s the role of hyper-dopaminergia in the pathogenesis of psychotic disorders was recognized when AP effectiveness was observed to be related to the drugs' affinity to DA receptors (Seeman & Lee, 1975). This hypothesis was amended in the beginning of the

'90s, when theorized that hyperdopaminergia in certain neurons induces positive symptoms whilst hypodopaminergia in others negative symptoms (Davis et al., 1991).

Howes and colleagues present dopaminergic dysregulation as the "final common pathway" to evoke psychotic symptoms (Howes et al., 2015; Howes & Kapur, 2009). The evidence for the latter has been found in various studies both in humans and animals, showing that dopaminergic neurotransmission increasing drugs can induce psychotic symptoms (McCutcheon et al., 2020). In this kind of drug-induced psychosis, however, pre-eminently positive psychotic symptoms (hallucinations, delusions, paranoia, conceptual disorganization) manifest and negative symptoms characterizing SSDs are atypical (Voce et al., 2019).

Differentiating specific circuits and localizations in the brain where the function of dopaminergic neurons is dysregulated is technically challenging. *In vivo* study methods are limited and, as the DA system responds to physiological changes, indirect methods of assessment are less reliable than in more static systems. Nevertheless, there is consistent evidence of altered striatal DA regulation in psychosis, that has been associated directly to the severity of positive psychotic symptoms across the spectrum of psychiatric disorders (Jauhar et al., 2017; R. McCutcheon et al., 2018).

In addition to inducing positive psychotic symptoms, numerous other effects that relate to the clinical manifestation of SSDs can arise from dopaminergic dysregulation. The DA system regulates perception and motivation. Increased DA activity leads to an aberrant valuation of stimuli including overemphasizing irrelevant or neutral ones causing improper associations and causal attributions (Sterzer et al., 2018). DA dysregulation may alter reward-associated behaviours in a way that reduces the motivational influence of stimuli and the appealing properties of rewards (thereby inducing anhedonia and lack of motivations), and affect working memory-related activation (Maia & Frank, 2017; Radua et al., 2015; Slifstein et al., 2015).

2.6.3. NMDA glutamate receptor model of schizophrenia

Beside the dopaminergic hypothesis of SCZ, another one closely linked to the aforementioned is the widely accepted and rapidly evolving glutamatergic hypothesis. In this, the role of ionotropic glutamate (Glu) NMDA receptors' dysfunction in the pathophysiology of psychotic illness is emphasized (Howes et al., 2015).

Both in humans and animal models, the administration of NMDA receptor antagonists has been repeatedly shown to induce symptoms similar to SCZ (Cheng et al., 2018; McCutcheon et al., 2020; Moghaddam & Javitt, 2012). In animals, these include sensorimotor errors, abnormal movements, social and cognitive impairment, and in humans a spectrum of positive, negative and cognitive symptoms that are also characteristic to SCZ (McCutcheon et al., 2020; Moghaddam & Javitt, 2012). Animal models with decreased levels of NMDA coagonist serine (Ser) have shown development of similar morphological changes

in animal subjects to the ones detected in SCZ patients' brains (Balu et al., 2013; Hu et al., 2015). Another observation supporting the mounting evidence, that NMDA receptor dysfunction plays a role in developing psychotic disorders, is the manifestation of psychiatric symptoms resembling SCZ in various cases of autoimmune encephalitis presenting anti-NMDA receptor antibodies (Al-Diwani et al., 2019; Pollak et al., 2020).

Similarly to the DA system, there are numerous limitations regarding techniques for studying the Glu system *in vivo*, thereby also to identify specific circuits and localizations in the brain where the function of glutaminergic neurons is dysregulated. Nevertheless, several brain regions with abnormal glutamatergic activity have been detected in SCZ patients (Merritt et al., 2016). Hypofunction of NMDA receptors is also hypothesized to lead to an aberrated interpretation of stimuli, as does redundant DA activity described previously (Sterzer et al., 2018). Compared to the DA system, however, dysregulation of the Glu system results in a broader variety of symptoms associated with psychotic disorders, especially negative and cognitive symptoms.

2.6.4. Linking the dopaminergic and NMDA receptor model of schizophrenia

Although both DA and Glu systems' dysfunction have the potency to evoke an extensive variety of symptoms independently, the conclusive pathophysiology of SSDs includes the interplay of the two. Reduced cortical levels of Glu may result in an excessively active glutamatergic mesolimbic projection to the striatum, that in turn leads to an enhanced DA synthesis in the ventral striatum (Gleich et al., 2015; Stahl, 2013; Schwartz et al., 2012). An analogous relationship between Glu in the cortex and DA in the striatum has been found in the studies of FEP patients (Jauhar et al., 2018).

A physiological balance between the inhibitory and the excitatory neuronal activity maintained in the brain is associated to cognitive processes (McCutcheon et al., 2020). The main inhibitory input in the central nervous system is provided by gamma-aminobutyric acid (GABA) interneurons (Xu & Wong, 2018). In the cortical and hippocampal regions, the decreased GABAergic neurotransmission due to any reason (for example, the dysfunction of NMDA receptors of GABA neuron) is likely to lead to increased pyramidal and hippocampal glutamatergic neuron activity that in turn leads to uncoordinated transmission to subcortical regions and excessive DA neuron stimulation (Coyle, 2006; Moghaddam & Javitt, 2012; Lieberman et al., 2018; Xu & Wong, 2018; Stahl, 2013). Therefore, GABA interneurons can mediate a glutamatergic dysfunction that instigates dopaminergic disinhibition leading to enhanced symptomatic manifestations.

2.7. Metabolomics and biomolecules associated to schizophrenia spectrum disorders

Shifts in the biomolecules' profile in the brain accompanying psychiatric manifestations are acknowledged. However, as the possibilities to directly estimate the biomolecules' concentrations in the human brain *in vivo* are highly limited, more indirect measures are commonly used. Differences maintained by the blood-brain barrier between cerebral and peripheral environment must be considered when comparing metabolite levels in the brain and in blood or other tissues. Fortunately, it has been shown that biomolecules' concentration shifts detected in the blood serum are in accordance with neuropsychiatric pathology and reflect the pathophysiological changes in the central nervous system (Varma et al., 2018). To assess a wide variety of biomolecules in a single sample simultaneously, metabolomics can be applied.

2.7.1. Metabolomics

Metabolomics is a field of research that comprises identification, quantitative assessment and characterization of small (<1500 Da) molecule metabolites in a particular cell, tissue, organ or organism (German et al., 2005; Wishart et al., 2007). As diseases, including psychiatric, cause alterations in biochemical pathways, they create more or less distinctive metabolic patterns that can be recognized using metabolomics approach (Sethi & Brietzke, 2015). Therefore, metabolomics has the potential to reveal pathophysiological mechanisms underlying psychopathology and to identify possible biomarkers. Latter can be clinically significant both diagnostically and therapeutically by predicting the risk of the disease, supporting the diagnose, helping to choose the most suitable AP medication and prognosticating the outcome (Tayeb et al., 2019). Emanating from models of SCZ pathophysiology, biomolecules in centre of interest regarding SSDs are cell membranes' lipids, AAs and BAs. For that reason, these metabolites are discussed in further detail and biomarkers for the metabolomic study chosen from them.

2.7.2. Lipids

Lipids have numerous functions in human cells. They serve as energy storage, essential components of cellular membranes and as messengers in signal transduction (van Meer et al., 2008). The main membrane-forming lipids are glycerophospholipids (GPLs), sphingolipids (SLs) and sterols (Harayama & Riezman, 2018). The variability of fatty acids (saturation, chain length) creates the diversity of GPLs and SLs, which, having different distribution patterns, in turn, enable the cell membrane composition variability (Harayama & Riezman, 2018). Lipid composition determines membranes' physical properties like membrane rigidity and fluidity and affects the membrane transport function and cell signalling (Harayama & Riezman, 2018).

2.7.2.1. Glycerophospholipids

GPLs are glycerol-based PLs that consist of a glycerol backbone esterified by two long-chain fatty acids at the sn-1 and sn-2 position and phosphoric acid at sn-3 (Harayama & Riezman, 2018). To the phosphate group additional groups may be esterified, allowing a high structural and functional diversity (Montealegre et al., 2014). Consisting of both hydrophobic fatty acid chains and hydrophilic phosphate group, GPLs are amphiphilic molecules. In cell membrane GPLs are organized in a bilayer in a manner to maintain the highest entropy – polar hydrophilic heads point to the aqueous environment and the non-polar hydrophobic tails inwards of the membrane (Meer & Kroon, 2011).

A high proportion of the outer leaflet of the plasma membrane is made up of phosphatidylcholine (PC) – a member of GPLs with choline as an alcohol head group esterified to the phosphate group – and sphingomyelin (SM) (Bevers & Williamson, 2016; Harayama & Riezman, 2018). The inner leaflet consists mainly of PLs phosphatidylserine (PS) and phosphatidylethanolamine (PE) (Bevers & Williamson, 2016). In the inner leaflet of the plasma membrane, hydrolysis of PLs generates many important second messengers. During the hydrolysis of PC by the phospholipase A_2 a fatty acid residue from the sn-2 position of membrane PL is removed and lysophosphatidylcholine (LysoPC) is formed (Mouchlis & Dennis, 2019).

2.7.2.2. Sphingolipids

SLs consist of a backbone of a hydrophobic sphingoid base (such as amino alcohol sphingosine) linked via an amide bond to a fatty acid residue (acyl chain) and O-linked to a head group such as phosphoethanolamine, oligosaccharides or phosphocholine (Harayama & Riezman, 2018). SMs are members of SLs family esterified by phosphocholine or phosphoethanolamine as a head group and extensively represented in the exoplasmic leaflet of cell membranes (Chen & Cao, 2017).

2.7.3. Shifts in lipidomic profile in schizophrenia spectrum disorders

Association between SCZ and metabolic syndrome is well known. Dyslipidemia is a common metabolic disturbance appertaining to metabolic syndrome and amplifying the risk of cardiovascular diseases related to premature mortality in SSD patients (Correll et al., 2017). Furthermore, insulin resistance, type 2 diabetes mellitus and non-alcoholic fatty liver disease, additionally to cardio-vascular diseases, are all prevalent comorbidities of SSDs grounded on metabolic disturbances (Suvitaival et al., 2016).

Changes in metabolic pathways leading to metabolic syndrome have been accredited to the adverse effects of AP medication (Chadda et al., 2013; Hua Jin

et al., 2004) and lifestyle descendance caused by the disease (Peet, 2004). Unfortunately, AP drugs do have metabolic adverse effects and the most efficacious of these medicaments (clozapine and olanzapine) seem to cause the most extensive metabolic deviances (Pillinger et al., 2020). However, there are AP pharmaceuticals that, in contrast, have shown beneficial effects to certain metabolic measures (Pillinger et al., 2020). Patients differ in their vulnerability to developing metabolic dysfunction and it has been suggested that metabolomics profiling could be used to recognise SSD patients most susceptible to metabolic co-morbidities (Suvitaival et al., 2016).

Growing evidence from studies including AP-naïve psychosis patients indicates that these undesirable metabolic alterations are not only provoked by AP treatment and lowered lifestyle but comprised in the pathogenesis of the illness itself (Chadda et al., 2013; Kirkpatrick et al., 2012). Meta-analysis representing 866 AP-naïve participants experiencing the first episode of non-affective psychosis revealed significantly lower levels of total cholesterol and high-density lipoprotein (HDL) as well as significantly higher levels of triglycerides in patients compared to control subjects, proving that alterations in lipid levels occur independently from AP treatment (Misiak et al., 2017). Furthermore, from analysing the lipid profiles of the patients, some lipid peroxidation metabolites, neuroactive steroids, PUFAs (especially arachidonic acid) and several PLs have emerged as potential SSD biomarkers (Davison et al., 2018; D. Wang et al., 2019).

As expected, knowing how influential metabolism of lipids in the context of psychotic disorders is, the composition of lipid metabolites in the central nervous system in SSD patients has been the target of interest. In post-mortem study of SCZ patients elevated PE22:1 level and lowered PC20:3n6, PE22:5n6 and PC22:5n6 levels in white matter have been found (Ghosh et al., 2017).* Another study revealed diminished levels of 16:0/20:4-phosphatidylinositol in the prefrontal cortex of SCZ patients compared to the control group (Matsumoto et al., 2017). *In vivo*, using nuclear magnetic resonance spectroscopy to assess lipid metabolite concentrations in SSD patients, altered phospho-choline (intermediate in the synthesis of PC), glycerophosphocholine and phosphoethanolamine levels have been found in basal ganglia and cortex (Weber-Fahr et al., 2013).

Findings from peripheral substrates seem to be consistent with those from the central nervous system. It has been detected that in fibroblasts of SCZ patients 6 out of 13 metabolites, which levels differed significantly from control subjects, were PCs and 2 PC-plasmalogens (Huang et al., 2017). In a study by Kaddurah-Daouk and colleagues (2007) over 300 lipid metabolites of SCZ patients' plasma samples were estimated before and after a short period of AP treatment. They demonstrated alterations in PE, PE-PUFA and PEn6 fatty acid family PE levels depending on patients treatment status (Kaddurah-Daouk et al., 2007). These findings are in concordance with a more recent study that found deviations

* When marking different fatty acids, structure (XX:YnZ) can be used, where XX marks the carbon number as fatty acids differ in chain length, Y marks the double bond number and Z the position of the first double bond from the omega end (Harayama & Riezman, 2018).

in several n3 PUFA levels differentiating between control subjects, first-episode and chronic SCZ patients, and were found to be modified by treatment (McEvoy et al., 2013). However, as there are not many studies targeting a wide variety of lipid metabolites and even less including AP-naïve patients, the results stay inconclusive and the dynamics of metabolomic changes during the evolvement of the disease and applying AP treatment is unclear.

The lipidomic approach enables to acquire more detailed knowledge on the pathophysiological mechanisms underlying the lipid metabolism deviances that have transpired in numerous studies engaging SSD patients (Sumit Sethi et al., 2017). This kind of metabolomics profiling could lead to identifying potential SSD biomarkers providing a much-needed diagnostic tool. It also could potentially be used to recognise patients susceptible to metabolic co-morbidities and AP side-effects. As brain lipids can be targeted by drugs, enzyme replacement therapy, gene therapy, or dietary manipulations, finding the ones to aim may even reveal new treatment options for SSD patients (Schneider et al., 2017). Therefore, further lipidomic studies of SSD patients, especially including the AP-naïve FEP patients, are promising to elevate the level of both preclinical and clinical understanding of SSDs.

2.7.4. Amino acids and biogenic amines

AAs are mainly used for protein synthesis. In human cells, there are 20 AAs coded by human genetic code that serve this function and are named proteinogenic AAs. However, in body AAs do not only act as building blocks but have numerous functions on their own. For example, a number of AAs are neurotransmitters or precursors for biomolecules like BAs that are essential, as described before, to maintain the physiological balance of excitatory and inhibitory impulses in the brain.

AAs are divided into two groups depending on how sufficiently, relative to metabolic needs, they can be synthesized *de novo* in human body cells. The ones that are not synthesized at all or are produced in amounts deficient to cover the metabolic demand are categorized as essential AAs and must be obtained from food (Hou & Wu, 2018). In humans, these include histidine (His), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), threonine (Thr), tryptophan (Trp), and valine (Val).

To understand better the role of certain AAs in the context of SSDs, a more thorough insight into their metabolism is needed.

DA is a catecholaminergic neurotransmitter that has a central role in the pathogenesis of SSDs. In the human brain, Phe is converted to tyrosine (Tyr) by catalysing Phe hydroxylase (Okusaga et al., 2014). Tyr hydroxylase (a rate limiting enzyme in the catecholamine synthesis) then converts Tyr to dihydroxyphenylalanine (DOPA) which in turn by aromatic AA decarboxylase is turned into DA (Daubner et al., 2011; Klein et al., 2019; Okusaga et al., 2014). From cytosol, DA is transported by vesicular monoamine transporter to synaptic vesicles from

where it can be further released to the synaptic cleft (McCutcheon et al., 2020). From synaptic cleft, DA can be bound to both postsynaptic and presynaptic receptors or be re-uptaken by DA transporter (McCutcheon et al., 2020).

Trp is a precursor for kynurenine (Kyn) and its derivate kynurenic acid (KYNA) but also to the neurotransmitter serotonin (Höglund et al., 2019). However, in normal conditions, Trp supply enters predominantly the kynurenic pathway where Trp is oxidized into N-formylkynurenine that is then deformylated into Kyn. In turn, Kyn can be transaminated into KYNA that is an NMDA and α7 nicotinic acetylcholine receptor antagonist (Erhardt et al., 2017; Fujigaki et al., 2017; Plitman et al., 2017; Rossi et al., 2019; Schwarcz et al., 2012). Alternatively, Kyn can be turned into other metabolites and finally through quinolinic acid, which is NMDA receptor agonist, into nicotinamide and nicotinamide adenine dinucleotide (NAD), that makes Kyn pathway important for cellular energetics (Rossi et al., 2019; Savitz, 2020). Enzymes catalysing Kyn transformations to more favourable metabolites than KYNA are Kyn mono-oxygenase and kynureninase (Rossi et al., 2019; Savitz, 2020). Kyn is transported to the brain through the blood-brain barrier by the large amino transporter. In mice models, Leu as a high-affinity competitive substrate for the same transporter has been shown to block Kyn entry to the brain, thereby preventing the production of neurotoxic Kyn metabolites (Walker et al., 2019).

AAs that are produced in human body cells are called nonessential and include alanine (Ala), arginine (Arg), asparagine (Asn), aspartate (Asp), cysteine (Cys), Glu, glutamine (Gln), glycine (Gly), ornithine (Orn), proline (Pro), Tyr, Ser.

Arg, depending on which enzyme catalyses the process – arginase, Arg decarboxylase or nitric oxide synthase – can be converted into Orn, agmatine or nitric oxide and citrulline respectively (Garip et al., 2019). Both agmatine and Orn may have substantial roles in the pathogenesis of SSDs. Agmatine is capable of inhibiting nitric oxide synthase and voltage-dependently NMDA receptor (Garip et al., 2019). Orn, on the other hand, is a precursor for spermine that has NMDA receptor modulating properties (Pegg, 2016).

Glu is the second major neurotransmitter in the centre of interest when discussing the pathogenesis of SSDs. The balance of Glu extracellular level is regulated by Glu/Gln cycle between neurons and astrocytes. In this cycle, extracellular Glu is transported to astrocytes, converted into Gln by glutamine synthetase to be then excreted from the glial cell and taken in by neurons (Brekke et al., 2015). In neurons, Gln is converted back to Glu by phosphate activated glutaminase (Brekke et al., 2015; McCutcheon et al., 2020). Glu can also be produced in association to tricarboxylic acid cycle, through several reactions from precursor citrate and it is itself a precursor for GABA synthesis (Brekke et al., 2015; Schousboe, 2019). Glu levels are regulated through another mechanism as well. In mitochondria, Pro is oxidized to Glu by Pro oxidase, the latter is encoded by the gene (PRODH) that has been associated with SCZ (Cappelletti et al., 2018; Kempf et al., 2008). This process determines the intracellular concentration of Pro and Glu and defects in enzyme function catalysing this reaction have been found to increase the susceptibility to SCZ (Cappelletti et al., 2018; Liu et al., 2002). It has also been

shown that elevated Pro levels (hypothetically through competitive inhibition of glutamate decarboxylase) lead to diminished GABA production, therefore contribute to disturbed balance in excitatory and inhibitory impulses, increasing the risk for psychotic disorders (Crabtree et al., 2016).

As said before, AAs have many functions. Asn is essential for growth and proliferation of neuronal cells and its synthetase deficiency, hence Asn deficiency results in progressive cerebral atrophy (Palmer et al., 2015). Asn needs a transporter to cross the blood-brain barrier, therefore its availability in neuronal cells depends on intracellular Asn synthetase (Palmer et al., 2015). Ser, as mentioned before, is an NMDA co-agonist (Hu et al., 2015). Genetic variations of the enzyme Ser racemase, that catalyses the D-Ser synthesis from L-Ser, have been associated with SCZ (Labrie et al., 2009).

BAs are biologically active compounds that contain at least one amine group and are generally synthesized by decarboxylation of AAs (Kaur et al., 2018). In previous paragraphs BAs DOPA, DA and Kyn have been mentioned. Alike AAs, BAs have various functions including neural transmission, inflammatory processes and growth regulation (Kaur et al., 2018). For example, taurine is a BA produced from Cys, which precursor, in turn, is Met. In central nervous system taurine acts as a neuroprotective agent that modulates neurodevelopment and neurogenesis and also as a neurotransmitter that inhibits NMDA receptor, activates the GABA and Gly insensitive chloride channels (O'Donnell et al., 2016). Spermine is another BA that can inhibit NMDA receptors but it also acts as its positive allosteric modulator selective to the GluN2B subunit (Hackos & Hanson, 2017). To sum up, additionally to AAs direct influence, they may have significant roles through their derivate BAs.

2.7.5. Shifts in amino acids and biogenic amines concentration profiles in schizophrenia spectrum disorders

As can be concluded from the previous section, AAs and BAs are through their metabolic pathways tightly connected to several determinants that are in the centre of SCZ pathogenesis. Naturally, alterations in AA and BA levels have been studied in the context of SSDs and abundant interesting findings have been made.

In animal studies on mice, a week of Met administration evoked behavioural changes similar to SSDs', including positive, negative and cognitive symptoms (Wang et al., 2015). Furthermore, in the same study by Wang and colleagues (2015), Met induced symptoms were overturned by AP treatment, making it a useful model for SCZ. In a study using zebrafish, pre-treatment with taurine prevented memory deficit and hyperlocomotion induced by non-competitive NMDA receptor antagonist, thereby showing potential neuroprotective role against Glu excitotoxicity (Franscescon et al., 2020). Spermine has also demonstrated its neurotransmission modulating capability in a study on rats (Zhang et al., 2018). On mice models, it has also been proven that peripherally administered Asp

efficiently crosses the blood-brain barrier and elevates Asp and Glu in the extracellular fluid of prefrontal cortex (Sacchi et al., 2017).

In several animal experiments, the relationship has been proven that high levels of alpha-aminoadipic acid (alpha-AAA) lead to decreased KYNA production and low levels of alpha-AAA to increased KYNA production in the brain (Okuno et al., 2011). As KYNA is the only known endogenous NMDA receptor antagonist, it is clear that disturbed balance in the Kyn pathway can have extensive consequences to the glutamatergic system and therefore psychosis predisposition (Savitz, 2020). Kyn pathway is a connection between the main findings in SSDs – glutamatergic, thereby dopaminergic dysregulation, inflammatory processes (Pedraz-Petrozzi et al., 2020; Savitz, 2020), and even energetic disbalance, mitochondrial dysfunction and oxidative stress (Castellano-Gonzalez et al., 2019; Savitz, 2020).

In a post-mortem study of SCZ patients' central nervous system, higher levels of Tyr hydroxylase have been found in the substantia nigra (Schoonover et al., 2017). As the substantia nigra projects to the corpus striatum and has the largest DA input to the brain, this supports the theory that DA production in SSDs is enhanced and Tyr can thereby be used more rapidly (Schoonover et al., 2017). In the grey matter of prefrontal cortex an increased arginase activity in SSD patients has been found in post-mortem studies (Liu et al., 2016). Furthermore, a positive correlation between the age of disease onset, duration of SCZ, arginase activity and Orn level was established (Liu et al., 2016). Also in the prefrontal cortex enhanced activity of Asp oxidase and decreased Asp levels in post-mortem study of SCZ patients have been found (Nuzzo et al., 2017).

For some metabolites, their concentration differs between the central nervous system and peripheral tissues, for some they still have to be specified (De Luca et al., 2008; Palmer et al., 2015). However, as the direct *in vivo* evaluation of the central nervous system's metabolic state is invasive and, for now, cannot be adopted as general clinical practice, indirect methods are commonly used. Metabolomics profiling from blood in SSD context is attaining more popularity and research results using this approach are published increasingly. Despite the growing interest, the number of these studies is still limited and their results sometimes contradictory as further underlined in the discussion. Often these studies include SSD patients from different age groups, with a different duration of the illness and a different treatment status. As the SSD onset can be sudden, there is a demand for the implementation of AP treatment without delay, and patients' cooperation may be problematic, more studies have been done with chronically ill patients as participants than with patients with recent onset of the disease. However, in this population of recurrent SSD patients, the variability of factors that could potentially affect the metabolomics profile of individuals is much higher and more difficult to control. Additionally, at the beginning of the illness, the changes in patients' symptoms are the most rapid (presumably reflecting rapid pathophysiological changes) to occur and more responsive to interventions (Drake et al., 2020). Therefore, to better understand the metabolomic changes underlying

patients' clinical manifestations, clear distinctions should be made in terms of patient selection regarding the duration of their illness.

2.8. Summary of reviewed literature

Although SSDs may vary in specific clinical manifestations, they share core characteristics and commonly the pattern of the disease progression. Etiology of SSDs is complex and the current understanding of it stays inconclusive. However, neurobiological approach with integrating data from different fields have revealed numerous aspects of pathophysiological mechanisms underlying the illness. These have provided the foundation for the main hypotheses of SCZ – the membrane PL, DA and NMDA Glu receptor theory. These hypotheses and additionally the metabolic effects of AP treatment have provoked studies on lipid metabolites, AAs and BAs in the context of psychotic disorders. Unfortunately, methods to estimate changes in metabolite levels in focus are limited. Despite animal experiments, *post mortem* research and visualizing studies that have given promising and rather concordant results, the methods stay insufficient for the clinical setting and the results inconclusive. Metabolomic approach has been proven beneficial in studying psychiatric conditions and enables the usage of peripheral blood as a substrate. However, the number of metabolomic studies of SSD patients is limited and factors presumably influential to the results (related to the treatment and phase of the illness) frequently overlooked. Hence, for better understanding of the pathophysiology of SSDs, metabolomic studies of biomolecules potentially associated to the disease (PLs, AAs, BAs) involving SSD patients with different illness duration and firm AP medication status could be of great benefit.

3. STUDY RATIONALE

The research, that the present dissertation is based on, started in 2008 as the first study of FEP patients in Estonia. By now, a number of recruited patients have been monitored since the onset of the psychosis for more than five years, making the research unique in Estonia and noteworthy worldwide. The research contributes to eliminating a gap in the literature related to the longitudinal evaluation of patients' AA, BA and lipid metabolite levels from blood plasma and metabolomic profile's change during the course of a psychotic illness and AP treatment. Therefore, leading to the development of more personal and compatible patient care.

4. AIMS OF THE THESIS

The main intention of this thesis is to characterize the profile of circulating GPLs, SLs, AAs and BAs, during the onset of a psychotic disorder before antipsychotic treatment and at different time points during the course of the illness after implementing AP treatment. Based on theoretical study, the hypothesis is that significant and associable alterations in the profile of these biomolecules occur during the course of SSDs and their treatment with AP medication.

Specific aims are the following:

- To characterize the profile of the circulating GPLs and SLs in FEP patients compared to the control subjects before AP treatment and after seven months of medication
- To characterize the profile of the circulating AAs and BAs in FEP patients before AP treatment, after seven months of medication and after five years of continuation of the disease and treatment compared to the control subjects
- To analyse the potential mechanisms underlying the alterations in metabolomics profile of circulating GPLs, SLs, AAs and BAs in patients with psychotic illness before treatment, after seven months of medication and after five years of continuation of the disease and treatment compared to the control subjects

5. SUBJECTS AND METHODS

5.1. Subjects

5.1.1. Ethical aspects

The research project for this study was approved by the Ethics Review Committee on Human Research, the University of Tartu, Estonia (initial approval No 177/T-2 issued on 15 Feb 2008 and follow-up approval No 211/M-22 issued 23 Jan 2012; No96/16 issued 21 Aug 2001 and No 176/T-4 issued 17 Nov 2008). All procedures complied with the ethical standards of the Helsinki Declaration of 1975, as revised in 2008. Prior to the study, all participants were informed about the aims of the study, planned interventions and of their freedom to decide if they want to participate and to leave the study at any time if they reconsider. The research had minimal burdens and risks and no restrictions for the received medical care for patients. Written informed consent was provided by all patients and control subjects.

5.1.2. Patients

Patients were recruited from the Psychiatry Clinic of Tartu University Hospital. Inclusion criteria to participate in the study were following: age between 18 and 45; experiencing the first psychotic episode; duration of untreated psychosis less than 3 years; no AP treatment received before the first contact with medical services regarding psychosis. Diagnoses of the FEP (F23 or F20 or F21) were based on the criteria of ICD-10 (World Health Organization, 1992). To obtain the diagnosis assessment of the patients' behaviour, clinical interviews with patient and collateral informants were performed and medical charts reviewed. This evaluation was done by experienced clinical psychiatrists.

Patients who had psychotic disorders owing to a general medical condition or substance-induced psychosis were excluded from the study. Additional exclusion criteria were previous organic brain damage, ongoing infections or severe systemic somatic illness.

As it was a naturalistic and longitudinal study, no restrictions were made in the usage of specific pharmacological agents and patients were treated with various AP medications according to clinically relevant circumstances. Furthermore, the pharmacological treatment of a single subject was permitted to be altered during the study and AP treatment was used in combination with other needed medications. Although there were no restrictions on medication, all participants were questioned about previously used pharmacological agents when recruited to the study. No additional tests were made to verify the information given by participants for this matter. AP medication use history was controlled by revision of patients' medical charts. Patients were allowed to receive anti-anxiety medication the night before the first blood sample was drawn.

5.1.3. General description of the study groups

To retain the naturalistic design, participants were not excluded from the study due to cigarette smoking and/or current or previous substance use. Information about smoking and substance use was not verified by any tests but relied on participants' reports.

At the beginning of the research when the first AA and BA analyses were conducted (Paper II) 38 AP-naïve FEP patients were initially recruited. As two of them refused AP treatment, they were excluded from the follow-up analyses; therefore, the follow-up analysis after 7 months of treatment included 36 patients. Considering AP medication, the mean theoretical chlorpromazine dose equivalent was 396 ± 154 (range 80–640) mg. There were 27 patients treated with APs only, nine patients needed mood stabilizers, antidepressants, or hypnotics in addition to AP treatment. Eight patients (21.1%, all men) were active cigarette smokers. Ten patients (eight of them current cigarette smokers) had used cannabis during their lifetime.

During the progression of the study, 15 patients were additionally recruited. Data from 53 AP-naïve FEP patients and 37 control subjects were obtained for the lipidomics analyses (Paper I). During the follow-up, two previously mentioned patients refused AP treatment and seven patients had lost contact with health-services, thereby the follow-up analyses after 7 months of treatment include 44 patients. Thirty-three patients were treated with AP only, 11 patients needed mood stabilizers, antidepressants, or hypnotics in addition to AP medication. At the time of the follow-up blood collection, the mean theoretical chlorpromazine dose equivalent was 363 ± 165 (range 80–780) mg.

As one patient discontinued the study and did not participate in either of the follow-up meetings, the 5-year follow-up AA and BA analyses include data from 52 patients (Paper III). During the monitoring period, 15 patients withdrew from the study (dropout rate of 29%). Main reasons for that were patients' non-compliance with the treatment (resulting in discontinuation of APs) and changing their place of residency. After an average of 7.2 months and 5.1 years of follow-up, patients' group comprised of 44 and 37 patients respectively. Nineteen patients (36%) reported that before the onset of psychotic disorder they had smoked cannabis, during the monitoring period seventeen patients (45%) continued cannabis consumption.

5.1.4. Control subjects

The control subjects of this study were recruited by advertisement by medical personnel from the same geographical area as the FEP patients. The exclusion criteria were neurological disorders, mental retardation or significant learning disorder, previous organic brain damage, ongoing infections or severe systemic somatic illness, major sight and hearing impairment and psychotic disorder among close relatives. To avoid the inclusion of subjects with mental disorders, control

subjects were interviewed by experienced psychiatrists. The control group consisted of 37 volunteers, attempted to match to the patients' group on body mass index (BMI), age, gender and smoking habit (yes/no).

5.2. Study design

5.2.1. Protocol

For FEP patients' fasting blood samples, clinical, demographic and BMI data were collected at three time-points. Firstly, in the enrolment phase on admission to hospital. Secondly, at the first follow-up after approximately 7 months (mean duration respectively 7.2 ± 0.7 months, 7.18 ± 0.73 months and 0.59 ± 0.06 years for papers I, II and III). Thirdly, at the second follow-up (paper III) roughly 5 years after the enrolment (mean duration 5.15 ± 1.25 years). The time between the first two evaluation points included about one month of initial stabilization of acute psychotic symptoms and six-months of continuous AP treatment. Control subjects' fasting blood samples, clinical, demographic and BMI data were collected at one time-point only. No tests were carried out to verify if the participants were indeed fasting prior to the blood sampling. We relied on the information given by participants as it would be done in common clinical settings as well.

Patients' symptoms severity was assessed using the Brief Psychiatric Rating Scale (BPRS) (Overall & Gorham, 1962) that evaluates the presence and severity of 18 symptoms on a seven-point Likert scale from "not present" to "extremely severe". A total score was used as the outcome.

5.2.2. Blood collection

Fasting blood samples from the study participants were collected between 9 a.m. and 11 a.m. into anticoagulant-free tubes using the standard venipuncture technique. Five ml blood samples were kept at 4 ºC for one hour for platelet activation before the serum was isolated (centrifugation at 2000 rpm for 15 minutes at 4 ºC). Serum samples were frozen and stored under identical conditions – firstly at –20 ºC, then at –80 ºC for longer storage until the determination of biomarkers.

5.2.3. Measurement of biomarkers

5.2.3.1. Measurement of amino acids and biogenic amines

Serum levels of AAs and BAs were measured applying the AbsoluteIDQ™ p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) using the flow injection analysis tandem mass spectrometry and high-performance liquid

chromatography technique (Agilent Infinity 1260 series HPLC with Sciex Qtrap4500 mass spectrometry) accordingly to the manufacturer's manual. The metabolomics data set contained 21 AAs and 21 BAs. Using multiple reactions monitoring with internal standards the AAs and BAs were identified and quantified. The calculation of metabolite concentrations was automatically performed by MetIDQ™ software (BIOCRATES Life Sciences AG).

5.2.3.2. Measurement of glycerophospholipids and sphingolipids

The concentrations of GPLs and SLs were assayed using the AbsoluteIDQ^{TM} p180 kit (BIOCRATES Life Sciences AG, Innsbruck, Austria) applying flow injection analysis tandem mass spectrometry as well as the high-performance liquid chromatography technique as described previously. The metabolomics data set contained 14 LysoPCs, 76 PCs and 15 SMs. When assumed to indicate a certain metabolic state or process, metabolite sums and ratios were calculated. The measurements were performed similarly to the AAs and BAs.

5.2.4. Statistical analyses

For the first and second paper, Shapiro-Wilk test was used to assess the normality of the distribution of the data. Consecutive analyzation using the Student's *t*-test was performed on normally distributed data (age, BMI, psychopathology score). Levels of the metabolomics markers were non-normally distributed. Hence, the Mann-Whitney U-test was used to establish preliminary metabolic profile differences between-groups of AP-naïve and treated patients vs. control subjects. Within group (AP-naïve vs. treated FEP patients) subjects were paired one by one and Wilcoxon Matched Pairs Test was used to display differences in biomarker levels. Due to the large number of simultaneous comparisons, meaningful differences were determined by using the Bonferroni correction. This resulted in a corrected critical *p*-value for between and within group level differences for the AAs and BAs of $p \leq 1e\text{-}03$ and for the GPLs and SMs $p \leq 5e\text{-}04$. For non-parametric test results that were identified as statistically significant, the effect sizes (eta^2) were calculated. Effect sizes with eta^2 ranging from 0.01–0.05 were considered as small, from 0.06–0.13 as moderate and ≥0.14 as large (Cohen, 1988). Considering GPLs and SMs non-parametric Levene's test was used to verify the equality of variances between and within the groups (Nordstokke & Zumbo, 2010).

As biomarker data are usually associated with the heterogeneity of variance, they were log-transformed before continuing with analyzation. To determine the differences in biomarker levels between the groups and within the group general linear models (GLM) were used. To see which model best fits the data categorical covariates and continuous covariates were used in the GLM to compare biomarker levels (dependent variables) between groups. Consequently, backward variable elimination was used to step-by-step eliminate the least significant

variable in the model until all remaining variables had individual *p*-values <5e-02. For further comparison of the linear models, *F*-tests were used and partial eta^2 values determined for the final models. Partial eta^2 values more than 0.26 were defined as large effects (Cohen, 1988).

For the third paper Student's *t*-test and repeated measure analysis of variance were performed on normally distributed data, and mean differences were tested with the Scheffé *post hoc* test. To manage unbalanced data sets, missing data due to some patients' discontinuation of the study and natural heterogeneity, linear mixed-effects models with random coefficients were used to analyse the metabolomic marker level changes between patients and control subjects at three different time points (Twisk et al., 2018; Laird & Ware, 1982). A random slope for time and intercept for participants were included for repeated measurements and variables' coefficients were compared to the model intercept representing control groups' biomarker levels. To validate the data, we applied a two-way analysis of variance. The false discovery rate procedure was used for multiple testing corrections. The *p*-values were adjusted using the Benjamini-Hochberg procedure by controlling the false discovery rate (Benjamini & Hochberg, 1995) and considered to be statistically significant when $p<1e-03$.

The statistical analyses were performed using Statistica (*TIBCO Software Inc., version 13*) software for Windows, the R statistical language version 3.5.0 (R Core Team, 2018) packages nlme (Pinheiro et al., 2018) and ggplot2 (Wickham, 2016) and analysis of a variance-type diagnostic test.

6. RESULTS

6.1. Description of the study groups

In terms of age, gender, tobacco use and mean values of BMI there were no statistically significant differences between AP-naïve FEP patients' group and control subjects' group. Characteristics of the study participants are summarized in more detail in Table 1.

7-month antipsychotic treatment resulted in significantly decreased (p<1e-04) psychopathology (BPRS) score in patients. Unfortunately, treatment also caused significant weight gain and increase in BMI (p<1e-04). Mean BMI gain in group of 36 patients was 3.0 kg/m^2 (± 2.2) during 7 months of treatment.

Table 1 Demographic and clinical features of study participants

	Lipid profile			Amino acid and biogenic amine profile						
	Paper I			Paper II			Paper III			
Demographic/ clinical variable	FEP_b (*n*=53)	$FEP_{(0.6\text{-year})}$ (*n*=44)	CS (*n*=37)	FEP_b (*n*=38)	$FEP_{(0.6\text{-year})}$ (*n* =36)	CS (*n*=37)	FEP_b (*n*=52)	$FEP_{(0.6\text{-year})}$ (*n*=44)	$FEP_{(5.1\text{-year})}$ (*n*=37)	CS (*n*=37)
Age, years (mean ± SD)	26.2 ± 6.0	27.7 ± 6.5	24.8 ± 5.3	25.4 ± 5.5	26.2 ± 5.6	24.8 ± 5.3	27.0 ± 6.1	27.7 ± 6.5	32.0 ± 5.9	24.8 ± 5.3
Female/Male (*n*)	21/32	18/26	21/16	17/21	16/20	21/16	21/31	18/26	14/23	21/16
Cigarette smoking (*n*, %)	19 (36%)	14 (32%)	7 (19%)	8 (21%)	8 (21%)	7 (19%)	17 (33%)	14 (32%)	18 (49%)	5 (14%)
BMI (mean ± SD)	22.9 ± 3.1	25.4 ± 4.1	23.0 ± 3.1	22.6 ± 2.9	25.6 ± 4.0	23.0 ± 3.1	22.8 ± 3.0	25.4 ± 4.0	27.8 ± 4.5	23.0 ± 3.1
BPRS (mean ± SD)	48.5 ± 15.5	23.3 ± 12.8	–	50.8 ± 14.9	23.1 ± 12.1	–	49.9 ± 15.5	23.3 ± 12.7	14.1 ± 10.6	–

FEPb – AP-naïve FEP patients
FEP(0.6-year) – FEP patients after 7-month treatment
FEP(5.1-year) – FEP patients after 5.1-year treatment
BMI – body mass index; BPRS – Brief Psychiatric Rating Scale

6.2. Changes in lipid profile (Paper I)

6.2.1. Differences in glycerophospholipids' and sphingomyelins' profile in untreated FEP patients compared to control subjects

The Mann-Whitney U-test was used to compare between the AP-naïve FEP patients and control subjects' GPL and SM levels. Levels of 90 circulating GPLs, 15 SLs and a number of ratios of them were analysed (Supplementary Table 1). Comparison revealed a significant reduction in the levels of 11 PC-aa-Cs, 5 PC-ae-Cs as well as one SM in FEP patients compared to the control subjects (Table 2). Furthermore, the sum of PC-aa (Total_PC-aa), ratio of Total_PC-aa/Total_ LysoPC-a and PC-aa-C36:3/PC-aa-C36:4 were also reduced. In contrast, the level of LysoPC (LysoPC-a-C20:4) and the ratio of LysoPC-a-C20:4/LysoPC-a-C20:3 were significantly elevated (Table 2).

The Bonferroni correction was then applied to determine meaningful changes. All the above-mentioned differences survived the correction and displayed a large effect (I^2 range 0.14–0.42) (Table 2).

Table 2 Comparison of serum levels of GPLs (μmol/l) and SMs (μmol/l) between the first-episode psychosis patients (n=53) at baseline (FEP_b) and control subjects (CS) (n=37)

	FEP_b	**CS**			
	Median (min – max)	**Median (min – max)**	**Z-value**	***p*-value**	**Effect size (I^2)**
Glycerophospholipids					
Lysophosphatidylcholine acyls					
LysoPC-a-C20:4	10.3 (6.43–19.8)	8.20 (3.81–15.9)	3.83	1e-04	0.16
Phosphatidylcholine diacyls					
PC-aa-C30:0	2.89 (1.27–4.95)	3.57 (2.14–10.7)	–4.15	3e-05	0.19
PC-aa-C32:1	8.87 (4.00–22.1)	12.4 (4.53–33.0)	–3.53	4e-04	0.14
PC-aa-C32:2	1.68 (0.19–3.77)	3.10 (1.59–8.09)	–6.10	<1e-05	0.41
PC-aa-C34:2	243 (136–350)	298 (187–473)	–3.95	8e-05	0.17
PC-aa-C34:3	7.94 (3.15–16.7)	12.5 (3.79–26.0)	–6.18	<1e-05	0.42
PC-aa-C34:4	0.58 (0.15–1.31)	1.10 (0.43–2.72)	–5.90	<1e-05	0.39

Table 2 (Continue)

	FEP_b	CS			
	Median (min – max)	**Median (min – max)**	**Z-value**	***p*-value**	**Effect size (η^2)**
PC-aa-C36:1	27.6 (14.8–49.8)	40.4 (24.6–59.4)	–5.07	<1e-05	0.29
PC-aa-C36:2	127 (54.3–209)	190 (89.1–285)	–5.76	<1e-05	0.37
PC-aa-C36:3	59.4 (24.8–97.5)	89.5 (45.1–164)	–5.64	<1e-05	0.36
PC-aa-C36:6	0.53 (0.20–1.22)	0.78 (0.38–1.53)	–4.39	1e-05	0.21
PC-aa-C38:3	22.7 (11.7–41.6)	30.2 (20.2–47.6)	–4.50	<1e-05	0.23
Total_PC-aa	914 (491–1435)	1096 (793–1735)	–4.25	2e-05	0.20
Phosphatidylcholine acyl-alkyls					
PC-ae-C34:2	6.73 (2.65–12.9)	9.47 (4.31–16.8)	–4.90	<1e-05	0.27
PC-ae-C36:2	12.1 (6.52–18.2)	15.3 (6.91–24.9)	–3.75	2e-04	0.16
PC-ae-C36:3	4.07 (1.59–7.39)	5.91 (2.88–9.89)	–4.97	<1e-05	0.27
PC-ae-C40:2	3.58 (1.04–6.91)	4.43 (2.05–7.21)	–3.48	5e-04	0.14
PC-ae-C40:4	5.84 (1.59–10.4)	7.33 (3.58–10.1)	–3.55	4e-04	0.14
Sphingolipids					
SM-C20:2	0.32 (0.04–0.62)	0.41 (0.24–0.85)	–3.69	2e-04	0.15
Ratios of biomarkers					
LysoPC-a-C20:4/ LysoPC-a-C20:3	3.71 (1.48–7.72)	2.67 (1.60–7.79)	3.76	2e-04	0.16
Total_PC-aa/ Total_LysoPC	2.32 (1.11–3.94)	2.92 (1.88–5.70)	–3.92	9e-05	0.17
PC-aa-C36:3/ PC-aa-C36:4	0.68 (0.31–1.24)	0.91 (0.30–1.34)	–3.67	3e-04	0.15

Z-adjusted values according to Mann-Whitney U-test (FEPb compared to CS)
Effect sizes ≥0.14 were interpreted as large
Effect sizes 0.06–0.13 were interpreted as moderate
Commentary: all measured values are 2.5…15 times higher than LOD

GLM were then used to determine significant main effects of the disease on the metabolite levels.

GLM revealed all the GPLs and SMs mentioned above as substantial biomarkers in the model. The final model demonstrated a large main effect of the disease ($F_{(18,67)}$=5.48, p<1e-05, partial eta^2=0.60). The presence of the disease was associated with shifts in 18 measured biomarkers (Table 3), with the strongest association appearing between the illness and reduced levels of five PCs (PC-aa-C32:2, PC-aa-C34:3, PC-aa-C34:4, PC-aa-C36:2, PC-aa-C36:3).

Table 3 Statistically significant regression coefficients (ß), confidence intervals (CI), and significance values of log-transformed biomarkers levels with disease, adjusted for gender, age, and smoking status

Biomarkers	**ß**	**ß (95% CI)**	***t*-value**	***p*-value**
Glycerophospholipids				
Lysophosphatidylcholine acyls				
LysoPC-a-C20:4	0.38	0.18, 0.58	3.83	2e-04
Phosphatidylcholine diacyls				
PC-aa-C30:0	–0.41	–0.61, –0.22	–4.17	7e-05
PC-aa-C32:1	–0.30	–0.50, –0.10	–3.03	3e-03
PC-aa-C32:2	–0.52	–0.70, –0.34	–5.72	<1e-05
PC-aa-C34:2	–0.40	–0.60, –0.21	–4.07	1e-04
PC-aa-C34:3	–0.56	–0.74, –0.38	–6.19	<1e-05
PC-aa-C34:4	–0.56	–0.74, –0.38	–6.30	<1e-05
PC-aa-C36:1	–0.52	–0.71, –0.33	–5.44	<1e-05
PC-aa-C36:2	–0.58	–0.76, –0.40	–6.40	<1e-05
PC-aa-C36:3	–0.57	–0.75, –0.39	–6.32	<1e-05
PC-aa-C36:6	–0.44	–0.63, –0.24	–4.54	2e-05
PC-aa-C38:3	–0.46	–0.66, –0.27	–4.66	1e-05
Phosphatidylcholine acyl-alkyls				
PC-ae-C34:2	–0.47	–0.65, –0.28	–4.92	<1e-05
PC-ae-C36:2	–0.41	–0.61, –0.21	–4.01	1e-04
PC-ae-C36:3	–0.47	–0.66, –0.28	–4.88	<1e-05
PC-ae-C40:2	–0.39	–0.59, –0.18	–3.77	3e-04
PC-ae-C40:4	–0.37	–0.56, –0.18	–3.89	2e-04
Sphingolipids				
SM-C20:2	–0.32	–0.50, –0.14	–3.46	9e-04

In brief, untreated FEP patients had reduced serum levels of 11 PC-aa-Cs, 5 PC-ae-Cs, Total PC-aa and SM-C20:2. On contrary, the level of LysoPC-a-C20:4 was elevated. All these changes were associated with the presence of the disease.

6.2.2. Differences in glycerophospholipids' and sphingomyelins' profile in FEP patients before and after 7 months of treatment

The Wilcoxon matched pairs test was used to compare the AP-naïve and treated FEP patients' levels of GPLs and SMs. Levels of two LysoPC-a-Cs, Total_PC-aa, and 9 PC-aa-Cs as well as the ratios between PC-aa-C36:3/PC-aa-C36:4, and Total_PC-aa/Total_SM showed significantly increased levels, whereas two SMs, and the ratios of LysoPC-a-C16:0/LysoPC-a-C16:1 and LysoPC-a-C20:4/LysoPC-a-C20:3 showed decreased levels after 7-month treatment compared to the AP-naïve patients' group (Table 4).

Next the Bonferroni correction was applied. All abovementioned shifts survived this procedure ($p \leq 5e\text{-}04$) and displayed a large effect ($\eta^2 = 0.14–0.31$) (Table 4).

Table 4 Comparison of serum levels of GPLs (μmol/l) and SMs (μmol/l) between the first-episode psychosis patients (n=44) at baseline (before treatment with antipsychotics (FEP_b)) and after 7-month treatment (FEP_f) (n=44) with antipsychotics

	FEP_b	FEP_f	Z-value	*p*-value	Effect size (η^2)
	Median (min – max)	Median (min – max)			
Glycerophospholipids					
Lysophosphatidylcholine acyls					
LysoPC-a-C14:0	4.05 (3.08–9.15)	5.23 (3.45–13.0)	4.26	2e-05	0.21
LysoPC-a-C20:3	2.82 (1.24–6.19)	3.44 (1.59–8.52)	3.73	2e-04	0.16
Phosphatidylcholine diacyls					
PC-aa-C32:2	1.73 (0.22–3.77)	2.90 (0.18–7.37)	4.59	<1e-05	0.24
PC-aa-C34:3	8.14 (3.15–16.7)	11.4 (4.86–39.6)	4.90	<1e-05	0.27
PC-aa-C34:4	0.56 (0.24–1.31)	0.94 (0.22–2.72)	4.89	<1e-05	0.27
PC-aa-C36:1	28.8 (14.8–49.8)	38.1 (18.9–82.2)	3.94	8e-05	0.18

Table 4 (Continue)

	FEP_b	FEP_f	Z-value	*p*-value	Effect size (η^2)
	Median (min – max)	**Median (min – max)**			
PC-aa-C36:2	133 (54.3 –209)	182 (80.2–348)	4.93	<1e-05	0.28
PC-aa-C36:3	57.2 (24.8–97.5)	85.9 (42.7–165)	4.77	<1e-05	0.26
PC-aa-C36:6	0.55 (0.26–1.22)	0.70 (0.16–1.91)	3.68	2e-04	0.15
PC-aa-C38:3	23.2 (11.7–41.6)	30.6 (13.7–64.8)	4.25	2e-05	0.21
PC-aa-C40:5	3.95 (1.88–8.61)	4.92 (1.92–17.0)	4.18	3e-05	0.20
Total_PC-aa	930 (546–1435)	1116 (599–2065)	3.97	7e-05	0.18
Sphingolipids					
SM-(OH)-C16:1	1.99 (0.57–3.56)	1.71 (0.52–3.01)	3.48	5e-04	0.14
SM-C18:0	14.8 (3.70–25.8)	12.0 (3.33–20.0)	3.50	5e-04	0.14
Ratios of biomarkers					
LysoPC-a-C16:0/ LysoPC-a-C16:1	49.8 (24.8–72.4)	40.3 (25.9–69.5)	4.06	5e-05	0.19
LysoPC-a-C20:4/ LysoPC-a-C20:3	3.80 (1.48–7.72)	2.63 (1.46–4.88)	5.20	<1e-05	0.31
PC-aa-C36:3/ PC-aa-C36:4	0.68 (0.31–1.24)	0.89 (0.61–1.54)	4.34	1e-05	0.21
Total_PC-aa/ Total_SM	5.97 (4.12–11.3)	7.94 (4.84–16.8)	5.18	<1e-05	0.31

Z-adjusted values according to Mann-Whitney U-test (FEPb compared to CS)
Effect sizes ≥0.14 were interpreted as large
Effect sizes 0.06–0.13 were interpreted as moderate
Commentary: all measured values are 2.5…15 times higher than LOD

GLM were then used to determine significant main effects of the 7-month AP treatment on the levels of serum GPLs and SMs. Backward elimination procedures' model revealed that AP treatment caused statistically significant elevations in two LysoPC-a-Cs (LysoPC-a-C14:0, LysoPC-a-C20:3), and 9 PCs (PC-aa-C32:2, PC-aa-C34:3, PC-aa-C34:4, PC-aa-C36:1, PC-aa-C36:2, PC-aa-C36:3, PC-aa-C36:6, PC-aa-C38:3, PC-aa-C40:5) (Table 5). The most prominent were the associations between PC-aa-C36:2 and PC-aa-C36:3 level elevation and treatment.

Table 5 Statistically significant regression coefficients (ß), confidence intervals (CI), and significance values of log_{10}-transformed biomarkers levels in first-episode patients' group before treatment compared to biomarkers values measured after 7-months treatment with antipsychotics

Biomarkers	**ß**	**ß (95% CI)**	***t*-value**	***p*-value**
Glycerophospholipids				
Lysophosphatidylcholine acyls				
LysoPC-a-C14:0	–0.30	–0.51, –0.10	–2.96	4e-03
LysoPC-a-C20:3	–0.33	–0.54, –0.13	–3.28	2e-03
Phosphatidylcholine diacyls				
PC-aa-C32:2	–0.37	–0.57, –0.17	–3.64	5e-04
PC-aa-C34:3	–0.47	–0.66, –0.28	–4.90	<1e-05
PC-aa-C34:4	–0.43	–0.62, –0.24	–4.40	3e-05
PC-aa-C36:1	–0.40	–0.59, –0.20	–4.00	1e-04
PC-aa-C36:2	–0.51	–0.69, –0.32	–5.46	<1e-05
PC-aa-C36:3	–0.50	–0.69, –0.32	–5.38	<1e-05
PC-aa-C36:6	–0.30	–0.50, –0.09	–2.91	5e-03
PC-aa-C38:3	–0.40	–0.59, –0.20	–4.00	1e-04
PC-aa-C40:5	–0.32	–0.52, –0.12	–3.13	2e-03

In brief, after 7 months of treatment levels of two LysoPC-a-Cs, Total_PC-aa, and 9 PC-aa-Cs had increased, whereas two SMs decreased in FEP patients. AP treatment was shown to be associated with these significant elevations in two LysoPC-a-Cs and 9 PC-aa-Cs.

6.2.3. Differences in glycerophospholipids' and sphingomyelins' profile in FEP patients after 7 months of treatment compared to control subjects

Although, after 7 months of treatment, serum levels of fourteen GPLs and two SMs differed when FEP patients and control subjects were compared, the found changes did not survive Bonferroni correction for multiple comparisons. Therefore, it can be said that seven months of AP treatment reverted GPL and SM serum levels of patients back to comparable with control group.

6.3. Changes in amino acid and biogenic amine profile (Paper II and Paper III)

6.3.1. Differences in amino acids' and biogenic amines' profile in untreated FEP patients compared to control subjects

For the primary study group of 38 AP-naïve FEP patients (Paper II), the Mann-Whitney *U*-test was used to compare AA and BA levels between patients and control subjects. 21 AAs, 20 BAs and their metabolically relevant ratios were analysed (Supplementary Tables 2, 3). Comparison revealed a trend of decrease in six AA levels (Ala, citrulline, His, Trp, Tyr, Val) when comparing FEP patients to control subjects (Supplementary Table 2). Statistically significant difference was found in the level of Pro (Z=–3.18, p=1e-03, η^2=0.14) and Tyr/Phe ratio (Z=–4.24, p=2e-05, η^2=0.24) that both were considerably reduced in patients' group. Circulating BA Kyn showed a decreasing trend, BA alpha-AAA level in patients was statistically significantly reduced (Z= –3.27, p=1e-03, η^2=0.14) compared to the control subjects. Contrarily, BAs' spermine and taurine levels in patients' group were significantly elevated (Z=3.20, p=1e-03, η^2=0.14; Z=5.56, p<1e-06, η^2=0.41, respectively) (Supplementary Table 3).

For the expanded study group of 52 patients (Paper III) AA, BA levels and their metabolically relevant ratios were analysed using a series of linear mixed-effects regression models.

In the enlarged study group, we confirmed our previous findings as the decrease in Pro (p=7e-05) and alpha-AAA levels (p=1e-06) and Tyr/Phe ratio (p=6e-04) were identified as significant when comparing patients with control subjects. In contrary, taurine level (p=2e-15) was significantly increased in AP-naïve patients compared to the control group. In addition to previous findings, Orn/Arg ratio (p=2e-06) was also found significantly increased in patients' group. These results are presented in more detail in supplementary tables (Supplementary Table 4, 5, 6).

In brief, untreated FEP patients had reduced serum levels of Pro, alpha-AAA and Tyr/Phe ratio. On contrary, the level of taurine and Orn/Arg ratio were elevated. All these changes were associated with the presence of the disease.

6.3.2. Differences in amino acids' and biogenic amines' profile in FEP patients before and after 7 months of treatment

In the primary study group Ala, Met, Tyr and Val showed an elevating trend whereas Asp showed a reducing trend after 7-month treatment compared to AP-naïve patients' group. His (Z=3.75, p=2e-04, η^2=0.20), Pro (Z=4.15, p=3e-05, η^2=0.24) and Tyr/Phe ratio (Z=4.46, p=8e-06, η^2=0.28) showed significantly increased levels after treatment (Supplementary Table 7).

On BAs level, Kyn/Trp ratio showed an elevating trend, contrary to spermine, methionine-sulfoxide (Met-So) and Met-So/Met ratio that were found to have a

reducing trend between patients after 7 months of treatment compared to AP-naïve patients' group. The levels of acetylornithine (Ac-Orn) (Z=3.41, p=7e-04, η^2=0.16), alpha-AAA (Z=3.33, p=9e-04, η^2=0.15) and Kyn (Z=3.59, p=3e-04, η^2=0.18) elevated significantly during the 7 months of treatment, whereas the level of taurine (Z=5,17, p<1e-06, η^2=0.37) was reduced compared to AP-naïve patients (Supplementary Table 8).

In brief, after 7 months of treatment levels of His, Pro, Ac-Orn, alpha-AAA, the Kyn and Tyr/Phe ratio had increased in FEP patients, whereas the serum level of taurine decreased in FEP patients.

6.3.3. Differences in amino acids' and biogenic amines' profile in FEP patients after 7 months of treatment compared to control subjects

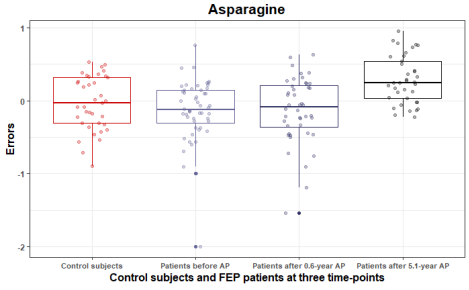
In the study group of 36 patients, 7 months of AP treatment resulted in AA levels returning to being comparable to control subjects. The BA Met-So level was significantly reduced (p=2e-02, η^2=0.07) hence the ratio between Met-So and Met was also significantly reduced (p=2e-02) in treated patients compared to the control subjects. The effect size of this change was moderate (η^2=0.08). The ratio between Kyn and Trp was elevated in treated patients compared to the control subjects (p=2e-03), the effect size was moderate (η^2=0.13).

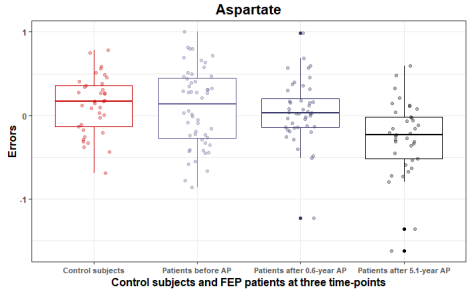
In 7 months of AP treatment in the expanded study group of 52 patients, all levels of previously altered AAs, BAs (Supplementary Table 9) and their ratios reverted to a level comparable to the control subjects'. This change was accompanied by reduction of psychotic symptoms.

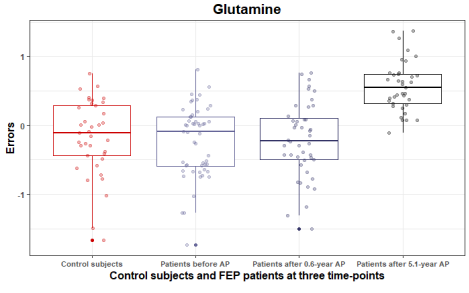
6.3.4. Differences in amino acids' and biogenic amines' profile in FEP patients after 5.1 years of treatment compared to control subjects

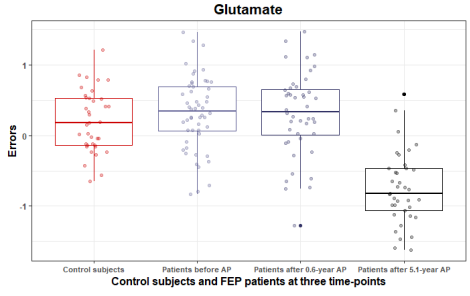
5.1 years after onset of SSD, patients' illness and AP medication usage were associated with significant alterations in six AAs'and two of their derivate BAs' levels (Supplementary Table 9) as well as several calculated biomarker ratios. In AAs' profile, persistence of the disease and treatment were found associated with the decrease in Asp ($t_{(71)}$=–5.26, p<1e-04) and Glu ($t_{(71)}$=–12.19, p<1e-04) levels. In contrast, Asn ($t_{(71)}$=4.73, p<1e-04), Gln ($t_{(71)}$=9.22, p<1e-04), Met ($t_{(71)}$=9.74, p<1e-04) and Orn ($t_{(71)}$=3.47, p<9e-04) levels were increased. A statistically significant decrease in alpha-AAA ($t_{(71)}$= –6.25, p<1e-04) and an increase in taurine ($t_{(71)}$=5.35, p<1e-04) level were detected in patients compared to the control subjects. Furthermore, the ratios of alpha-AAA/Kyn ($t_{(71)}$=–5.71, p<1e-04), Asp/Asn ($t_{(71)}$= –8.26, p<1e-04), and Glu/Gln ($t_{(71)}$= –12.24, p<1e-04) were also decreased. The ratio between Orn and Arg ($t_{(71)}$=5.43, p<1e-04) was, contrarily, increased. Therefore, the profile of AAs and BAs, after 5.1 years of ongoing illness and treatment, was shifted back towards pre-treatment status compared to 7-month treatment that showed more resemblance to the control groups (Figures 1, 2).

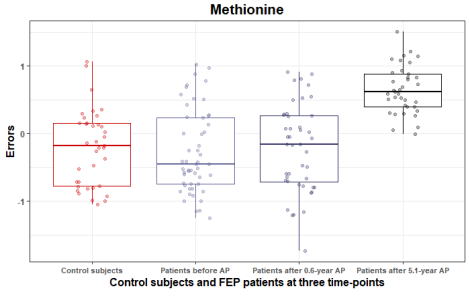
Amino acids

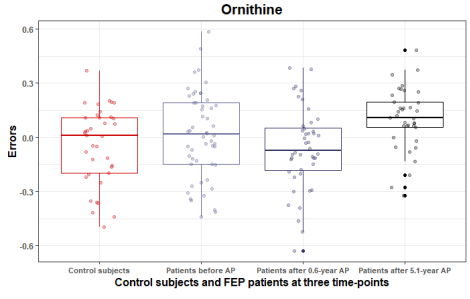

Asparagine
Errors
Control subjects
Patients before AP
Patients after 0.6-year AP
Patients after 5.1-year AP
Control subjects and FEP patients at three time-points

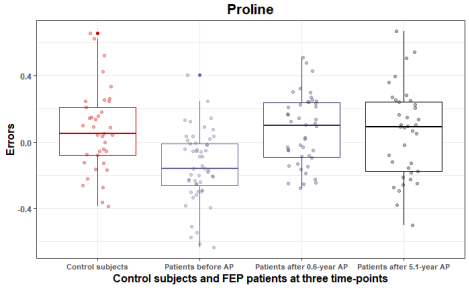

Aspartate
Errors
Control subjects
Patients before AP
Patients after 0.6-year AP
Patients after 5.1-year AP
Control subjects and FEP patients at three time-points


Glutamine
Errors
Control subjects
Patients before AP
Patients after 0.6-year AP
Patients after 5.1-year AP
Control subjects and FEP patients at three time-points


Glutamate
Errors
Control subjects
Patients before AP
Patients after 0.6-year AP
Patients after 5.1-year AP
Control subjects and FEP patients at three time-points


Methionine
Errors
Control subjects
Patients before AP
Patients after 0.6-year AP
Patients after 5.1-year AP
Control subjects and FEP patients at three time-points


Ornithine
Errors
Control subjects
Patients before AP
Patients after 0.6-year AP
Patients after 5.1-year AP
Control subjects and FEP patients at three time-points


Proline
Errors
Control subjects
Patients before AP
Patients after 0.6-year AP
Patients after 5.1-year AP
Control subjects and FEP patients at three time-points

Biogenic amines

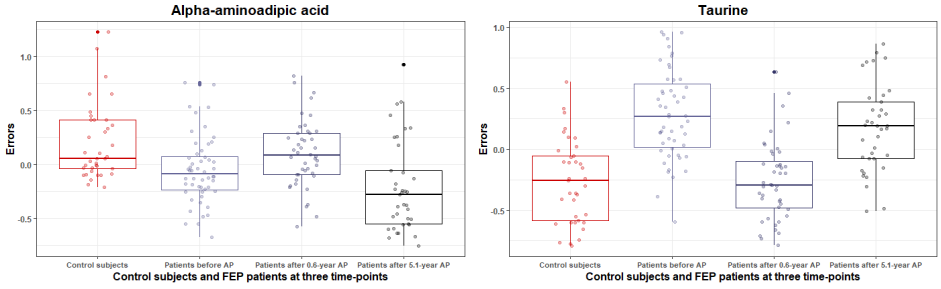


Figure 1 Boxplots of the statistically significant variation of the errors (residuals) of log-transformed amino acid and biogenic amine levels (derived by regressing out covariate effects) for control subjects and first-episode (FEP) patients at baseline (before treatment with APs), after 0.6-year, and after 5.1-year treatment with APs. The solid black horizontal line in each box represents the median. The area above and below the line represents the 50th and the 25th to the 50th percentiles, respectively. The whiskers extend to the highest and lowest values contained within 1.5 times the interquartile range of the data. Each calculated error variance is represented as a dot

Biomarker ratios

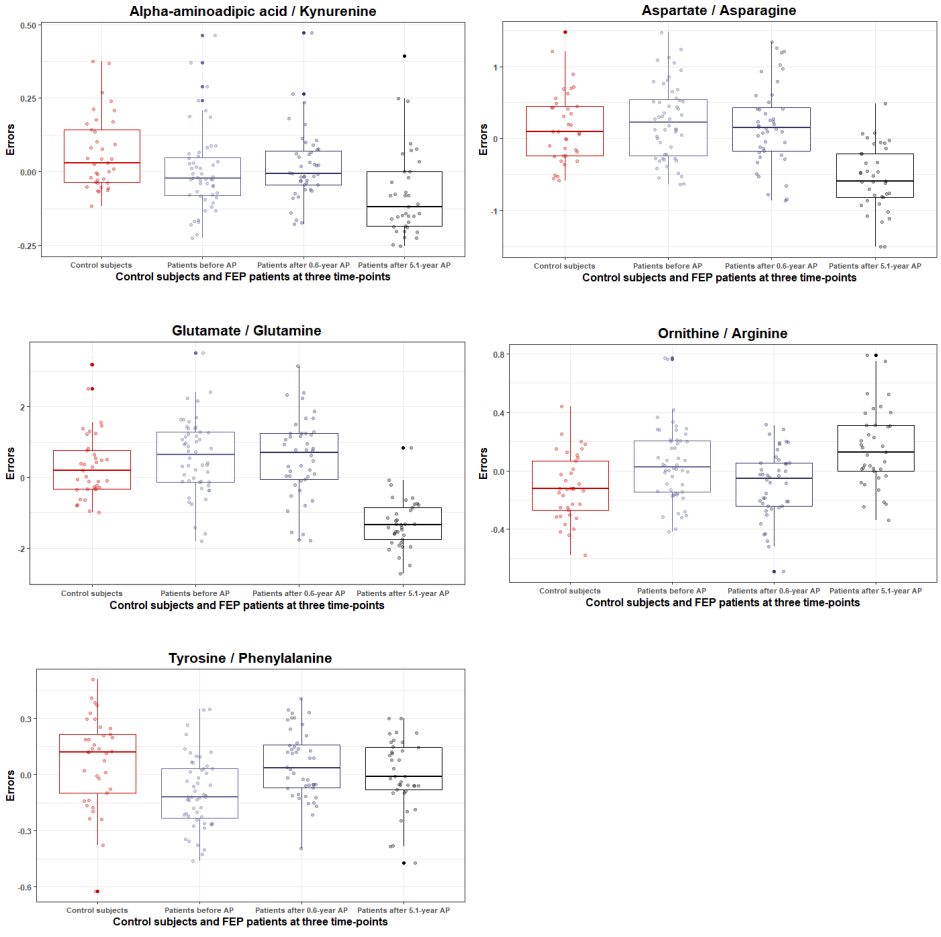


Figure 2 Boxplots of the statistically significant variation of the errors (residuals) of log-transformed biomarker ratios (derived by regressing out covariate effects) for control subjects and first-episode (FEP) patients at baseline (before treatment with APs), after 0.6-year, and after 5.1-year treatment with APs

In brief, patients treated for 5.1 years had reduced serum levels of Asp, Glu, alpha-AAA and Glu/Gln, Asp/Asn and alpha-AAA/Kyn ratio. In contrast, the levels of Asn, Gln, Met, Orn, taurine and Orn/Arg ratio were elevated. Therefore, the profile of AAs and BAs was significantly altered and not only compared to control subjects but to untreated FEP patients and patients after short-term AP treatment.

7. DISCUSSION

SSDs are a diverse class of psychiatric conditions that, to present knowledge, greatly share a biochemical background. To understand the pathophysiology underlying the symptoms of SSDs better, the dynamics of metabolic changes during the different stages of the illness should be researched. As the processes at the onset of the disease are known to have a crucial role in determining further prognosis (Drake et al., 2020), the early course of these conditions is in the focus of our interest. Derived from the main hypotheses of SSD pathophysiology we concentred our study to lipid and AA-related changes. We used metabolomics approach to evaluate GPL, SL, AA and BA levels in SSD patients' blood serum compared to control subjects. Assessments were conducted at different time-points of the illness – AP-naïve state in the first psychotic episode, after the stabilisation of patients' condition in 7 months of AP treatment and for AA and BA levels again in approximately 5 years after the onset of the disease and treatment initiation. The aim was to characterise the changes in GPLs', SLs', AAs' and BAs' metabolomic profiles during the early course of SSD and AP medication use.

7.1. Changes in lipid profile in first psychotic episode (Paper I)

In this part of the study, we detected statistically significant alterations in numerous lipid metabolite levels in AP-naïve FEP patients' group compared to the control group. Eleven PC diacyls and the calculated total PC-aa showed diminished concentrations in patients' group. Additionally, five PC acyl-alkyls' and SL SM-C20:2 levels were decreased in patients experiencing the onset of SSD. However, LysoPC-a-C20:4 level, in contrast, was elevated in patients compared to the control subjects. All these changes demonstrated a strong main effect of the disease in GLM modelling, with the strongest effect on PC-aa-C32:2, PC-aa-C34:3, PC-aa-C34:4, PC-aa-C36:2 and PC-aa-C36:3 concentrations.

Heightened LysoPC levels have also been found in a study involving 55 AP-naïve FEP SCZ and bipolar disorder patients (Costa et al., 2019). However, Costa and colleagues (2019), in contrast to our findings, saw an increase in patients' PC blood plasma levels compared to the control group (Costa et al., 2019). In another study including 20 SCZ onset patients, the AP-naïve patients demonstrated increased SM, triacylglycerol and cholesteryl ester levels, whilst their plasmenyl-PC, plasmenyl-PE, PC, LysoPC, LysoPE and acylcarnitine levels were reduced (Yan et al., 2018). Wang and colleagues (2019) found in their sample of 119 SCZ patients including 20 first-episode patients, that at least a month off-treatment recurrent SCZ patients show similar lipid profiles to AP-naïve FEP patients with most PC, LysoPC, and all PE serum levels decreased compared to control subjects. Most LysoPE and SM levels, however, were found to have increased (Wang et al., 2019). These findings support our results as decreased PC levels in SCZ

patients seem to be eminent in most studies. As PCs are an important component of HDL, our find of diminished PC levels may also be associated with lower levels of HDL found in first-episode non-affective psychosis patients (Misiak et al., 2017).

The vast majority of cell membrane PCs are diacyls containing two long-chain fatty acid residues (van der Veen et al., 2017). Previously we have demonstrated the elevation of circulating long-chain acylcarnitines' (C16:0, C16:1, C16:1-OH, C18:1, and C18:2) serum levels in AP-naïve FEP patients (Kriisa et al., 2017). These findings suggest that in FEP a lipidomic shift occurs that is characterized by increased disintegration of PCs leading to an elevated concentration of acylcarnitines. A similar mechanism may explain the elevated LysoPC-a-C20:4 as lecithin-cholesterol acyltransferase can transfer PC's fatty acid to the free cholesterol in plasma, producing cholesteride and LysoPC (Rousset et al., 2009). When PC contains C20:4 residues then the decomposition would result in a lowered PC and a heightened LysoPC-a-C20:4 level. Described lipidomic shifts may refer to cell membrane damage in FEP.

Potential factors that can induce differences between the results of different studies are numerous, starting with a selection of subjects in relation to their duration of the illness and age ending with varied methodological and statistical approaches. These partially contradictive findings indicate that lipid metabolism in FEP patients may be altered by factors of which present studies do not give thorough comprehension yet. Further investigation is needed and more emphasis on patients' illness duration, age and medication history could clarify the emerged inconsistencies.

7.2. Changes in lipid profile after seven months of antipsychotic treatment (Paper I)

After 7 months of treatment statistically significant elevation in 9 PC and two LysoPC levels emerged. GLM modelling verified the AP treatment as its cause and treatment effect was the strongest on PC-aa-C36:2 and PC-aa-C36:3 levels. Contrary to PCs, SM-(OH)-C16:1 and SM-C18:0 serum concentrations showed a decrease. Although other changes in lipid profile subsequent to the treatment did not demonstrate such salience when comparing treated patients' group to the control group, after applying the Bonferroni correction for multiple comparisons, the two groups did not differ significantly in their lipid metabolite concentrations. Therefore, 7 months of AP treatment was sufficient to reverse GPL and SL serum level changes accompanying FEP.

In contrast to our results, studying 36 FEP patients, Suvitaival and colleagues found that in patients' group the serum levels of studied PCs were reduced during the follow-up period of 1 year (Suvitaival et al., 2016). This contradiction, however, may be the result of a difference in the duration of the illness between patients' groups. In the study of Yan and colleagues, 8 weeks after starting the

AP medication SCZ patients' lipid profiles showed four different alteration patterns (Yan et al., 2018). Firstly, some lipid levels were elevated in AP-naïve patients and decreased by treatment, these were cholesteryl esters and triacylglycerols. Secondly, plasmenyl-PC, PC and LysoPC levels were decreased in AP-naïve patients and further diminished after two months of treatment. Third, glucosylceramide, ceramide and fatty acid levels that were not altered in AP-naïve patients but decreased during 8 weeks of treatment. Lastly, plasmenyl-PE, LysoPE and acylcarnitine levels were altered in AP-naïve patients' group compared to control subjects, but these alterations had no significant response to treatment (Yan et al., 2018). In our study, the evaluation of lipid profile changes related to AP use was performed 7 months after initiating the treatment. Taking into consideration the variety of results depending on the treatment and illness duration, it can be assumed that further changes in the lipid profile may occur after a longer follow-up period.

Considering SM levels in our study, in AP-naïve patients only one discriminated patients from control subjects significantly and that was SM-C20:2, the level of which was decreased in patients. After 7 months of treatment, levels of SM-(OH)-C16:1 and SM-C18:0 were diminished. Previous studies on SM metabolism changes in the context of SSDs are very limited and focused mainly on the central nervous system findings. Relating to peripheral tissues, Tessier and colleagues (2016) noticed in their study of SCZ patients' red blood cell membrane lipidomic composition that patients divide into two groups with respect to SM membrane content – first group demonstrated prominent alterations in membrane lipids including low levels of SMs, second groups' results were similar to control subjects. Furthermore, perturbations in membrane lipid composition were accompanied by more severe psychopathology, suggesting its association with DA signalling (Tessier et al., 2016). Similarly to our finding, SM blood plasma level decrease after a period of AP medication has been detected in a group of SCZ patients before, however, in that study AP-naïve patients demonstrated elevated SM concentrations contrary to ours (Yan et al., 2018).

The subject of peripheral lipidomic patterns in SSDs need further investigation as current studies are few in numbers and provide limited data. Nevertheless, relying on available information and our study results, AP treatment seems to reverse alterations in GPL and SL profiles during 7 months of follow-up. What kind of lipidomic changes accompany psychotic illness and AP treatment in long term, what are the potential influential factors and how do they relate to the course of the illness need to be specified.

7.3. Changes in amino acid and biogenic amine profile in first psychotic episode (Paper II and Paper III)

In our study, when FEP patients' and control subjects' AA and BA metabolomic profiles from serum were compared, several statistically significant differences were observed. AP-naïve patients demonstrated a decrease in Pro levels, Tyr/Phe ratio and alpha-AAA levels. An increase in taurine level was eminent in FEP patients compared to the control subjects. Additionally, Orn/Arg ratio elevation was detected in the extended group of SCZ patients, although it was not significant in the primary group of 38 patients.

Alterations in Pro concentrations, as Pro is the precursor of Glu synthesis and potentially has several other effects on neurotransmission, have been studied in SSDs context (Clelland et al., 2016; Volk et al., 2016). However, data on peripheral Pro levels in FEP patients are extremely limited. To our knowledge only one relevant study including 40 drug-naïve FEP patients exists and, in contrast to our results, it showed elevated plasma levels of Pro in comparison to control subjects (Garip & Kayir, 2019). However, recent findings indicate, that plasma prolidase levels alter significantly in different stages of SCZ (Bolu et al., 2021), therefore, patient cohort differences may contribute to the inconsistent findings. Nevertheless, further investigation of Pro in the context of FEP is necessary to clarify its role in the pathogenesis of SSDs.

We identified a shift in favour of Phe in the ratio between AAs Tyr and Phe. To our knowledge, there are no previous publications related to Phe blood concentrations or Phe and Tyr relation changes in FEP. However, in a study measuring Phe and Tyr plasma concentrations in over 900 SCZ patients, in accordance with our finding in FEP patients, an elevated Phe and Phe/Tyr ratio has been described (Mathai et al., 2016; Okusaga et al., 2014). The relative elevation of Phe can be the result of the inhibition of Phe hydroxylase by acquired immune activated state, which is well known to accompany SSDs (Upthegrove & Khandaker, 2020; Haring et al., 2015; Mathai et al., 2016). Knowing that Tyr is a substrate for Tyr hydroxylase in producing DA, a relatively lower level of Tyr may reflect the amplified production of DA in dopaminergic neurons, associated with the manifestation of psychotic symptoms.

Heightened levels of taurine were apparent and it had the most distinct relation to the FEP compared to the other observed metabolites in our study. Elevated taurine levels in FEP patients are in line with a study by Cao and colleagues (2019) of 29 drug-naïve first-episode patients and 84 recurrent SCZ patients who had been medication-free for at least a month. In this study, the authors did not find any significant differences in metabolic profiles between FEP drug-naïve patients and recurrent SCZ patients (Cao et al., 2019). Elevated plasma levels of taurine in patients compared to the control subjects were demonstrated and Cao and colleagues (2019) stated, that excessive taurine may contribute to oxidative stress, neurotransmitter disorders and even to the dysfunction of the NMDA receptor. However, administering taurine in addition to the AP medication has

shown greater improvements in psychopathology scores in FEP patients than placebo, presumably owing to its antioxidant properties (O'Donnell et al., 2016). Taurine's potential role in oxidative stress and neuroprotective processes is discussed in more detail in a previous article by our research group, where taurine and epidermal growth factor are proposed to be FEP biomarkers and taurine emphasized as one of the most important endogenous antioxidant (Koido et al., 2016).

Our study demonstrated a reduction in BA alpha-AAA levels in AP-naïve FEP patients. Alpha-AAA concentration in human physiology is tightly connected to Kyn concentration. As a competitive substrate, alpha-AAA in high levels prevents Kyn from reacting with Kyn aminotransferase II (a prevalent isoform of Kyn aminotransferases in the brain) and thereby transaminating into KYNA (Hallen et al., 2013; Passera et al., 2011). High levels of alpha-AAA lead to decreased KYNA production from Kyn and low levels of alpha-AAA lead to increased KYNA production. Both reduced alpha-AAA and Kyn peripheral concentrations may indicate enhanced KYNA production in the central nervous system. Although in our patients' group of 38 people we found the trend of diminishing Kyn levels, in the extended patients' group it was not observed. In drug-naïve first-episode SCZ patients' decreased Kyn plasma levels compared to the control group have been demonstrated before (Joaquim et al., 2018). Low levels of Kyn in the periphery may be the result of intensified Kyn use in the central nervous system. This is in accordance with the reduced alpha-AAA found in our study. Decreased alpha-AAA would lead to increased KYNA production from Kyn, thereby depleting the levels of both alpha-AAA and Kyn.

In the group of 52 patients, we exposed a significant elevation of Orn/Arg ratio. So far only very limited data on Arg metabolism in SSD patients have been available. In a case-control study of FEP patients, no significant difference was found in Orn plasma levels between drug-naïve patients and controls, nor did the Orn level change after 10 weeks of AP treatment (Garip et al., 2019). Again, as the matter has been studied very little, the reason for inconsistent results is unclear. However, the shift we found towards Orn may reflect the activity of arginase, the increase of which has been associated with SSDs (Liu et al., 2016).

Orn/Arg ratio alteration may be linked to the metabolomic changes observed in SSD in yet another way. Orn is the precursor for the synthesis of spermine. In our primary FEP patients group, the elevation of spermine levels was found, whereas the same pattern did not occur in the extended study group. Although, to our knowledge, there are no previous studies presenting spermine levels in FEP drug-naïve patients, its significantly elevated plasma levels have been found in SCZ and bipolar disorder patients compared to the control subjects (Baytunca & Öngür, 2020). It is hypothesized that heightened spermine levels may serve as a compensatory mechanism attempting to recover NMDA receptor dysfunction, as it has several NMDA receptors modulating properties (Baytunca & Öngür, 2020; Hackos & Hanson, 2017). These findings of Orn/Arg and spermine levels that, at first glance, are not in line with our primary and extended study groups, may, in fact, reflect a nuanced interplay and fragile balance in Arg metabolic pathway. It

can be hypothesized that in the case of increased arginase activity the Orn/Arg ratio will incline towards Orn. At some point, during the course of FEP, the Orn's relative excess will enhance spermine production that lowers Orn levels and presumably also modulates NMDA receptor dysfunction. Further research of Arg pathway alterations in SSD patients has to be done, to explain seemingly contradictive findings and reveal the potential underlying mechanisms that shift the balance of the metabolic pathway and mediate the NMDA receptor dysfunction.

7.4. Changes in amino acid and biogenic amine profile after seven months of antipsychotic treatment (Paper II and Paper III)

As expected, 7 months of AP treatment reduced psychotic symptoms markedly in FEP patients. Unfortunately, it was accompanied by a significant increase in BMI, a probable sign of developing metabolic syndrome. After 7 months of AP treatment metabolomic changes described above relating AAs and BAs were reverted and their concentrations had shifted back to the level comparable to CS.

An exception emerged when analysing primary patients' group. The Ac-Orn level was also elevated by the treatment but unlike the other BAs mentioned, its levels were not significantly changed in drug-naïve FEP patients compared to CS. Therefore, Ac-Orn concentration ascendance presumably reflects its connection with drug intake rather than the shifts related to the psychotic illness itself. However, Ac-Orn has not been observed to be an influential molecule in the central nervous system, nor has it been associated with AP treatment in general. In mouse brains, an increase of Ac-Orn level has been found to accompany prolonged administration of haloperidol (McClay et al., 2015). The cause of its elevation in our study is unknown.

Taurine levels after 7 months of AP treatment were decreased, whereas alpha-AAA and Kyn levels increased to the level comparable to the control subjects'. Levels of AAs Pro and His were elevated and the ratio between Tyr and Phe shifted in favour of Tyr. The latter could be a consequence of an increased DA demand resulting from D_2 receptor blockage by AP drugs (Ichikawa et al., 2001), therefore, heightened use of its precursor may occur.

Although not all changes in AA and BA levels survived the Bonferroni correction, the trend in AA and BA levels indicate that the metabolomic changes accompanying FEP were reversed towards the normalisation of the metabolomic profile after 7 months of treatment. Repeated measures GLM was used to estimate the main effect of the treatment on the AA and BA level changes and BMI. It revealed that AP treatment was strongly linked to the decrease of taurine, spermine and Asp and increase of Pro, His, Ala, alpha-AAA, Kyn levels and BMI. The changes in taurine and Pro concentrations showed the most distinguished association with AP treatment.

7.5. Changes in amino acid and biogenic amine profile after 5.1 years of ongoing psychotic illness and treatment (Paper II and Paper III)

In the follow-up after an average of 5.1 years of ongoing treatment and SSD, results regarding the AA and their derivate BA metabolomic profiles in patients were pathologic. Out of 31 metabolites measured, eight showed altered levels compared to the control subjects. We detected increased Asn, Gln, Orn, taurine, Met and decreased Asp, Glu and alpha-AAA levels. Consequently, Asp/Asn, Glu/Gln, alpha-AAA/Kyn ratios had lowered and the Orn/Arg ratio heightened (Figures 1, 2).

Metabolism of AAs Asp, Gln and Asn, Glu is tightly linked. Asp and Gln are substrates for Asn synthetase that, in adenosine triphosphate (ATP) dependent reaction, converts Asp and Gln into Asn and Glu (Lomelino et al., 2017). In accordance to our findings, heightened Asn levels have been shown by Garip and Kayir in a study involving 40 FEP patients, however, their follow-up period was only 10 weeks and during the treatment period, the level of Asn started to normalize, analogously to our short-term treatment results (Garip & Kayir, 2019). It has been proposed that the excessive peripheral Asn levels may result in an inhibited uptake of other AAs due to the competition for limited transporters (Lomelino et al., 2017).

Our study revealed decreased Asp levels, increased Asn levels and therefore decreased Asp/Asn ratio in the sample of SSD patients compared to the control subjects. Diminished Asp plasma levels in patients with recurrent SCZ compared to the first-episode patients have also been shown (Cao et al., 2018). According to mice models, peripherally increased Asp concentrations elevate Asp central levels as it efficiently crosses the blood-brain barrier (Sacchi et al., 2017). Asp is an important regulator of adult neurogenesis, an NMDA receptor agonist and, additionally, can activate NMDA, and several different presynaptic receptors, thereby stimulating Glu release in specific areas of the brain (Genchi, 2017; Errico et al., 2018; Sacchi et al., 2017). Established changes reflect an altered metabolic pathway that involves not only Asn and Asp but Glu as well.

As increased Gln and decreased Glu serum concentrations occurred in patients, a decreased Glu/Gln ratio was consequently detected in SSD patients compared to the control group. Madeira and colleagues (2018) have found that patients with illness onset within 5 years showed Gln/Glu ratio elevation, whereas chronic SCZ patients (in their study mean illness duration 23.6 years) had a decreased Gln/Glu ratio when compared to the control subjects. This is in accordance with our findings that likewise showed Glu and Gln ratio to be inclined in favour of Gln. This diversion in Glu and Gln ratio demonstrates dynamic metabolomic changes putatively related to pathogenesis and pharmacological treatment of SSDs (Madeira et al., 2018). In a study involving 15 SCZ patients, heightened breath ammonia levels were detected in patients compared to the control subjects (Popa et al., 2015). This phenomenon was hypothesised by authors to be related to the

deficiency of AAs required for ammonia detoxification in the liver and reduced kidney function both as the result of medication. The main route for ammonia detoxification in the brain is by Gln synthesis by Gln synthetase but it also can be bound into Ala by the collaboration of Glu dehydrogenase and Ala aminotransferase (Dadsetan et al., 2013). Both of these mechanisms demand Glu and produce Gln. Gln in turn, as described before, is a substrate for Asn synthesis. Therefore, it can be hypothesised that the elevation of Gln and Asn (with a concomitant decrease in Glu and Asp levels) in SSD patients is the result of an intensified ammonia detoxification derived from AP medication use. Considering the variable findings amongst patient groups with different durations of the SSD, it is clear that Glu and Gln levels are affected by the illness progression and further investigation with emphasis on the disease duration in relation to Asp, Asn, Gln and Glu metabolism is needed.

We detected increased Met level in SSD patients with illness and treatment duration of 5.1 years compared to controls. In the first-episode stage of the illness, this alteration was not salient. Nevertheless, heightened Met levels have been previously associated with SSDs. Methylation, as an important regulator of numerous metabolic pathways and biologic processes including gene expression through DNA methylation, has been found to be impaired in SSD patients (Li et al., 2019; Wang et al., 2015). In the '60s and '70s, several studies showed that Met treatment of SSD patients concluded in exacerbation of the symptoms (Cohen et al., 1974). Although the mechanisms through which Met provokes psychotic manifestations in not entirely clear, alterations in peripheral Met level should be considered as a potential expression of underlying pathological processes.

After 5.1 years of disease and treatment, the SSD patients in our study demonstrated an increased level of Orn and therefore Orn/Arg ratio similarly to the state at the onset of the disease. To our knowledge, there are no previous studies that could validate or confute this finding. A significant correlation between Orn serum concentration and the duration of illness has been found in SCZ patients, giving ground to a hypothesis that long term AP medication may induce Orn concentration (Tomiya et al., 2007). A study involving 168 recurrent SCZ patients, who had been off-medication at least for a month, and 40 drug-naïve FEP patients, found diminished Orn plasma levels in patients compared to the control subjects (Cao et al., 2018). Taking into consideration the substantial differences between the patients' cohorts in Cao and colleagues' and our study, especially in terms of previous treatment and illness duration variability, the results are hardly comparable, and their controversy is not unexpected.

In accordance with our findings, increased taurine serum concentration in SSD patients has been found in a study including 113 SCZ patients (Cao et al., 2019). However, the aforementioned limitations in the comparability of the studies apply again, as the study by Cao and colleagues (2019) included both recurrent off-medication and drug-naïve first-episode SCZ patients. Taurine upregulation may be associated to its beneficial properties as anti-inflammatory, antioxidant and glucose and lipid metabolism modulating agent and thereby function as a compensatory mechanism (Koido et al., 2016; O'Donnell et al., 2016; Rosa et al., 2014).

Decreased alpha-AAA level, therefore reduced alpha-AAA/Kyn ratio reappeared during the 5-year duration of the SSD. Limited data are available regarding alpha-AAA blood level changes in SSD patients. However, Kyn pathway has several potential modulating effects on SSD development as Kyn is the precursor for KYNA, an antagonist of Glu receptors (Joaquim et al., 2018). Low-grade inflammatory processes accompanying SSDs have been shown by Joaquim and colleagues (2018) to incline Trp metabolism towards the production of Kyn instead of serotonin. Therefore, Kyn prevalence in relation to alpha-AAA may also reflect the upregulation of Kyn production. Further investigation is needed for more conclusive explanations relating to Kyn pathway alterations and SSD pathogenesis putative associations.

7.6. Strengths and limitations of the study

The main limitation of our study is the modest number of participants. Nevertheless, it is possible to successfully identify metabolomic profiles in the sample sizes including 30–50 subjects per group (Kohler et al., 2017) and our previous results from metabolite studies on study groups of the same size have so far gotten confirmed by other researches. A fundamental reason for the rather small patient number is the relatively low incidence of FEP compared to more prevalent illnesses. Secondly, data from control subjects were gathered at one time point and the control group did not undergo longitudinal monitoring contrary to patients' group. It can be discussed that relying on patients' statements relating to substance use, fasting status before blood collection or similar subjects is a limitation. However, in everyday clinical practice additional analyses would not be conducted to verify these statements either, therefore it is in accordance with our naturalistic approach. As the pharmacological agents' use among study patients was not restricted but derived from clinically relevant circumstances, it was enabled to be altered during the study, and in addition to different AP medication, anxiolytic, mood stabilizing and antidepressant drugs were permitted to be used. Therefore, we were not able to assess the effect of specific active substances on evaluated metabolites. However, the naturalistic approach has several beneficial aspects. Firstly, we can assume that the outcomes achieved are truly relevant in routine clinical practice. Secondly, as the study-related additional interventions and restrictions were minimal, so was the distress caused by them to patients. The latter is possibly a reason for our study's relatively low (considering the nature of the condition) dropout rates both in 7 months and 5 years perspective. Other strengths of our study include simultaneous determination of numerous biomolecules and the strict selection of patients in considering their diagnoses. However, the most important strength of our study is the longitudinal design. Starting the evaluation in the first psychotic outbreak in AP-naïve state and following the patients up to 5 years and more makes this study a unique source of information for understanding the dynamic metabolomic changes during the lengthy course of the SSDs.

7.7. Summary of the discussion and future directions

SSDs are characterised by numerous metabolomic changes. Our study concentrated on GPL, SL, AA and BA levels that were measured in FEP patients before initiating AP treatment, 7 months into the treatment and ongoing of the disease and finally for AAs and BAs again in 5 years after the onset of the disease and initiation of treatment. Visual summary of our findings can be seen in Figure 3.

We detected the following alterations in drug-naïve FEP patients' lipidomic profiles: a diminished level of eleven PC diacyls, calculated total PC-aa, five PC acyl-alkyls, one SM and an elevated level of one LysoPC. These changes may reflect cell membrane damage and energetic dysregulation. Seven months of AP treatment reversed the observed metabolomic changes seen in drug-naïve FEP patients, referring that in this early stage of the disease, the pathological processes influencing metabolic pathways can be managed to a certain extent. However, as a common adverse effect, a significant BMI elevation accompanied the AP medication use, showing that the lipid metabolism disturbances exceed the range of GPLs and SLs studied.

For more conclusive insight into the lipid metabolism in context of SSDs, a wider variety of lipid subtypes should be studied simultaneously and measurements made at more numerous time points during the course of the disease. The first question of interest would be how fast the positive effects of AP treatment seen in the first seven months emerge and for how long do they last in the context of lipid metabolism, as we demonstrated that it is limited in the case of AAs and BAs.

Concerning AA and BA levels we identified a decrease in Pro and alpha-AAA levels, Tyr/Phe, Orn/Arg ratio and an increase in taurine level in drug-naïve FEP patients compared to the control subjects. These changes can be related to neurotransmission dysregulation (many through NMDA receptor dysfunction), immune activation due to low grade inflammation and oxidative stress – all well-known constituents of SSDs pathogenesis. Seven months of AP treatment reversed the observed metabolomic changes seen in drug-naïve FEP patients. However, several AA and BA level alterations reappeared after 5.1 years of treatment and illness continuation. It seems that the positive effect AP treatment initially established for metabolomic aberrations diminishes over time and pathogenetic mechanisms overpower the treatment. However, there are metabolomic changes that may reflect the activation of compensatory mechanisms instead.

Our results, former data and the controversial findings in overviewed studies indicate, that biomolecule alterations in SSDs are dynamic. Specific metabolomic patterns characterise different stages of the disease and the illness and AP treatment duration have significant influence to the metabolomic profile. As the number of studies taking these variables into account are few in number, it is rather difficult to make conclusive deductions based on the existing data. Currently, the knowledge, which changes accompany the pathogenetic processes of the illness, which are compensatory, which follow the patients' lifestyle changes and which can be attributed to pharmacological effects, is limited. Shifts in

metabolite levels can be associated to contemporary theories of SSDs pathogenetic mechanisms and help to give further direction to the research. More thorough investigation is needed to clarify the factors educing alterations in metabolomic profiles of patients, reveal potential biomarkers and targets for treatment.

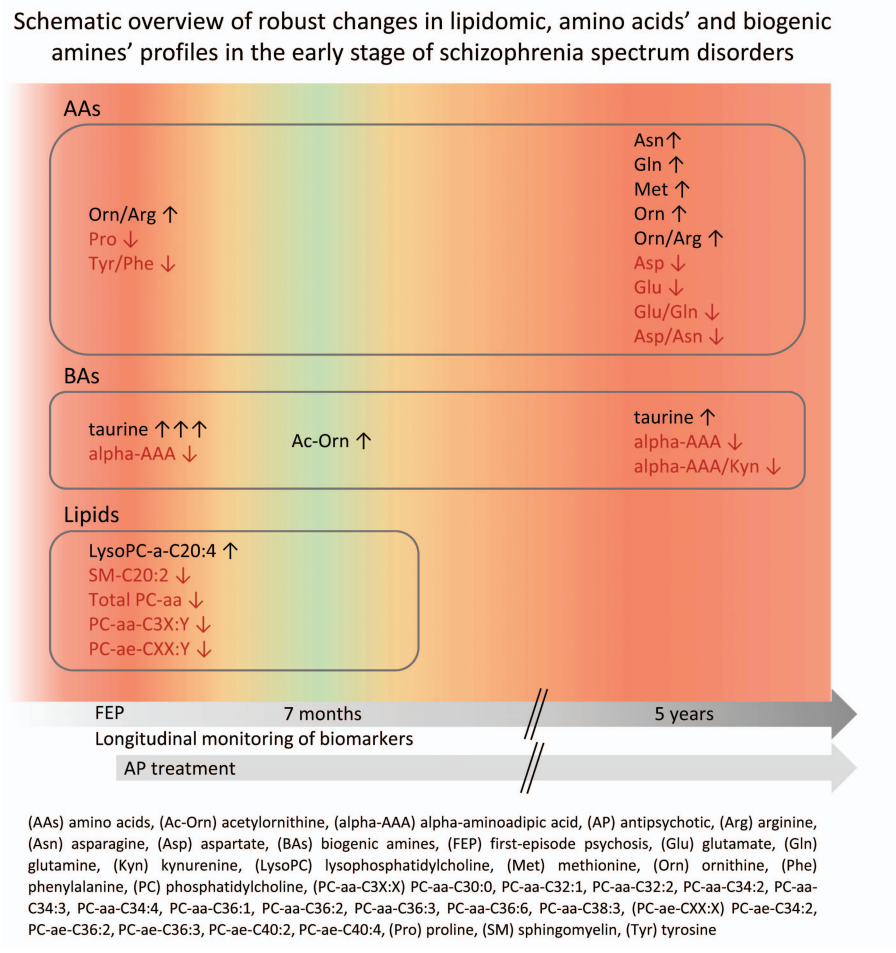


Figure 3 Visual summary of changes found in this study in lipidomic, amino acid, and biogenic amine profiles during three timepoints of early stage schizophrenia spectrum disorders

8. CONCLUSIONS

The hypothesis that significant and associable alterations in the profile of circulating GPLs, SLs, AAs and BAs occur during SSD was confirmed. Furthermore, connected altered metabolic pathways cause "domino effects" in synthesis, transport and elimination of the metabolites, leading to several functional disturbances. This thesis posed three main objectives. Following our research, the results imply the following conclusions:

- Before AP treatment FEP patients demonstrate diminished serum concentrations of eleven PC diacyls (PC-aa-C30:0, PC-aa-C32:1, PC-aa-C32:2, PC-aa-C34:2, PC-aa-C34:3, PC-aa-C34:4, PC-aa-C36:1, PC-aa-C36:2, PC-aa-C36:3, PC-aa-C36:6, PC-aa-C38:3), calculated total PC-aa and five PC acyl-alkyls (PC-ae-C34:2, PC-ae-C36:2, PC-ae-C36:3, PC-ae-C40:2, PC-ae-C40:4) when comparing their circulating GPL serum levels to the control groups'. Additionally, SL SM-C20:2 level is decreased in patients when comparing their circulating SLs levels to the control subjects'. In contrast, LysoPC-a-C20:4 serum level is elevated in patients compared to the control subjects. After seven months of AP medication statistically significant elevations in nine PC (PC-aa-C32:2, PC-aa-C34:3, PC-aa-C34:4, PC-aa-C36:1, PC-aa-C36:2, PC-aa-C36:3, PC-aa-C36:6, PC-aa-C38:3, PC-aa-C40:5) and two LysoPC (LysoPC-a-C14:0, LysoPC-a-C20:3) levels emerge. Contrary to PCs, SM-(OH)-C16:1 and SM-C18:0 serum concentrations show a decrease. After 7 months of AP treatment, when applying the Bonferroni correction for multiple comparisons in comparing treated patients' group to the control group, the two groups do not differ significantly in their lipid metabolite concentrations. Therefore, our study proved the dynamic alterations in specific lipid metabolites' profile of SSD patients during the course of illness and treatment compared to the control subjects.
- Before AP treatment FEP patients demonstrate a decrease in Pro levels, Tyr/Phe ratio and alpha-AAA levels. An increase in taurine level is seen in FEP patients compared to the control subjects. Additionally, an Orn/Arg ratio elevation is present when comparing SSD patients' circulating AA and BA serum levels to the control subjects'. After seven months of AP medication, metabolomic changes relating AAs and BAs are reverted and their concentrations shift back to the level comparable to CS. Exceptionally, after seven months of AP medication of FEP patients, Ac-Orn level is elevated in patients, although its level is not significantly different in drug-naïve FEP patients compared to the control subjects. After five years of continuation of the disease and AP treatment increased Asn, Gln, Orn, taurine, Met and decreased Asp, Glu and alpha-AAA circulating serum levels are detectable in patients compared to control subjects. Consequently, Asp/Asn, Glu/Gln, alpha-AAA/Kyn ratios are lowered and Orn/Arg ratio heightened. Therefore, our study proved the dynamic alterations in specific AA and BA profiles of SSD patients during the course of illness and treatment compared to control subjects.

- The alterations in metabolomic profile of circulating GPLs, SLs, AAs and BAs in AP-naïve FEP SSD patients indicate an extensive metabolomic shift induced by the psychotic illness. Changes in GPL and SL levels in drug-naïve patients refer to a lipidomic shift characterized by an increased disintegration of PCs that may reflect the damage of cell membranes and underly impaired HDL metabolism. Observed alterations in AAs' and BAs' levels, predominantly seem to be connected to metabolic pathways synthesising or modulating the effect of the key neurotransmitters (Glu, DA) associated with psychotic symptoms. Seven months of AP treatment in case of FEP are sufficient to reverse GPLs, SLs, AAs and BAs related metabolomic alterations induced by the onset of psychosis. However, during five years of AP medication use and ongoing SSD, the AAs and BAs metabolomic profiles aberrate again. The mechanisms of this appear to be related not only to previously mentioned neurotransmitter functions but more widely to inflammatory, oxidative and energetic processes known to be impaired in SSDs. Therefore, our study proved the dynamic alterations in the metabolomic profile of SSD patients during the course of illness and treatment that are in concordance with the previous theoretical knowledge.

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10. SUMMARY IN ESTONIAN

Lipiidide, aminohapete ja biogeensete amiinide metaboloomilise profiili muutused skisofreenia spektri häirete varajases kulus

10.1. Sissejuhatus

Skisofreenia spektri häired mõjutavad ülemaailmselt otseselt 20 miljonit inimset (James et al., 2018) ning on suureks koormaks mitte ainult haigetele ja nende lähedastele vaid kogu ühiskonnale. Kuigi skisofreenia spektri häirete spetsiifilised kliinilised avaldused võivad ulatuslikult varieeruda, on neil ühised tuumsümptomid ning tavaliselt ka haiguse progressiooni muster. Skisofreenia spektri häirete etioloogia on kompleksne ning kaasaegsed sellekohased teadmised ei ole ammendavad. Haiguse avaldumist mõjutavad nii geneetilised, arengulised, keskkondlikud kui meditsiinilised faktorid (Stilo & Murray, 2019). Neurobioloogiline lähenemine, integreerides andmeid erinevatelt kitsamatelt teadusaladelt on aga ilmsiks toonud arvukaid haiguse aluseks olevate patofüsioloogiliste mehhanismide aspekte. Need on andnud aluse peamisteks skisofreenia patogeneesi hüpoteesideks – rakumembraanide fosfolipiidide teooriale, dopamiiniteooriale ning N-metüül-D-aspartaat (NMDA) glutamaadi retseptori teooriale.

Rakumembraanide fosfolipiidide hüpoteesi kohaselt tuleneb psühhootiliste sümptomite avaldumine rakumembraanide lipiidkompositsiooni muutustest, mille tagajärjel hälbib membraanide retseptorite funktsioon. Dopamiini hüpoteesi kohaselt kutsub teatud aju regioonide hüperdopaminergia esile psühhoosi positiivsed sümptomid ning hüpodopaminergia negatiivsed sümptomid. NMDA glutamaadi retseptori teooria kohaselt on psühhootiliste sümptomite väljakujunemise aluseks NMDA glutamaadi retseptori düsfunktsioon, mille tagajärjel muutub ka dopaminergilise närviülekande toimimine. Glutamaadi ja dopamiini ringet seob omavahel gamma-aminovõihappe vahendatud närviülekanne ning kõrvalekalded nendes süsteemides toovad endaga kaasa inhibitoorsete ja eksitatoorsete impulsside düsbalansi ajus, millega võivad kaasneda psühhootilised avaldused.

Kirjeldatud hüpoteesid ning antipsühhootiliste preparaatide metaboolsed efektid on kannustanud lipiidmetaboliitide, aminohapete ning biogeensete amiinide uuringuid psühhootiliste häirete kontekstis. Kahjuks on meetodid, millega psühhootiliste häirte fookuses olevaid metaboliite määrata aga limiteeritud. Kuigi loomkatsed, *post mortem* aju uuringud ning visualiseerivad aju uuringud on andnud lubavaid ning pigem heas kooskõlas olevaid tulemusi, jääb nende meetodite tõhusus hetkel kliinilises praktikas ebapiisavaks ning senised tulemused mitteammendavateks.

Metaboloomiline lähenemine on osutunud psühhiaatriliste häirete uurimisel tulusaks ning võimaldab perifeerse vere substraadina kasutamist. Skisofreenia spektri häiretega patsientidel tehtud metaboloomiliste uuringute hulk on väike ning faktorid, mis oletatavasti tulemusi mõjutada võivad (seoses haiguse staadiumi

ning raviga) pahatihti tähelepanuta jäetud. Seega, skisofreenia spektri häirete patofüsioloogia selgitamisel võiksid potentsiaalselt haigusega seotud biomolekulide (fosfolipiidide, aminohapete, biogeensete amiinide) metaboloomilised uuringud olulist kasu tuua, kuid seda eeskätt juhul, kui patsientide kaasamisel võetakse arvesse nende haiguskestust ning ravistaatust.

10.2. Uurimistöö eesmärgid

Käesoleva doktoritöö peamiseks eesmärgiks on iseloomustada tsirkuleerivate glütserofosfolipiidide, sfingolipiidide, aminohapete ning biogeensete amiinide profiili psühhootilise häire vallandumisel (enne antipsühhootilise ravi alustamist) ning erinevatel ajahetkedel haiguse kulu vältel antipsühhootilise ravi foonil. Alaeesmärgid on järgmised:

- Iseloomustada esmase psühhoosiepisoodiga patsientide tsirkuleerivate glütserofosfolipiidide ja sfingolipiidide profiili võrreldes kontrollgruppi kuuluvate isikutega enne antipsühhootilise ravi alustamist ning pärast seitset kuud medikamentoosset ravi
- Iseloomustada esmase psühhoosiepisoodiga patsientide tsirkuleerivate aminohapete ja biogeensete amiinide profiili võrreldes kontrollgruppi kuuluvate isikutega enne antipsühhootilise ravi alustamist, pärast seitset kuud medikamentoosset ravi ning pärast viit aastat jätkuvat medikamentoosset ravi ja haiguse jätkumist
- Analüüsida tsirkuleerivate glütserofosfolipiidide, sfingolipiidide, aminohapete ja biogeensete amiinide metaboloomilise profiili muutuste aluseks olevaid potentsiaalseid mehhanisme psühhootilise häirega patsientidel, võrreldes kontrollgruppi kuuluvate isikutega enne antipsühhootilise ravi alustamist, pärast seitset kuud medikamentoosset ravi ning pärast viit aastat jätkuvat medikamentoosset ravi ja haiguse jätkumist

10.3. Uuritavad je meetodid

Uuringu läbiviimiseks andis loa Tartu Ülikooli inimuuringute eetika komitee ning see on kooskõlas Helsingi deklaratsiooni eetiliste standarditega. Kõik uuringus osalejad andsid selleks kirjaliku informeeritud nõusoleku.

Patsiendid kaasati uuringusse Tartu Ülikooli Kliinikumis, kuhu nad olid pöördunud ravile esmase psühhootilise episoodi vallandumise tõttu. Uuringudisain oli naturalistlik ning sellest lähtuvalt ei piiratud uuringu vältel osalejate medikamentoosse ravi võimalusi. Patsiendid said lähtuvalt kliiniliselt olulistest asjaoludest ravi erinevate antipsühootiliste farmakonide, nende kombinatsioonide ja vajadusel täiendavate preparaatidega ning raviskeemi võis uuringu vältel ka muuta. Kontrollgruppi värvati inimesed sarnasest geograafilisest piirkonnast. Uuringus osales kokku 53 patsienti ning 37 kontrollgrupi kuuluvat isikut.

Patsientidelt koguti demograafilised andmed, kliinilised andmed ning analüüsideks vajalikud vereseerumi proovid kolmel ajahetkel – esmase psühhoosiepisoodiga haiglasse saabumisel enne antipsühhootilise ravi alustamist, seitse kuud pärast antipsühhootilise raviga alustamist ning viis aastat pärast psühhootilise häire vallandumist ja antipsühhootilise raviga alustamist. Patsientide psühhopatoloogiat hinnati „Psühhiaatrilise lühiskaala" (*Brief Psychiatric Rating Scale* (BPRS)) alusel (Overall & Gorham, 1962). Patsientide diagnoosid lähtuvad Rahvusvahelise Haiguste klassifikatsiooni 10. väljaande (WHO, 1992) diagnostilistest kriteeriumitest ning on püstitatud baseerudes kliinilisele intervjuule, patsientide käitumise hindamisele ning terviseandmete läbivaatusele kogenud psühhiaatrite poolt. Kontrollgruppi kuuluvatelt isikutelt koguti vajalikud andmed ning vereproovid ühekordselt uuringusse kaasamisel ning neid intervjueerisid samuti kogenud psühhiaatrid, vältimaks psühhiaatriliste häiretega isikute sattumist kontrollgruppi.

Biokeemiliste markerite määramisel ning nende seerumitasemete mõõtmisel kasutati voogsisestus tandem-mass-spektromeetria ning vedelik kromatograafia meetodit ning statistlilises analüüsis andmete tüübile vastavaid kaasaegseid statistilisi meetodeid.

10.4. Tulemused ja järeldused

- Tsirkuleerivate glütserofosfolipiidide seerumtasemete võrdlemisel avaldus esmase psühhoosiepisoodiga patsientidel enne antipsühhootilise ravi rakendamist üheteistkümne fosfatidüülkoliin diatsüüli (PC-aa-C30:0, PC-aa-C32:1, PC-aa-C32:2, PC-aa-C34:2, PC-aa-C34:3, PC-aa-C34:4, PC-aa-C36:1, PC-aa-C36:2, PC-aa-C36:3, PC-aa-C36:6, PC-aa-C38:3), arvutusliku kogu fosfatidüülkoliin diatsüüli ning viie fosfatidüülkoliin atsüül-alküüli (PC-ae-C34:2, PC-ae-C36:2, PC-ae-C36:3, PC-ae-C40:2, PC-ae-C40:4) vähenenud seerumkontsentratsioonid võrreldes kontrollgrupiga. Lisaks sellele on patsientidel alanenud ka sfingolipiid SM-C20:2. LysoPC-a-C20:4 seerumi tase, vastupidiselt, on pantsientidel kontrollgruppi kuuluvate isikutega võrreldes aga tõusnud. Pärast seitset kuud antipsühhootilist ravi ilmnes üheksa fosfatidüülkoliini (PC-aa-C32:2, PC-aa-C34:3, PC-aa-C34:4, PC-aa-C36:1, PC-aa-C36:2, PC-aa-C36:3, PC-aa-C36:6, PC-aa-C38:3, PC-aa-C40:5) ning kahe lüsofosfatidüülkoliini taseme statistiliselt oluline tõus. Vastupidiselt fosfatidüülkoliinidele SM-(OH)-C16:1 ja SM-C18:0 seerumi kontsentratsioonid langesid. Bonferroni korrektsiooni rakendamisel ei erinenud pärast seitset kuud antipsühhootilist ravi patsientide grupi ning kontrollgrupi lipiidmetaboliitide kontsentratsioonid üksteisest statistiliselt olulisel määral. Seega tõendas meie uurimus skisofreenia spektri häiretega patsientide spetsiifiliste lipiidmetaboliitide profiili dünaamiliste muutuste olemasolu haiguse kulu ja ravi vältel, võrreldes kontrollgruppi kuuluvate isikutega.

- Tsirkuleerivate aminohapete ja biogeensete amiinide seerumtasemete võrdlemisel ilmestus esmase psühhoosiepisoodiga patsientidel enne antipsühhootilise ravi rakendamist proliini taseme, türosiin/fenüülalaniini suhte ja alfa-aminoadipiinhappe taseme vähenemine ning tauriini taseme oluline suurenemine võrreldes kontrollgrupiga. Lisaks sellele on patsientidel tõusnud ornitiin/arginiini suhe patsientidel. Pärast seitset kuud antipsühhootilist ravi on aminohappeid ja biogeenseid amiine puudutavad metaboloomilised muutused tagasi pöördunud ning nende kontsentratsioonid nihkunud tagasi tasemele, mis on võrreldavad kontrollgruppi kuuluvate isikute samade näitajatega. Erandlikult on pärast seitset kuud ravi antipsühhootikumidega esmase psühhoosiepisoodiga patsientide atsetüülornitiini tase tõusnud, kuigi selle tase ravim-naiivsetel patsientidel ei ole oluliselt erinev kontrollgrupi kuuluvate iskute omast. Pärast viis aastat kestnud haigust ning antipsühhootilist ravi on patsientidel võrreldes kontrollgruppi kuuluvate isikutega tuvastatavad kõrgenenud asparagiini, glutamiini, ornitiini, tauriini, metioniini ja alanenud aspartaadi, glutamaadi ja alfa-aminoadipiinhappe seerumitasemed. Sellest tulenevalt on alanenud aspartaat/asparagiini, glutamaat/glutamiini, alfa-aminoadipiinhape/künureniini suhted ning kõrgenenud ornitiin/arginiini suhe. Seega tõendas meie uurimus skisofreenia spektri häiretega patsientide spetsiifiliste aminohapete ja biogeensete amiinide profiili dünaamiliste muutuste olemasolu haiguse kulu ja ravi vältel, võrreldes kontrollgruppi kuuluvate isikutega.
- Muutused antipsühhootikum-naiivsete esmase psühhoosiepisoodiga skisofreenia spektri häirega patsientide tsirkuleerivate glütserofosfolipiidide, sfingolipiidide, aminohapete ja biogeensete amiinide metaboloomilises profiilis näitavad ulatusliku psühhootilise haiguse poolt indutseeritud metaboloomilise nihke olemasolu. Ravim-naiivsetel patsientidel avaldunud muutused glütserofosfolipiidide ja sfingolipiidide tasemes viitavad lipidoomilisele nihkele, mida iseloomustab fosfatidüülkoliinide suurenenud lagunemine, mis võib peegeldada rakumembraanide kahjustust ning olla aluseks suure tihedusega lipoproteiinide häirunud metabolismile. Aminohapped ja biogeensed amiinid, mille tasemete muutusi täheldati, näivad eeskätt olevat seotud metaboolsete radadega, mis sünteesivad psühhootiliste sümptomitega seostatud võtme neurotransmittereid (glutamaadi, dopamiini) või moduleerivad nende toimet. Seitse kuud vältav antipsühhootiline ravi esmase psühhoosiepisoodi korral on piisav, et psühhoosi vallandumisest indutseeritud glütserofosfolipiidide, sfingolipiidide, aminohapete ning biogeensete amiinidega seotud metaboloomilised muutused tagasi pöörata. Aminohapete ja biogeensete amiinide metaboloomilised profiilid viie aasta pikkuse antipsühhootilise ravi tarvitamise ning jätkuva skisofreenia spekti häire kestel hälbivad aga taas. Selle mehhanism paistab olevat seotud mitte ainult eelmainitud neurotransmitterite funktsiooniga, vaid laialdasemalt põletikuliste, oksüdatiivsete ning energeetiliste protsessidega, mis skisofreenia spektri häirete korral teadaolevalt häirunud on. Seega tõendas meie uurimus skisofreenia spektri häiretega patsientide metaboloomilise profiili dünaamiliste muutuste olemasolu haiguse kulu ja ravi vältel, mis on kooskõlas varsemate teoreetiliste teadmistega.

Meie uuringu tulemused viitavad, et haiguse ning antipsühhootilise ravi kestusel on oluline mõju skisofreenia spekti häirega patsientide metaboloomilisele profiilile. Kuna uuringuid, mis neid muutujaid arvesse võtaks on vähe, on olemasolevatele andmetele tuginedes raske lõplikke järeldusi teha. Meie tuvastatud nihked metaboliitide tasemes on seostatavad kaasaegsete skisofreenia spektri häirete patogeneetiliste mehhanismide teooriatega ning aitavad seada edasisi sihte uurimistööks. Vajalik on veel enam süvitsi minev metaboloomiliste muutuste uurimine skisofreenia spektri häirete kontekstis, et nende patogeneesi paremini mõista, leida potentsiaalseid biomarkereid ning teha kindlaks märklaudu ravivõimaluste parandamiseks.

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SUPPLEMENTARY MATERIALS

Table 1 Comparison of serum levels of glycerophospholipids (μmol/l) and sphingolipids (μmol/l) between the first-episode psychosis patients (n=53) at baseline (FEP_b) and control subjects (CS) (n=37)

	FEP_b	**CS**	**Z-value**	***p*-value**
	Median (min – max)	**Median (min – max)**		
Glycerophospholipids				
Lysophosphatidylcholine acyls				
LysoPC-a-C14:0	4.13 (3.08–11.7)	4.69 (3.05–7.70)	–0.72	0.47
LysoPC-a-C16:0	215 (132–388)	208 (107–248)	1.66	0.10
LysoPC-a-C16:1	4.41 (2.33–7.92)	4.50 (2.24–7.18) *	0.22	0.83
LysoPC-a-C17:0	3.89 (1.89–7.66)	3.94 (1.35–5.16)	0.52	0.60
LysoPC-a-C18:0	60.3 (31.8–117)	62.2 (26.5–87.1)	–0.52	0.60
LysoPC-a-C18:1	42.1 (22.5–71.6)	40.2 (19.8–56.7)	0.78	0.44
LysoPC-a-C18:2	48.2 (21.6–96.2)	53.0 (19.2–107)	–0.50	0.62
LysoPC-a-C20:3	2.83 (1.24–6.19)	2.87 (1.31–5.45)	–0.64	0.53
LysoPC-a-C20:4	10.3 (6.43–19.8)	8.20 (3.81–15.9)	3.83	**1e-04**
LysoPC-a-C24:0	0.63 (0.16–1.81)	0.57 (0.30–2.12)	0.13	0.90
LysoPC-a-C26:0	1.19 (0.28–5.69)	1.02 (0.55–4.25)	0.13	0.90
LysoPC-a-C26:1	0.48 (0.15–2.33)	0.45 (0.24–2.80)	0.00	1.00
LysoPC-a-C28:0	0.77 (0.13–3.59)	0.82 (0.37–2.52)	–1.07	0.29
LysoPC-a-C28:1	0.76 (0.17–2.80)	0.75 (0.43–3.24)	–1.09	0.28
Total_LysoPC	404 (257–687)	398 (200–527)	1.07	0.28
Phosphatidylcholine diacyls				
PC-aa-C24:0	0.57 (0.19–1.82)	0.47 (0.24–1.83)	1.15	0.25
PC-aa-C26:0	1.72 (0.80–10.30)	1.44 (0.82–8.40)	0.975	0.33

Table 1 (Continue)

	FEP$_b$	CS	Z-value	*p*-value
	Median (min – max)	**Median (min – max)**		
PC-aa-C28:1	2.68 (1.14–4.44)	2.75 (1.59–5.18)	–1.065	0.29
PC-aa-C30:0	2.89 (1.27–4.95)	3.57 (2.14–10.7)	–4.15	**<1e-05**
PC-aa-C30:2	0.28 (0.00–0.88)	0.30 (0.03–1.13)	–0.69	0.49
PC-aa-C32:0	10.0 (4.68–15.0)	11.2 (6.23–20.4)	–1.67	0.10
PC-aa-C32:1	8.87 (4.00–22.1)	12.4 (4.53–33.0)	–3.53	**4e-04**
PC-aa-C32:2	1.68 (0.19–3.77)	3.10 (1.59–8.09)	–6.10	**<1e-05**
PC-aa-C32:3	0.42 (0.14–0.65)	0.49 (0.28–1.30)	–2.12	0.03
PC-aa-C34:1	146 (87.8–230)	165 (117–256)	–2.57	0.01
PC-aa-C34:2	243 (136–350)	298 (187–473)	–3.95	**8e-05**
PC-aa-C34:3	7.94 (3.15–16.7)	12.5 (3.79–26.0)	–6.18	**<1e-05**
PC-aa-C34:4	0.58 (0.15–1.31)	1.10 (0.43–2.72)	–5.90	**<1e-05**
PC-aa-C36:0	3.21 (1.44–6.77)	3.95 (1.68–9.95)	–1.62	0.11
PC-aa-C36:1	27.6 (14.8–49.8)	40.4 (24.6–59.4)	–5.07	**<1e-05**
PC-aa-C36:2	127 (54.3–209)	190 (89.1–285)	–5.76	**<1e-05**
PC-aa-C36:3	59.4 (24.8–97.5)	89.5 (45.1–164)	–5.64	**<1e-05**
PC-aa-C36:4	84.9 (32.9–175)	106 (50.8–167)	–2.46	0.01
PC-aa-C36:5	9.23 (2.45–37.7)	13.0 (5.56–31.9)	–2.63	9e-03
PC-aa-C36:6	0.53 (0.20–1.22)	0.78 (0.38–1.53)	–4.39	**1e-05**
PC-aa-C38:0	2.52 (1.32–4.41)	3.01 (1.15–6.66)	–2.63	9e-03
PC-aa-C38:1	2.09 (0.82–6.84)	2.44 (0.58–5.25)	–1.76	0.08
PC-aa-C38:3	22.7 (11.7–41.6)	30.2 (20.2–47.6)	–4.50	**<1e-05**
PC-aa-C38:4	45.5 (20.0–114)	50.8 (29.2–93.1)	–2.58	0.01

Table 1 (Continue)

	FEP$_b$	CS	Z-value	*p*-value
	Median (min – max)	**Median (min – max)**		
PC-aa-C38:5	21.4 (8.08–50.9)	26.8 (13.3–100)	–2.59	0.01
PC-aa-C38:6	30.2 (10.0–94.0)	36.0 (15.5–138)	–1.03	0.30
PC-aa-C40:1	1.00 (0.39–2.27)	1.06 (0.36–2.08) *	–0.78	0.44
PC-aa-C40:2	2.45 (0.33–5.88)	2.57 (0.89–4.00)	–0.29	0.77
PC-aa-C40:3	2.31 (0.54–5.65)	2.42 (0.84–3.77)	–0.59	0.56
PC-aa-C40:4	4.09 (2.27–8.16)	4.52 (2.25–8.09)	–1.38	0.17
PC-aa-C40:5	3.89 (1.88–8.61)	4.50 (2.73–10.5)	–2.60	0.01
PC-aa-C40:6	9.45 (3.93–26.3)	12.0 (5.38–49.0)	–1.78	0.07
PC-aa-C42:0	0.74 (0.37–1.27)	0.70 (0.31–1.79)	0.61	0.54
PC-aa-C42:1	0.52 (0.22–1.00)	0.49 (0.17–1.05)	0.34	0.73
PC-aa-C42:2	0.67 (0.21–1.37)	0.68 (0.27–1.41)	0.17	0.86
PC-aa-C42:4	0.91 (0.12–2.06)	1.01 (0.41–1.60)	–1.66	0.10
PC-aa-C42:5	0.55 (0.32–1.05)	0.64 (0.26–1.26)	–2.54	0.02
PC-aa-C42:6	0.69 (0.35–1.28)	0.69 (0.43–2.53)	–0.54	0.59
Total_PC-aa	914 (491–1435)	1096 (793–1735)	–4.25	**2e-05**
Phosphatidylcholine acyl-alkyls				
PC-ae-C30:0	0.42 (0.22–0.88)	0.45 (0.25–1.20)	–1.07	0.28
PC-ae-C30:1	0.35 (0.01–1.51)	0.40 (0.12–1.72)	–0.83	0.41
PC-ae-C30:2	0.26 (0.12–0.49)	0.25 (0.12–0.67)	0.07	0.94
PC-ae-C32:1	2.33 (0.87–3.78)	2.43 (1.24–4.67)	–0.97	0.33
PC-ae-C32:2	0.70 (0.24–1.22)	0.70 (0.40–1.98)	–0.93	0.35
PC-ae-C34:0	0.89 (0.40–1.37)	1.03 (0.47–2.26)	–2.35	0.02
PC-ae-C34:1	7.35 (3.36–13.0)	8.20 (4.80–12.7)	–2.38	0.02

Table 1 (Continue)

	FEP$_b$	CS	Z-value	*p*-value
	Median (min – max)	**Median (min – max)**		
PC-ae-C34:2	6.73 (2.65–12.9)	9.47 (4.31–16.8)	–4.90	**<1e-05**
PC-ae-C34:3	4.58 (1.52–9.47)	5.95 (3.10–11.2)	–3.30	1e-03
PC-ae-C36:0	0.60 (0.00–1.15)	0.33 (0.00–1.30)	2.96	3e-03
PC-ae-C36:1	25.8 (5.00–50.6)	29.0 (12.8–55.4)	–2.02	0.04
PC-ae-C36:2	12.1 (6.52–18.2)	15.3 (6.91–24.9)	–3.75	**2e-04**
PC-ae-C36:3	4.07 (1.59–7.39)	5.91 (2.88–9.89)	–4.97	**<1e-05**
PC-ae-C36:4	7.24 (2.48–16.2)	9.40 (5.36–18.3)	–2.98	3e-03
PC-ae-C36:5	4.61 (1.64–11.0)	5.42 (2.62–10.6)	–1.93	0.05
PC-ae-C38:0	1.17 (0.56–2.07)	1.30 (0.89–5.67)	–2.63	8e-03
PC-ae-C38:1	8.30 (0.49–19.8)	11.6 (4.14–23.5)	–3.17	2e-03
PC-ae-C38:2	9.10 (1.63–22.1)	13.3 (5.27–24.5)	–3.06	2e-03
PC-ae-C38:3	17.4 (3.12–32.9)	20.1 (10.1–36.6)	–2.60	9e-03
PC-ae-C38:4	8.94 (4.11–15.1)	11.2 (5.97–18.8)	–3.09	2e-03
PC-ae-C38:5	8.09 (3.21–17.0)	8.99 (5.18–18.2)	–1.85	0.07
PC-ae-C38:6	2.90 (0.84–6.18)	3.49 (1.76–13.0)	–2.00	0.05
PC-ae-C40:1	2.22 (1.08–4.27)	2.03 (1.09–5.35)	0.22	0.82
PC-ae-C40:2	3.58 (1.04–6.91)	4.43 (2.05–7.21)	–3.48	**5e-04**
PC-ae-C40:3	7.42 (0.85–13.5)	9.04 (3.79–14.0)	–3.12	2e-03
PC-ae-C40:4	5.84 (1.59–10.4)	7.33 (3.58–10.1)	–3.55	**4e-04**
PC-ae-C40:5	8.56 (2.23–20.8)	9.74 (5.20–19.5)	–2.05	0.04
PC-ae-C40:6	2.65 (0.99–5.77)	2.84 (1.40–9.83)	–1.07	0.28
PC-ae-C42:0	0.91 (0.56–1.70)	0.95 (0.69–2.15)	–1.82	0.07

Table 1 (Continue)

	FEP$_b$	CS	Z-value	*p*-value
	Median (min – max)	**Median (min – max)**		
PC-ae-C42:1	1.47 (0.51–3.56)	1.29 (0.53–3.02)	0.58	0.56
PC-ae-C42:2	1.18 (0.57–2.27)	1.11 (0.47–2.52)	0.07	0.94
PC-ae-C42:3	1.71 (0.79–2.97)	1.67 (0.77–3.45)	0.63	0.53
PC-ae-C42:4	1.41 (0.72–2.29)	1.75 (0.94–2.85)	–2.97	3e-03
PC-ae-C42:5	2.92 (1.62–5.84)	3.23 (1.98–6.75)	–2.10	0.04
PC-ae-C44:3	0.53 (0.19–1.08)	0.52 (0.22–1.17)	–0.25	0.80
PC-ae-C44:4	0.44 (0.24–0.86)	0.48 (0.29–0.80)	–0.58	0.56
PC-ae-C44:5	1.11 (0.60–2.04)	1.06 (0.65–2.07)	0.47	0.64
PC-ae-C44:6	0.73 (0.39–1.34)	0.77 (0.39–2.44)	–0.01	0.99
Sphingolipids				
SM-(OH)-C14:1	3.78 (0.92–6.10)	3.83 (1.89–7.02)	–0.96	0.34
SM-(OH)-C16:1	1.84 (0.44–3.56)	1.78 (0.84–3.86)	–0.18	0.86
SM-(OH)-C22:1	6.36 (1.78–11.2)	7.17 (3.45–13.1)	–1.92	0.05
SM-(OH)-C22:2	5.59 (1.28–9.96)	6.16 (2.94–13.2) *	–1.96	0.05
SM-(OH)-C24:1	0.73 (0.15–1.54)	0.68 (0.24–1.58)	–0.21	0.84
SM-C16:0	65.0 (19.1–96.3)	70.5 (29.3–108) *	–1.56	0.12
SM-C16:1	7.92 (2.43–13.5)	9.09 (4.71–16.5)	–1.36	0.17
SM-C18:0	13.1 (3.09–25.8)	13.2 (5.84–32.1)	–0.32	0.75
SM-C18:1	5.36 (1.45–11.6)	5.86 (2.59–14.0)	–0.44	0.66
SM-C20:2	0.32 (0.04–0.62)	0.41 (0.24–0.85)	–3.69	**2e-04**
SM-C22:3	1.40 (0.00–3.32)	1.47 (0.33–3.61)	–1.01	0.31
SM-C24:0	11.1 (3.35–17.7)	12.2 (5.77–19.8)	–2.16	0.03
SM-C24:1	26.9 (9.70–51.8)	28.3 (11.3–58.2)	–0.52	0.61

Table 1 (Continue)

	FEP$_b$	CS	Z-value	*p*-value
	Median (min – max)	**Median (min – max)**		
SM-C26:0	0.11 (0.00–0.28)	0.15 (0.01–0.47)	–2.73	6e-03
SM-C26:1	0.41 (0.02–0.85)	0.51 (0.12–0.92)	–3.00	3e-03
Total_SM	155 (48.2–239)	166 (70.7–270)	–0.94	0.35
Ratios of biomarkers				
LysoPC-a-C16:0/ LysoPC-a-C16:1	48.5 (24.8–72.4)	45.6 (27.99–108)	1.48	0.14
LysoPC-a-C20:4/ LysoPC-a-C20:3	3.71 (1.48–7.72)	2.67 (1.60–7.79)	3.76	**2e-04**
PC-aa-C24:0/ LysoPC-a-C24:0	0.90 (0.6 1–2.07)	0.85 (0.60–1.35)	1.47	0.14
PC-aa-C26:0/ LysoPC-a-C26:0	1.72 (0.81–4.80)	1.52 (0.82–2.90)	1.98	0.05
PC-aa-C28:1/ LysoPC-a-C28:1	3.47 (1.14–12.9)	3.37 (1.60–5.79)	0.62	0.53
Total_PC-aa/ Total_LysoPC	2.32 (1.11–3.94)	2.92 (1.88–5.70)	–3.92	**9e-05**
Total_PC-aa/ Total_SM	6.32 (4.12–16.0)	7.52 (3.41–12.6)	–2.59	0.01

Z-adjusted values according to Mann-Whitney *U*-test (FEPb compared to CS).
p-values less than or equal to 0.0005 after Bonferroni correction are marked in bold.
* *p*-values less than 0.05 according to Levene's test for homogeneity of variances (df=1,70).
Commentary: all measured values are 2.5…15 times higher than LOD.

Table 2 Comparison of serum levels of amino acids (μmol/l) between the first-episode psychosis (FEP) patients (n=38) at baseline (FEP_b) and control subjects (CS) (n=37)

Amino acids	**FEP_b**	**CS**	**Z-value**	***p*-value**	**Effect size (I^2)**
	Median (min – max)	**Median (min – max)**			
Alanine (Ala)	342.50 (206.00–673.00)	405.00 (232.00–716.00)	–2.10	0.04	0.06
Arginine (Arg)	147.50 (88.00–216.00)	152.00 (94.00–225.00)	–1.66	0.10	–
Asparagine (Asn)	36.80 (19.50–83.30)	33.90 (15.00–60.40)	–0.34	0.73	–
Aspartate (Asp)	38.70 (18.80–62.90)	34.20 (15.90–65.20)	1.07	0.28	–
Citrulline (Citr)	22.40 (12.60–38.10)	27.40 (11.00–48.90)	–2.95	3e-03	0.12
Glutamine (Gln)	377.00 (118.00–813.00)	308.00 (77.00–683.00)	0.68	0.49	–
Glutamate (Glu)	210.00 (59.60–381.00)	183.00 (114.00–550.00)	0.42	0.67	–
Glycine (Gly)	273.50 (153.00–420.00)	250.00 (123.00–443.00)	1.48	0.14	–
Histidine (His)	82.60 (61.50–106.00)	92.10 (58.30–138.00)	–2.29	0.02	0.07
Isoleucine (Ile)	84.95 (42.70–130.00)	85.40 (50.10–179.00)	–0.56	0.57	–
Leucine (Leu)	164.50 (73.00–273.00)	166.00 (79.60–409.00)	–0.16	0.87	–
Lysine (Lys)	183.50 (117.00–279.00)	202.00 (107.00–309.00)	–1.35	0.18	–
Methionine (Met)	7.75 (4.46–26.30)	9.08 (4.43–35.20)	0.79	0.43	–
Ornithine (Orn)	57.25 (30.70–115.00)	56.80 (23.40–91.40)	0.56	0.57	–
Phenylalanine (Phe)	72.00 (41.80–101.00)	67.10 (38.20–115.00)	0.73	0.47	–
Proline (Pro)	166.00 (83.30–381.00)	215.00 (123.00–479.00)	–3.18	**1e-03**	0.14
Serine (Ser)	170.50 (99.40–293.00)	160.00 (69.30–363.00)	0.92	0.36	–
Threonine (Thr)	140.00 (84.70–214.00)	154.00 (74.10–373.00)	–1.65	0.10	–
Tryptophan (Trp)	64.75 (30.30–89.30)	73.20 (32.80–120.00)	–2.45	0.01	0.08
Tyrosine (Tyr)	58.55 (35.80–88.70)	63.20 (33.70–159.00)	–2.38	0.02	0.08
Valine (Val)	197.50 (112.00–299.00)	220.00 (126.00–401.00)	–2.17	0.03	0.06

Table 2 (Continue)

Amino acids	FEP_b	CS	Z-value	*p*-value	Effect size (η^2)
	Median (min – max)	**Median (min – max)**			
Citr/Arg	0.16 (0.09–0.31)	0.16 (0.08–0.33)	–1.35	0.18	–
Tyr/Phe	0.82 (0.61–1.26)	1.03 (0.49–1.57)	–4.24	**2e-05**	0.24

Z-adjusted values according to Mann-Whitney U-test (FEPb compared to CS).

p-values less than or equal to 0.001 after Bonferroni correction (bolded) were considered statistically significant.
Effect sizes ≥0.14 were interpreted as large.
Effect sizes 0.06–0.13 were interpreted as moderate.
Commentary: all measured values are higher than LLOQ.

Table 3 Comparison of serum levels of biogenic amines (μmol/l) between the the first-episode psychosis (FEP) patients (n=38) at baseline (FEP_b) and control subjects (CS) (n=37)

Biogenic amines	FEP_b	CS	Z-value	*p*-value	Effect size (η^2)
	Median (min – max)	**Median (min – max)**			
Acetylornithine (Ac-Orn)	0.56 (0.18–1.06)	0.59 (0.18–2.03)	–1.44	0.15	–
Asymmetric dimethylarginine (ADMA)	0.43 (0.30–0.67)	0.43 (0.19–0.60)	–0.50	0.62	–
Alpha-Aminoadipic-acid (alpha-AAA)	0.56 (0.25–1.34)	0.76 (0.45–1.98)	–3.27	**1e-03**	0.14
c4-OH-Pro	0.25 (0.00–0.34)	0.00 (0.00–0.39)	1.79	0.07	–
Carnosine	0.00 (0.00–0.13)	0.00 (0.00–0.12)	–0.66	0.51	–
Creatinine	69.65 (42.30–123.00)	68.50 (35.00–112.00)	0.37	0.72	–
l-DOPA	0.12 (0.00–0.26)	0.15 (0.00–0.26)	–0.71	0.48	–
Kynurenine (Kyn)	2.20 (1.39–5.42)	2.70 (1.37–3.89)	–2.91	4e-03	0.11
Histamine	0.45 (0.37–0.46)	0.38 (0.37–0.46)	1.56	0.12	–
Methionine-sulfoxide (Met-SO)	10.35 (2.11–24.90)	10.80 (3.04–23.10)	–0.98	0.33	–
Putrescine	0.07 (0.02–0.19)	0.08 (0.03–0.20)	–1.61	0.11	–
Symmetric-dimethy-larginine (S-DMA)	0.57 (0.39–0.93)	0.53 (0.39–0.81)	1.22	0.22	–
Serotonin (5-HT)	0.57 (0.08–1.69)	0.65 (0.19–1.47)	–0.92	0.36	–

Table 3 (Continue)

Biogenic amines	FEP_b	CS	Z-value	*p*-value	Effect size (I^2)
	Median (min – max)	**Median (min – max)**			
Spermine	0.27 (0.17–0.43)	0.23 (0.16–0.28)	3.20	**1e-03**	0.14
t4-OH-Pro	0.42 (0.00–15.30)	0.61 (0.00–20.70)	–0.70	0.49	–
Taurine	76.45 (32.40–172.00)	47.10 (25.80–116.00)	5.56	**<1e-06**	0.41
total-DMA	0.70 (0.49–1.08)	0.73 (0.37–0.96)	0.39	0.70	–
Met-SO/ Methionine (Met)	1.35 (0.11–4.39)	1.44 (0.16–4.29)	–0.86	0.39	–
Kyn/ Tryptophan (Trp)	0.03 (0.02–0.08)	0.04 (0.03–0.05)	–1.21	0.22	–
5-HT/Trp	0.01 (0.00–0.03)	0.01 (0.00–0.02)	0.27	0.79	–

Z-adjusted values according to Mann-Whitney *U*-test (FEP_b compared to CS).
p-values less than or equal to 0.001 after Bonferroni correction (bolded) were considered statistically significant.
Effect sizes ≥0.14 were interpreted as large.
Effect sizes 0.06–0.13 were interpreted as moderate.
Commentary: ADMA, creatinine, Kyn, Met-So, 5-HT, spermine, taurine, and total-DMA values are higher than LLOQ. Ac-Orn, alpha-AAA, histamine, S-DMA values were at least 1.5 to 3 times higher than LOD.

Table 4 Serum mean levels, standard deviations (SD), and range of amino acids (μmol/l) for control subjects (CS, n=37), first-episode psychosis (FEP) patients at baseline (before treatment with antipsychotics, $FEP_{b,}$ n=52), after 0.6-year treatment ($FEP_{0.6\text{-year,}}$ n=44), and after 5.1-year treatment ($FEP_{5.1\text{-year,}}$ n=37) with antipsychotics. Comparison between reduced and unrestricted, *p*-values have been multiple test corrected according to false discovery rate (FDR) method. The unrestricted regression model is designated as True (i.e. more complex set of predictor variables explained more effectively biomarker level alterations over time) or False (i.e. unrestricted model did not provide more explanatory power than a simple one)

Regression equations for estimating biomarker level or biomarkers ratio alterations.	
Reduced model	log(biomarker)~Age+BMI+Gender+Smoking+(1|Patient)
Unrestricted model	log(biomarker)~Age+BMI+Gender+Smoking+Visit+Time1+Time2+(1|Patient)

BMI = body mass index, Visit = biomarker measurements at three time points in patients' group, Time1 = time difference between FEP_b and $FEP_{0.6\text{-year}}$, Time2 = time difference between $FEP_{0.6\text{-year}}$ and $FEP_{5.1\text{-year}}$, (1|Patient) = random effects of patients.

Amino acids	**CS**	**FEP_b**	**$FEP_{(0.6\text{-year})}$**	**$FEP_{(5.1\text{-year})}$**	**Comparison between models**	
	Mean ± SD (range)	**Mean ± SD (range)**	**Mean ± SD (range)**	**Mean ± SD (range)**	**Adjusted *p*-value**	**True (T) or false (F)**
Alanine	425 ± 118 (232–716)	387 ± 112 (206–673)	462 ± 134 (290–750)	520 ± 171 (289–953)	0.03	T
Arginine	163 ± 36.1 (94.1–225)	144 ± 32.6 (75.9–216)	155 ± 35.5 (93.0–263)	143 ± 49.8 (72.5–327)	0.08	F
Asparagine	37.2 ± 13.0 (15.0–65.2)	33.8 ± 12.6 (4.80–83.3)	35.7 ± 14.1 (8.06–75.5)	53.2 ± 17.4 (30.2–87.4)	2e-07	T
Aspartate	35.3 ± 10.7 (15.9–65.2)	33.8 ± 14.3 (10.6–62.9)	29.9 ± 11.5 (7.86–57.4)	19.0 ± 7.14 (5.11–39.6)	7e-06	T
Citrulline	27.8 ± 8.37 (11.0–48.9)	22.5 ± 5.77 (12.1–38.1)	25.0 ± 6.66 (11.5–39.4)	29.1 ± 13.7 (14.1–76.7)	9e-03	T
Glutamine	342 ± 153 (76.5–683)	328 ± 148 (80.4–813)	365 ± 176 (103–810)	854 ± 308 (495–1760)	4e-18	T
Glutamate	216 ± 99.2 (114–550)	250 ± 116 (59.6–627)	241 ± 121 (57.2–529)	74.1 ± 40.1 (18.7–172)	1e-25	T
Glycine	257 ± 72.1 (123–443)	278 ± 65.1 (153–474)	287 ± 81.9 (149–597)	297 ± 81.5 (187–518)	0.41	F
Histidine	94.0 ± 20.9 (58.3–138)	86.4 ± 14.8 (61.5–135)	97.7 ± 16.1 (73.3–136)	107 ± 21.1 (66.4–157)	9e-03	T

Table 4 (Continue)

Amino acids	CS	FEP_b	$FEP_{(0.6\text{-year})}$	$FEP_{(5.1\text{-year})}$	Comparison between models	
	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)	Mean ± SD (range)	Adjusted *p*-value	True (T) or false (F)
Isoleucine	92.2 ± 30.2 (50.1–179)	86.8 ± 22.7 (42.7–158)	95.4 ± 29.5 (43.9–190)	96.1 ± 28.0 (46.4–164)	0.95	F
Leucine	176 ± 68.9 (79.6–409)	166 ± 47.6 (73.0–279)	174 ± 56.8 (85.5–364)	200 ± 78.4 (77.0–443)	0.69	F
Lysine	203 ± 46.4 (107–309)	205 ± 51.8 (117–314)	217 ± 56.0 (103–399)	231 ± 55.6 (124–393)	0.69	F
Methionine	10.2 ± 6.37 (4.43–35.2)	9.89 ± 6.36 (3.95–26.3)	12.0 ± 7.38 (3.23–33.5)	27.7 ± 7.76 (13.2–43.0)	1e-18	T
Ornithine	55.8 ± 17.2 (23.4–91.4)	66.7 ± 25.2 (30.7–143)	63.3 ± 20.0 (28.4–125)	81.5 ± 22.1 (39.9–136)	8e-03	T
Phenylalanine	71.0 ± 19.7 (38.2–115)	70.7 ± 12.5 (41.8–101)	68.9 ± 15.2 (38.2–108)	77.0 ± 20.1 (43.6–134)	0.44	F
Proline	229 ± 79.2 (123–479)	184 ± 55.5 (83.3–381)	237 ± 55.4 (140–362)	261 ± 91.2 (111–459)	7e-05	T
Serine	170 ± 52.3 (69.3–363)	174 ± 43.9 (99.4–293)	168 ± 34.4 (115–253)	159 ± 55.2 (88.9–346)	0.69	F
Threonine	165 ± 60.2 (74.1–373)	144 ± 35.0 (71.6–214)	160 ± 38.1 (71.0–280)	151 ± 62.3 (82.7–430)	0.44	F
Tryptophan	74.2 ± 17.0 (32.8–120)	66.9 ± 16.7 (30.3–120)	71.7 ± 18.4 (34.2–121)	82.4 ± 25.9 (46.1–157)	0.02	T
Tyrosine	73.0 ± 27.9 (33.7–159)	60.7 ± 13.8 (35.8–99.8)	70.7 ± 19.4 (40.6–121)	77.2 ± 23.6 (40.4–138)	0.09	F
Valine	233 ± 62.1 (126–401)	219 ± 61.7 (112–413)	244 ± 67.0 (136–448)	305 ± 83.7 (155–490)	7e-03	T

Table 5 Serum mean levels, standard deviations (SD), and range of biogenic amines (μmol/l) for control subjects (CS, n=37), first-episode psychosis (FEP) patients at baseline (before treatment with antipsychotics, $FEP_{b,}$ n=52), after 0.6-year treatment ($FEP_{0.6\text{-year,}}$ n=44), and after 5.1-year treatment ($FEP_{5.1\text{-year,}}$ n=37) with antipsychotics. Comparison between reduced and unrestricted, *p*-values have been multiple test corrected according to false discovery rate (FDR) method. The unrestricted regression model is designated as True (i.e. more complex set of predictor variables explained more effectively biomarker level alterations over time) or False (i.e. unrestricted model did not provide more explanatory power than a simple one)

Biogenic amines	**CS**	**FEP_b**	**$FEP_{(0.6\text{-year})}$**	**$FEP_{(5.1\text{-year})}$**	**Comparison between models**	
	Mean ± SD (range)	**Mean ± SD (range)**	**Mean ± SD (range)**	**Mean ± SD (range)**	**Adjusted p-value**	**True (T) or false (F)**
Acetylornithine	0.68 ± 0.36 (0.18–2.03)	0.69 ± 1.24 (0.00–8.98)	0.67 ± 0.47 (0.00–2.63)	0.74 ± 0.68 (0.00–2.68)	0.95	F
Alpha-aminoadipic acid	0.90 ± 0.40 (0.45–1.98)	0.55 ± 0.34 (0.00–1.34)	0.75 ± 0.38 (0.00–1.54)	0.40 ± 0.45 (0.00–1.59)	1e-06	T
Asymmetric dimethylarginine	0.42 ± 0.08 (0.19–0.60)	0.44 ± 0.10 (0.28–0.67)	0.44 ± 0.09 (0.29–0.63)	0.58 ± 0.19 (0.26–1.19)	8e-03	T
Creatinine	69.4 ± 18.4 (35.0–112)	68.3 ± 16.2 (39.1–123)	69.7 ± 17.6 (42.0–124)	79.9 ± 32.5 (33.2–187)	0.51	F
Histamine	0.41 ± 0.04 (0.37–0.46)	0.34 ± 0.14 (0.12–0.46)	0.36 ± 0.12 (0.12–0.45)	0.28 ± 0.21 (0.00–0.57)	0.27	F
Kynurenine	2.71 ± 0.52 (1.37–3.89)	2.47 ± 0.85 (1.39–5.42)	3.02 ± 0.74 (1.77–4.88)	3.50 ± 2.05 (1.86–14.9)	3e-04	T
Putrescine	0.09 ± 0.04 (0.03–0.20)	0.11 ± 0.06 (0.02–0.32)	0.09 ± 0.04 (0.03–0.21)	0.14 ± 0.04 (0.06–0.23)	5e-03	T
Serotonin	0.76 ± 0.35 (0.19–1.47)	0.70 ± 0.40 (0.08–1.82)	0.65 ± 0.38 (0.05–1.48)	0.79 ± 0.47 (0.02–1.84)	0.25	F
Symmetric dimethylarginine	0.55 ± 0.10 (0.39–0.81)	0.56 ± 0.13 (0.28–0.93)	0.53 ± 0.12 (0.25–0.80)	0.65 ± 0.25 (0.32–1.49)	0.21	F
Taurine	49.0 ± 18.1 (25.8–116)	90.1 ± 31.6 (32.4–172)	51.4 ± 17.4 (28.2–119)	85.4 ± 31.8 (38.4–159)	2e-15	T

Table 6 Serum mean levels, standard deviations (SD), and range of biomarker ratios for control subjects (CS, n=37), first-episode psychosis (FEP) patients at baseline (before treatment with antipsychotics, $FEP_{b,}$ n=52), after 0.6-year treatment ($FEP_{0.6\text{-year,}}$ n=44), and after 5.1-year treatment ($FEP_{5.1\text{-year,}}$ n=37) with antipsychotics. Comparison between reduced and unrestricted, *p*-values have been multiple test corrected according to false discovery rate (FDR) method. The unrestricted regression model is designated as True (i.e. more complex set of predictor variables explained more effectively biomarker level alterations over time) or False (i.e. unrestricted model did not provide more explanatory power than a simple one)

Biomarker ratios	**CS**	**FEP_b**	**$FEP_{(0.6\text{-year})}$**	**$FEP_{(5.1\text{-year})}$**	**Comparison between models**	
	Mean ± SD (range)	**Mean ± SD (range)**	**Mean ± SD (range)**	**Mean ± SD (range)**	**Adjusted p-value**	**True (T) or false (F)**
Alpha-aminoadipic acid / Kynurenine	0.33 ±0.13 (0.15–0.70)	0.24 ± 0.15 (0.00–0.65)	0.25 ± 0.14 (0.00–0.79)	0.12 ± 0.15 (0.00–0.61)	4e-05	T
Aspartate / Asparagine	1.07 ± 0.54 (0.43–3.07)	1.11 ± 0.57 (0.36–2.71)	0.98 ± 0.54 (0.27–2.44)	0.38 ± 0.15 (0.15–0.77)	2e-15	T
Glutamate / Glutamine	1.01 ±1.35 (0.20–7.18)	1.18 ± 1.37 (0.09–7.80)	0.99 ± 0.98 (0.08–5.14)	0.09 ± 0.05 (0.02–0.25)	2e-26	T
Ornithine / Arginine	0.35 ± 0.11 (0.16–0.66)	0.48 ± 0.20 (0.22–1.24)	0.42 ± 0.13 (0.23–0.81)	0.61 ± 0.21 (0.30–1.14)	2e-06	T
Tyrosine / Phenylalanine	1.03 ±0.23 (0.49–1.57)	0.86 ± 0.16 (0.61–1.28)	0.95 ± 0.49 (0.37–2.36)	1.01 ± 0.18 (0.61–1.32)	6e-04	T

Table 7 Comparison of serum levels of amino acids (μmol/l) between the first-episode psychosis (FEP) patients (n=36) at baseline (before treatment with antipsychotics, FEP_b) and after 7-month treatment (FEP_f) (n=36) with antipsychotics

Amino acids	FEP_b	FEP_f	Z-value	*p*-value	Effect size (I^2)
	Median (min–max)	Median (min–max)			
Alanine (Ala)	342.50 (206.00–673.00)	418.00 (294.00–750.00)	2.66	8e-03	0.10
Arginine (Arg)	147.50 (88.00–216.00)	153.00 (93.00–218.00)	0.44	0.66	–
Asparagine (Asn)	36.80 (19.50–83.30)	37.90 (17.70–75.50)	1.21	0.23	–
Aspartate (Asp)	38.70 (18.80–62.90)	28.95 (17.70–57.40)	2.50	0.01	0.09
Citrulline (Citr)	22.40 (12.60–38.10)	24.55 (15.50–38.70)	1.54	0.12	–
Glutamine (Gln)	377.00 (118.00–813.00)	372.50 (162.00–810.00)	1.52	0.13	–
Glutamate (Glu)	210.00 (59.60–381.00)	206.50 (57.20–498.00)	1.17	0.24	–
Glycine (Gly)	273.50 (153.00–420.00)	267.00 (149.00–597.00)	0.23	0.82	–
Histidine (His)	82.60 (61.50–106.00)	93.10 (73.30–132.00)	3.75	**2e-04**	0.20
Isoleucine (Ile)	84.95 (42.70–130.00)	94.85 (43.90–190.00)	1.78	0.07	–
Leucine (Leu)	164.50 (73.00–273.00)	173.00 (85.50–364.00)	0.41	0.68	–
Lysine (Lys)	183.50 (117.00–279.00)	207.00 (103.00–306.00)	1.47	0.14	–
Methionine (Met)	7.75 (4.46–26.30)	12.50 (4.53–33.50)	2.50	0.01	0.09
Ornithine (Orn)	57.25 (30.70–115.00)	57.50 (28.40–91.90)	0.90	0.37	–
Phenylalanine (Phe)	72.00 (41.80–101.00)	65.55 (38.20–108.00)	1.35	0.18	–
Proline (Pro)	166.00 (83.30–381.00)	236.00 (140.00–362.00)	4.15	**3e-05**	0.24
Serine (Ser)	170.50 (99.40–293.00)	157.50 (115.00–246.00)	1.18	0.24	–
Threonine (Thr)	140.00 (84.70–214.00)	147.50 (71.00–280.00)	1.71	0.09	–
Tryptophan (Trp)	64.75 (30.30–89.30)	70.45 (34.20–121.00)	1.68	0.09	–
Tyrosine (Tyr)	58.55 (35.80–88.70)	63.25 (40.60–121.00)	2.99	3e-03	0.12

Table 7 (Continue)

Amino acids	FEP_b Median (min–max)	FEP_f Median (min–max)	Z-value	*p*-value	Effect size (I^2)
Valine (Val)	197.50 (112.00–299.00)	231.50 (136.00–390.00)	2.92	3e-03	0.12
Citr/Arg	0.16 (0.09–0.31)	0.17 (0.10–0.31)	1.81	0.07	–
Tyr/Phe	0.82 (0.61–1.26)	1.01 (0.77–1.44)	4.46	**8e-06**	0.28

Z-values according to Wilcoxon Matched Pairs Test (FEP_b compared to FEP_f).
p-values less than or equal to 0.001 after Bonferroni correction (bolded) were considered statistically significant.
Effect sizes ≥0.14 were interpreted as large.
Effect sizes 0.06–0.13 were interpreted as moderate.

Table 8 Comparison of serum levels of biogenic amines (μmol/l) between the first-episode psychosis (FEP) patients (n=36) at baseline (FEP_b) (before treatment with antipsychotic) and after 7-month treatment (FEP_f) (n=36) with antipsychotics

Biogenic amines	FEP_b Median (min – max)	FEP_f Median (min – max)	Z-value	*p*-value	Effect size (I^2)
Acetylornithine (Ac-Orn)	0.56 (0.18–1.06)	0.61 (0.24–1.47)	3.41	**7e-04**	0.16
Asymmetric dimethylarginine (ADMA)	0.43 (0.30–0.67)	0.41 (0.29–0.61)	0.20	0.84	–
Alpha aminoadipic acid (alpha-AAA)	0.56 (0.25–1.34)	0.81 (0.33–1.54)	3.33	**9e-04**	0.15
c4-OH-Pro	0.25 (0.00–0.34)	0.00 (0.00–0.38)	1.72	0.09	–
Carnosine	0.00 (0.00–0.13)	0.00 (0.00–0.15)	2.03	0.04	0.06
Creatinine	69.65 (42.30–123.00)	71.65 (45.70–124.00)	0.03	0.98	–
l-DOPA	0.12 (0.00–0.26)	0.14 (0.00–0.30)	1.05	0.30	–
Kynurenine (Kyn)	2.20 (1.39–5.42)	2.86 (1.77–4.74)	3.59	**3e-04**	0.18
Histamine	0.45 (0.37–0.46)	0.38 (0.37–0.45)	1.89	0.06	–
Methioninesulfoxide (Met-SO)	10.35 (2.11–24.90)	8.72 (1.69–20.30)	2.05	0.04	0.06
Putrescine	0.07 (0.02–0.19)	0.07 (0.03–0.21)	0.07	0.94	–

Table 8 (Continue)

Biogenic amines	FEP_b Median (min – max)	FEP_f Median (min – max)	Z-value	*p*-value	Effect size (I^2)
Symmetric-dimethylarginine (S-DMA)	0.57 (0.39–0.93)	0.52 (0.39–0.80)	1.34	0.18	–
Serotonin (5-HT)	0.57 (0.08–1.69)	0.58 (0.05–1.33)	1.56	0.12	–
Spermine	0.27 (0.17–0.43)	0.19 (0.16–0.27)	2.79	5e-03	0.11
t4-OH-Pro	0.42 (0.00–15.30)	0.60 (0.00–27.10)	0.51	0.61	–
Taurine	76.45 (32.40–172.00)	46.60 (28.20–119.00)	5.17	**<1e-06**	0.37
total-DMA	0.70 (0.49–1.08)	0.73 (0.52–0.98)	0.50	0.62	–
Met-SO/Methionine (Met)	1.35 (0.11–4.39)	0.66 (0.05–3.55)	2.14	0.03	0.06
Kyn/Tryptophan (Trp)	0.03 (0.02–0.08)	0.04 (0.03–0.06)	2.70	7e-03	0.10
5-HT/Trp	0.01 (0.00–0.03)	0.01 (0.00–0.02)	1.17	0.24	–

Z-values according to Wilcoxon Matched Pairs Test (FEP_b compared to FEP_f).
p-values less than or equal to 0.001 after Bonferroni correction (bolded) were considered statistically significant.
Effect sizes ≥0.14 were interpreted as large.
Effect sizes 0.06–0.13 were interpreted as moderate.
Commentary: ADMA, creatinine, Kyn, Met-So, 5-HT, spermine, taurine, and total-DMA values are higher than LLOQ. Ac-Orn, alpha-AAA, histamine, S-DMA values were at least 1.5 to 3 times higher than LOD.

Table 9 Estimated effects of complex set of predictor variables on biogenic amines serum concenrations between control subjects (CS, n=37), first-episode psychosis (FEP) patients at baseline (before treatment with antipsychotics, $FEP_{b,}$ n=52), after 0.6-year treatment ($FEP_{0.6\text{-year,}}$ n=44), and after 5.1-year treatment ($FEP_{5.1\text{-year,}}$ n=37) with antipsychotics: results from linear mixed-effects model

						Disease and treatment effect			Time between $FEP_{(b)}$ and $FEP_{(0.6\text{-year})}$	Time between $FEP_{(0.6\text{-year})}$ and $FEP_{(5.1\text{ year})}$
Biogenic amines	**Intercept**	**Age**	**Gender**	**Body mass index**	**Smoking status**	**FEP patients before treatment**	**FEP patients after 0.6-year treatment**	**FEP patients after 5.1-year treatment**		
	Estimate (Std. error), *p*-value									
Acetyl-ornithine	–0.05 (0.49), ns	0.01 (0.01), ns	–0.06 (0.14), ns	0.02 (0.02), ns	–0.36 (0.15), *p*=0.02	0.04 (0.17), ns	–0.01 (0.18), ns	–0.01 (0.21), ns	0.42 (1.69), ns	–0.09 (0.10), ns
Alpha- amino-adipic acid	0.46 (0.23), ns	0.002 (0.006), ns	–0.24 (0.07), *p*=4e-04	0.02 (0.009), *p*=0.01	–0.07 (0.07), ns	**–0.37 (0.08), *p*<1e-04**	–0.25 (0.09), *p*=0.006	**–0.65 (0.10), *p*<1e-04**	0.81 (0.71), ns	–0.01 (0.05), ns
Asymmetric dimet-hylarginine	–1.00 (0.16), *p*<1e-04	0.0004 (0.004), ns	–0.02 (0.05), ns	0.005 (0.006), ns	0.08 (0.05), ns	0.03 (0.06), ns	0.03 (0.06), ns	0.23 (0.07), *p*=0.002	0.24 (0.50), ns	0.01 (0.03), ns
Creatinine	4.45 (0.17), *p*<1e-04	–0.006 (0.004), ns	–0.27 (0.05), *p*<1e-04	0.003 (0.006), ns	–0.07 (0.05), ns	–0.02 (0.06), ns	–0.02 (0.06), ns	0.11 (0.07), ns	0.23 (0.48), ns	–0.005 (0.04), ns
Histamine	0.53 (0.09), *p*<1e-04	–0.002 (0.002), ns	–0.03 (0.03), ns	–0.002 (0.003), ns	–0.03 (0.03), ns	–0.06 (0.03), *p*=0.04	–0.06 (0.03), ns	–0.09 (0.04), *p*=0.03	0.19 (0.17), ns	–0.006 (0.02), ns
Kynurenine	0.73 (0.17), *p*=1e-04	–0.005 (0.004), ns	–0.14 (0.06), *p*=0.006	0.02 (0.006), *p*=0.003	0.04 (0.05), ns	–0.14 (0.06), *p*=0.02	0.04 (0.06), ns	0.11 (0.08), ns	0.44 (0.54), ns	–0.07 (0.04), ns
Putrescine	–2.59 (0.31), *p*<1e-04	–0.002 (0.008), ns	–0.17 (0.09), ns	0.01 (0.01), ns	0.09 (0.10), ns	–0.003 (0.11), ns	–0.10 (0.11), ns	0.33 (0.13), *p*=0.02	–0.26 (0.96), ns	–0.04 (0.06), ns
Serotonin	0.03 (0.45), ns	0.007 (0.01), ns	–0.13 (0.13), ns	–0.02 (0.02), ns	0.31 (0.14), *p*=0.03	–0.24 (0.15), ns	–0.32 (0.17), ns	–0.21 (0.19), ns	0.72 (1.54), ns	–0.16 (0.09), ns
Symmetric dimet-hylarginine	–0.67 (0.16), *p*=1e-04	–0.003 (0.004), ns	–0.06 (0.05), ns	0.007 (0.006), ns	–0.05 (0.05), ns	0.02 (0.06), ns	–0.04 (0.06), ns	0.12 (0.07), ns	0.20 (0.57), ns	–0.01 (0.03), ns
Taurine	3.79 (0.21), *p*<1e-04	0.0002 (0.005), ns	–0.03 (0.06), ns	0.002 (0.008), ns	0.06 (0.07), ns	**0.59 (0.08), *p*<1e-04**	0.02 (0.08), ns	**0.51 (0.10), *p*<1e-04**	–0.87 (0.77), ns	–0.03 (0.05), ns

Significant differences in the biomarker levels over time in the patients group compared to CS are marked in bold.
ns: $p \geq 0.05$.

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Parksepp, M., **Leppik, L.**, Koch, K., Uppin, K., Kangro, R., Haring, L., Vasar, E., & Zilmer, M. (2020). Metabolomics approach revealed robust changes in amino acid and biogenic amine signatures in patients with schizophrenia in the early course of the disease. *Scientific Reports*, 10(1), 13983. https://doi.org/10.1038/s41598-020-71014-w

Leppik, L., Parksepp, M., Janno, S., Koido, K., Haring, L., Vasar, E. & Zilmer, M. (2020). Profiling of lipidomics before and after antipsychotic treatment in first-episode psychosis. *European Archives of Psychiatry and Clinical Neuroscience,* 270, 59–70 https://doi.org/10.1007/s00406-018-0971-6

Leppik, L., Kriisa, K., Koido, K., Koch, K., Kajalaid, K., Haring, L., Vasar, E., & Zilmer, M. (2018). Profiling of Amino Acids and Their Derivatives Biogenic Amines Before and After Antipsychotic Treatment in First-Episode Psychosis. *Frontiers in Psychiatry*, 9. https://doi.org/10.3389/fpsyt.2018.00155

Kriisa, K., **Leppik, L.**, Balõtšev, R., Ottas, A., Soomets, U., Koido, K., Volke, V., Innos, J., Haring, L., Vasar, E., & Zilmer, M. (2017). Profiling of Acylcarnitines in First Episode Psychosis before and after Antipsychotic Treatment. *Journal of Proteome Research*, 16(10), 3558–3566. https://doi.org/10.1021/acs.jproteome.7b00279

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Parksepp, M., **Leppik, L.**, Koch, K., Uppin, K., Kangro, R., Haring, L., Vasar, E., & Zilmer, M. (2020). Metabolomics approach revealed robust changes in amino acid and biogenic amine signatures in patients with schizophrenia in the early course of the disease. *Scientific Reports*, 10(1), 13983. https://doi.org/10.1038/s41598-020-71014-w

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Leppik, L., Kriisa, K., Koido, K., Koch, K., Kajalaid, K., Haring, L., Vasar, E., & Zilmer, M. (2018). Profiling of Amino Acids and Their Derivatives Biogenic Amines Before and After Antipsychotic Treatment in First-Episode Psychosis. *Frontiers in Psychiatry*, 9. https://doi.org/10.3389/fpsyt.2018.00155

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