Transcriptome studies in the context of disturbed connectivity in schizophrenia

Estudos transcriptômicos no contexto da conectividade perturbada em esquizofrenia

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Abstract

Schizophrenia is a severe neurobiological disease with genetic and environmental factors playing a role in the pathophysiology. Several brain regions have been implicated in the disease process and are connected in complex neuronal circuits. On the cellular and molecular level, affected connectivity between these regions, involving dysfunctional myelination of neuronal axons, as well as alterations on the synaptic level and energy metabolism of neurons leading to disturbances in synaptic plasticity are major findings in *post-mortem* studies. Microarray studies investigating genome-wide gene expression have contributed to the findings of alterations in complex pathways in relevant brain regions in schizophrenia. Moreover, first laser-capture microdissection studies allowed the investigation of gene expression in specific groups of neurons. However, it must be kept in mind that in *post-mortem* studies confounding effects of medication, mRNA quality as well as the capability of the brain for neuroplastic regenerative mechanisms in individuals with a lifetime history of schizophrenia may influence the complex pattern of alterations on the molecular level. Despite these limitations, hypothesis-free transcriptome studies in brain tissue from schizophrenia patients offer a unique possibility to learn more about underlying mechanisms, leading to new insights in the pathophysiology of the disease.

Resumo

Esquizofrenia é uma severa doença neurobiológica com fatores genéticos e ambientais desempenhando um papel na fisiopatologia. Diversas regiões cerebrais têm sido implicadas no processo da doença e estão conectadas em complexos circuitos neuronais. Nos níveis molecular e celular, a conectividade afetada entre essas regiões, envolvendo mielinização disfuncional dos axônios neuronais, bem como as alterações no nível sináptico e metabolismo energético levando a distúrbios na plasticidade sináptica, são os maiores achados em estudos *post-mortem*. Estudos de microarranjos investigando a expressão gênica contribuíram para os achados de alterações em vias complexas em regiões cerebrais relevantes na esquizofrenia. Além disso, estudos utilizando microdissecção e captura a *laser* permitiram a investigação da expressão gênica em grupos específicos de neurônios. Entretanto, deve ser mantido em mente que em estudos *post-mortem*, confusos efeitos de medicação, qualidade de RNAm, bem como capacidade de mecanismos regenerativos neuroplásticos do cérebro em indivíduos com história de vida de esquizofrenia, podem influenciar o complexo padrão de alterações no nível molecular. Apesar dessas limitações, estudos transcriptômicos livres de hipóteses em tecido cerebral de pacientes esquizofrênicos oferecem uma possibilidade única para aprender mais sobre os mecanismos subjacentes, levando a novas ópticas da fisiopatologia da doença.

Introduction

Schizophrenia is a debilitating, chronic neurocognitive disorder affecting mostly young adults. Its prevalence is 1% world-wide independently of cultural identity or ethnicity. The disorder is characterized by various abnormal cognitive, affective and motor behavioral features. Most of the affected subjects (> 80%) are unable to maintain a self-sustaining professional life[1] and their life expectancy is shortened at an average of 10 years[2]. Along with frequent hospitalizations and the progressive neurocognitive impairment it deeply impacts the lives of those afflicted and their families. The cumulated financial burden of European social and health systems is high (€ 35 229 000 000 annually)[3].

Despite tremendous advances in science and medicine, the disorder remains mysterious. In the absence of consistent neurobiological markers for schizophrenia, diagnosis still relies on subjective assessment of a cluster of signs and symptoms, based on ICD-10 or DSM-IV-R criteria, and the current pharmacological treatments in most cases are empirically based. The identification of the pathophysiology associated with biological parameters deployable to unequivocally diagnose or evaluate the course and treatment remains challenging.

To date, the hypothesis of schizophrenia basing on multifactorial interactions which impact both early and late brain developmental changes prevails. Several lines of evidence support this neurodevelopmental hypothesis; among them are the association between obstetric complications such as the common factor hypoxia and schizophrenia[4,5]. There is strong evidence for a genetic basis for schizophrenia with functional polymorphisms in the promoter region of a gene, resulting in quantitative changes of the amount of gene expression or polymorphisms in the encoding region resulting in qualitative alterations of the gene product. However, abnormal gene-environmental interaction during development might also be due to epigenetic mechanisms on chromatin remodeling, which regulates gene transcription in complex neuronal pathways, and such mechanisms have been demonstrated in schizophrenia[6,7].

Neuronal circuits involved in schizophrenia

Recent investigations suggest that not only a deficit in one circumscribed region is underlying cognitive or positive/negative symptoms in schizophrenia, but alterations in neuronal networks control cognition and higher sensory processing.

Meta-analyses of structural magnetic resonance imaging (MRI) studies reveal gray matter volume deficits in a number of different brain regions in schizophrenic patients. Affected regions are the medial temporal lobe including the hippocampus and entorhinal cortex, the heteromodal association cortex including the prefrontal, parietal, and superior temporal cortex. The degree of grey matter reduction is in the range of 5%-10% in the frontotemporolimbic network[8,9].

Structural MRI and *post-mortem* studies have shown volume loss in the medial temporal lobe, especially in the hippocampus, as one of the most consistent structural abnormalities, along with deficits in spatial and verbal memory[10]. Additionally, *post-mortem* studies showed volume loss in hippocampal subregions which have been related to positive symptoms[11,12]. Newer developments in neuroimaging techniques like diffusion tensor imaging (DTI) afforded the investigation of disturbances in connectivity and neuronal networks. DTI studies of white matter tracts of the fornix body and hippocampus in schizophrenia show decreased fractional anisotropy and support the hypothesis of structural and functional dysconnectivity[13-16] which is correlated with deficits in cognitive function, *e.g.* verbal declarative memory[17].

The prefrontal cortex has been regarded as a key region for the pathophysiology of the disease due to its involvement in executive function and working memory, which are affected in schizophrenia[18]. MRI investigations uncovered reductions mainly in the dorsolateral prefrontal cortex in 70% of the studies reporting decreased volumes[19,20]. A further sign of early developmental deficit consists in the disturbed gyrification index as a measure of disturbed cortical folding[21-25]. A decreased prefrontal glucose uptake and hypofrontality has been related to negative symptoms and cognitive dysfunction[26,27]. The anterior cingulate cortex is part of to the medial prefrontal cortex and receives sensory information from insular, temporal and parietal association cortices plus emotional information from the amygdala and orbitofrontal cortex. This brain region is modulator of the dorsolateral prefrontal cortex[28,29] and has been shown to mediate urban upbringing as an environmental risk factor in schizophrenia[30]. The prefrontal cortex, as much as the superior temporal gyrus, anterior cingulate and inferior parietal cortex, belongs to the heteromodal association cortex, which is responsible for cognitive processing and influences complex behavior[31]. A dysfunction in these cortices has been described in chronic schizophrenia patients living in home residencies, a population which serves as basis for most of the *post-mortem* investigations[32]. A meta-analysis of fMRI studies of executive function in schizophrenia revealed reduced activation of the prefrontal cortex, anterior cingulate cortex, and thalamus[33].

A reduction in thalamic volumes in schizophrenia has been shown in structural MRI studies[34,35]. The thalamus acts as a central relay station, transferring peripheral sensory inputs to the cortex. It plays a critical role in filtering sensory information, in regulating cognitive input to the cortex, and mediating cortico-cortical connections between areas particularly implicated in schizophrenia, such as frontal and temporal regions. In schizophrenia patients, positron emission tomography (PET) studies show a dysfunction of the cortico-cerebellar-thalamic-cortical neuronal circuit contributing to "cognitive dysmetria", i.e. impaired cognition and other symptoms of the disease[36,37]. In addition, a disturbed prefronto-parietal-thalamic network has been shown to be involved in disease-related working memory deficits in schizophrenia[38]. In summary, alterations of connectivity are present in complex neuronal circuits, causing not only a dysfunction in specific brain regions but also basing on deficits in complex pathways on the molecular level.

Principles of gene expression methods in *post-mortem* tissue

To unravel the neurobiological disturbances underlying altered activation of volume loss of brain regions, during the last years, transcriptome studies using a hypothesis-free approach with microarray investigations of thousands of genes, and hypothesis-driven studies using quantitative real-time polymerase chain reaction (qRT-PCR) or *in situ* hybridization have been conducted. Each of these methods provides advantages in molecular biology: the high quantity of genes detectable with microarray studies and higher sensitivity in the detection of a limited number of genes in qRT-PCR studies, which makes this method suitable for verification of altered gene expression in microarray experiments. The spatial resolution of subregions, cortical layers or cell populations is the advantage of *in situ* hybridization investigations. The development of high-throughput technologies as genome-wide microarray, proteomics or recently next-generation sequencing has improved the understanding of the involvement of complex molecular pathways in the pathophysiology of the disease, thus enhancing knowledge about the dysfunction of single molecules[39]. New microscopic techniques like laser-capture microdissection, used for collection of neuronal cell populations or subregions with preserved mRNA integrity in histological stained sections, provide new insights in the understanding of interactions between cellular and molecular networks[40,41].

However, many samples stemming from human autopsies are in sub-optimal condition for gene expression studies and multiple factors influence the quality of extracted mRNA from brain tissue. They mainly include the agonal state with hypoxia, cause of death and the *post-mortem* delay. The tissues' pH-level is influenced by these factors and may serve as a marker of subsequent RNA quality. Additionally, individual mRNA transcripts may exhibit different decay rates and half-lifes. Thus, *post-mortem* delay and pH should be documented and mRNA quality of the tissue should always be determined for example using the Agilent 2100 BioAnalyzer (Agilent, Palo Alto, CA)[42,43]. Additionally, most of the chronic schizophrenia patients had been treated with antipsychotics for decades, so effects of antipsychotic doses calculated in chlorpromazine equivalents should be correlated statistically to individual mRNA levels[44].

Disturbed macroconnectivity: evidence from cellular and molecular studies

Disturbed connectivity in schizophrenia is associated with deficits in myelination of neuronal axons. Myelination plays an important role in nerve cell propagation and also integrates the brain's structural synchrony, resulting in functional and structural connectivity. Oligodendrocytes are the myelin-building glia cells in the brain. Other oligodendrocyte functions are trophic signaling to adjacent neurons, synthesis of growth factors, neuronal survival and development, neurotransmission, and synaptic function[45]. Reduced number, dysfunction or death of oligodendrocytes may influence neuronal integrity. In several stereological studies, numbers of oligodendrocytes have been reported to be decreased in the prefrontal cortex and CA4 subregion of the hippocampus[46-48]; density maps of oligodendrocytes show lesser clustering in the prefrontal cortex[47]. Structural abnormalities of myelin sheath and regressive changes in oligodendrocytes in the prefrontal cortex and hippocampus may be based on neurodevelopmental disturbances or apoptotic cell death[49-51]. Interestingly, the heteromodal association cortex has its main myelination period in young adulthood with first symptoms of schizophrenia appearing[52]. On the molecular level, evidence for disordered myelination has been provided by several microarray studies in brain regions of schizophrenia patients[53-57]. Quantitative RT-PCR studies[57,58] confirm the implication of oligodendrocyte and myelin in the pathogenesis by revealing down-regulation of multiple oligodendrocyte and/or myelin-related genes in different brain areas[59]. Additionally, proteomic studies reported oligodendrocyte dysfunction and related disturbances of myelination. Myelin basic

protein (MBP) is the major constituent of the myelin sheath produced by oligodendrocytes and the myelin oligodendrocyte glycoprotein (MOG) was found to be downregulated in the prefrontal and temporal cortex, hippocampus and entorhinal cortex, where it was associated with migrational abnormalities of pre-alpha cells[60-63]. Additionally the oligodendrocyte marker 2`,3` cyclic nucleotide,3`-phosphodiesterase (CNP) was downregulated in these regions and reduced cell number of CNPase-stained neurons has been reported in the prefrontal cortex[47]. Other commonly altered myelin-related genes are peripheral myelin protein 22 (PMP22), myelin-associated Gylcoprotein (MAG), Myelin and leucozytes protein (MAL), transferrin (TF), claudin 1, claudin 5, Proteolipid Protein (PLP), Gelsolin (GSN), Plasmolipin (PLLP, TM4SF11), and quaking (QKI)[56,57,59], which play an important role in neurodevelopment, growth, and maintenance of myelin und axons[64]. These proteins might also be potential biomarkers for schizophrenia[65]. The oligodendrocyte protein ermin was found to be upregulated in the prefrontal cortex and downregulated in the temporal cortex in two used shotgun proteomic studies[66,67]. Brockschnieder *et al.*[68] concluded that ermin probably contributes to the maintenance and stabilization of the myelin sheath in the adult brain.

Schizophrenia as a disorder of disturbed synaptic plasticity

'Synapses are located on dendritic spines which have been reported to be reduced in the prefrontal cortex of schizophrenic patients[69]. Possibly a hypofunction of the glutamatergic NMDA receptor acting on long-term potentiation disturbs synaptic plasticity[70]. On the molecular level there is strong support for decreased synaptic plasticity, mainly affecting the expression of presynaptic vesicle proteins including the synaptosome-associated protein 25 (SNAP-25) and syntaxin forming the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex[71-79]. The trimeric SNARE complex is involved in membrane fusion and exocytosis of neurotransmitters. Levels of SNAP-25 mRNA have been reported to be increased in the superior temporal cortex of the younger group of schizophrenic patients[79]. A microarray study in the prefrontal cortex showed altered expression of genes associated with synaptic vesicle recycling in schizophrenia[80]. Accordingly, on the protein level reduced synaptophysin and SNAP-25 immunoreactivity have been detected in the prefrontal cortex[60,69,81-83]. However, results are not consistent and vary between different brain regions[83,84]. In the terminal fields of entorhinal cortex projections, SNAP-25 expression was decreased[85]. In the hippocampus it was shown that neuron populations had fewer dendritic spines and reduced dendritic arborizations[86]. This observation was supported by studies detecting molecular markers like microtubule-associated protein 2 (MAP2) and spinophilin[87,88]. Evidence for reduced presynaptic markers was also reported. A more consistent finding within the hippocampus is abnormal synaptic connectivity, as shown by decreased expression of the presynaptic proteins synapsin, synaptophysin and SNAP-25[73]. In addition, disturbances of complexins in the hippocampus were associated with the severity of *ante-mortem* cognitive impairment[78]. Further insights in disturbed synaptic plasticity provided a microarray study of the superior temporal cortex, showing that decreased expression of immune-related genes may affect synaptic strength and transmission[44]. Altogether, when combining findings on the mRNA and protein level, this strongly supports the notion of regionally distinct expression of synaptic genes.

Altered expression of cytoskeletal proteins in schizophrenia-related brain regions may influence synaptic plasticity during neurodevelopment and in adulthood, with consequences on neurotransmission. In nerve terminals, crystalline mu modulates cytoskeletal proteins[89]. In a proteomic study, this cytoskeletal protein has been reported to be upregulated in the prefrontal cortex in schizophrenia[66]. Increased expression of crystalline mu in schizophrenia has also been reported in transcriptome studies[53,90]. However, a more detailed investigation of cytoskeletal genes in *post-mortem* brain regions is

needed. Interestingly, a laser capture microdissection (LCM) study in isolated dentate granular neurons reported decreased expression of genes related to cytoskeletal proteins, synaptic plasticity and energy metabolism[91], thus supporting the hypothesis of disturbed microconnectivity.

Disturbed energy metabolism: evidence from transcriptome studies

In schizophrenia, several altered genes are involved in energy metabolism-related processes of glucose metabolism. For example, hexokinase was found to be upregulated in prefrontal and superior temporal cortex. It participates in different cellular processes, be it glycolysis or fructose, galactose, sucrose, and mannose metabolism[65]. Some other proteins reported to be altered on the protein level are also described as differentially expressed in transcriptome studies. They are involved in energy metabolism such as the mitochondrial precursor of ATP synthase alpha chain, transferrin, and aldolase C[53,92]. Neuronal mitochondria are known to produce most of the cell energy. Therefore, alterations in mitochondrial proteins, caused by endogenous or exogenous factors, can result in dysregulation of energy production. Pathways involved in energy metabolism entail internal processes as glycolysis, the Krebs cycle, and oxidative phosphorylation. Changes in mitochondrial oxidative phosphorylation in the brain[93] and in platelets[94] of schizophrenic patients have already been reported. Genes associated with mitochondrial functions essential for the normal development of the brain and synaptic function have also been shown to be abnormal[95,96]. Ben-Shachar *et al.*[95] found a pathophysiological link between mitochondrial function and schizophrenia. This was demonstrated at the level of mitochondrial function, mitochondrial respiration and complex I activity and at the level of gene and protein expression. The disease also exhibits an abnormal interaction between dopamine and the mitochondria[95,97]. Alterations in prohibitin were hypothesized to be involved in disturbed synaptic plasticity[98]. It is known to control histone deacetylation[99] and can be localized in the inner membrane of mitochondria.

Conclusions

Transcriptome studies state strong evidence for a synaptopathy, deficits in oligodendrocyte function and energy metabolism underlying cognitive deficits and symptom domains in schizophrenia. These may be fundamental for volume deficits in distinct neuronal circuits as well as altered brain activation during cognitive tasks. However, despite these pathophysiological insights, in *post-mortem* studies confounding effects of medication, mRNA quality, cause of death as well as the capability of the brain for neuroplastic regenerative mechanisms in individuals with a lifetime history of interacting mechanisms by the disease process itself, but also by physical and cognitive activation, may lead to a complex pattern of brain alterations. Despite these limitations, transcriptome and proteome studies of *post-mortem* tissue offer the unique opportunity of identifying disturbances in complex pathways to unravel the complex picture of the schizophrenia pathophysiology and to develop new neuroprotective treatment strategies.

References

1. Lindstrom E, Eberhard J, Neovius M, Levander S. Costs of schizophrenia during 5 years. Acta Psychiatr Scand Suppl. 2007;(435):33-40
2. Saha S, Chant D, McGrath J. A systematic review of mortality in schizophrenia: is the differential mortality gap worsening over time? Arch Gen Psychiatry. 2007;64(10):1123-31.
3. Andlin-Sobocki P, Rossler W. Cost of psychotic disorders in Europe. Eur J Neurol. 2005;12(Suppl 1):74-7.
4. McNeil TF, Cantor-Graae E, Ismail B. Obstetric complications and congenital malformation in schizophrenia. Brain Res Brain Res Rev. 2000;31(2-3):166-78.

5. Fendt M, Lex A, Falkai P, Henn FA, Schmitt A. Behavioural alterations in rats following neonatal hypoxia and effects of clozapine: implications for schizophrenia. Pharmacopsychiatry. 2008;41(4):138-45.

6. Roth TL, Lubin FD, Sodhi M, Kleinman JE. Epigenetic mechanisms in schizophrenia. Biochim Biophys Acta. 2009;1790(9):869-77.

7. Fischer A, Sananbenesi F, Mungenast A, Tsai LH. Targeting the correct HDAC(s) to treat cognitive disorders. Trends Pharmacol Sci. 2010;31(12):605-17.

8. Wright IC, Rabe-Hesketh S, Woodruff PW, David AS, Murray RM, Bullmore ET. Meta-analysis of regional brain volumes in schizophrenia. Am J Psychiatry. 2000;157(1):16-25.

9. Shenton ME, Dickey CC, Frumin M, McCarley RW. A review of MRI findings in schizophrenia. Schizophr Res. 2001;49:1-52.

10. Heckers S. Neuroimaging studies of the hippocampus in schizophrenia. Hippocampus. 2001;11:520-8.

11. Bogerts B, Falkai P, Haupts M, Greve B, Ernst S, Tapernon-Franz U, et al. Post-mortem volume measurements of limbic system and basal ganglia structures in chronic schizophrenics. Initial results from a new brain collection. Schizophr Res. 1990;3:295-301.

12. Bogerts B, Lieberman JA, Ashtari M, Bilder RM, Degreef G, Lerner G, et al. Hippocampus-amygdala volumes and psychopathology in chronic schizophrenia. Biol Psychiatry. 1993;33:236-46.

13. Kalus P, Buri C, Slotboom J, Gralla J, Remonda L, Dierks T, et al. Volumetry and diffusion tensor imaging of hippocampal subregions in schizophrenia. Neuroreport. 2004;15:867-71.

14. Kuroki N, Kubicki M, Nestor PG, Salisbury DF, Park HJ, Levitt JJ, et al. Fornix integrity and hippocampal volume in male schizophrenic patients. Biol Psychiatry. 2006;60:22-31.

15. White T, Kendi AT, Lehericy S, Kendi M, Karatekin C, Guimaraes A, et al. Disruption of hippocampal connectivity in children and adolescents with schizophrenia – a voxel-based diffusion tensor imaging study. Schizophr Res. 2007;90:302-7.

16. Zhou Y, Shu N, Liu Y, Song M, Hao Y, Liu H, et al. Altered resting-state functional connectivity and anatomical connectivity of hippocampus in schizophrenia. Schizophr Res. 2008;100:120-32.

17. Lim KO, Ardekani BA, Nierenberg J, Butler PD, Javitt DC, Hoptman MJ. Voxelwise correlational analyses of white matter integrity in multiple cognitive domains in schizophrenia. Am J Psychiatry. 2006;163:2008-10.

18. John JP. Fronto-temporal dysfunction in schizophrenia: a selective review. Indian J Psychiatry. 2009;51(3):180-90.

19. Cannon TD, Thompson PM, Van Erp TG, Toga AW, Poutanen VP, Huttunen M, et al. Cortex mapping reveals regionally specific patterns of genetic and disease-specific gray-matter deficits in twins discordant for schizophrenia. Proc Natl Acad Sci U S A. 2002;99(5):3228-33.

20. Schmitt A, Weber-Fahr W, Jatzko A, Tost H, Henn FA, Braus DF. Aktueller überblick über strukturelle magnetresonanztomographie bei schizophrenie. Fortschr Neurologie Psychiatrie. 2001;69(3):105-15.

21. Vogeley K, Tepest R, Schneider-Axmann T, Hütte H, Zilles K, Honer WG, et al. Automated image analysis of disturbed cytoarchitecture in Brodmann area 10 in schizophrenia. Schizophr Res. 2003;62:133-40.

22. Sallet PC, Elkis H, Alves TM, Oliveira JR, Sassi E, Campi de Castro C, et al. Reduced cortical folding in schizophrenia: an MRI morphometric study. Am J Psychiatry. 2003;160(9):1606-13.

23. Falkai P, Honer WG, Kamer T, Dustert S, Vogeley K, Schneider-Axmann T, et al. Disturbed frontal gyrification within families affected with schizophrenia. J Psychiatry Res. 2007;41(10):805-13.

24. Stanfield AC, Moorhead TW, Harris JM, Owens DG, Lawrie SM, Johnstone EC. Increased right prefrontal cortical folding in adolescents at risk of schizophrenia for cognitive reasons. Biol Psychiatry. 2008;63(1):80-5.

25. McIntosh AM, Moorhead TW, McKirdy J, Hall J, Sussmann JE, Stanfield AC, et al. Prefrontal gyral folding and its cognitive correlates in bipolar disorder and schizophrenia. Acta Psychiatr Scand. 2009;119(3):192-8.

26. Carter CS, Perlstein W, Ganguli R, Brar J, Mintun M, Cohen JD. Functional hypofrontality and working memory dysfunction in schizophrenia. Am J Psychiatry. 1998;155(9):1285-7.

27. Schröder J, Buchsbaum MS, Siegel BV, Geider FJ, Lohr J, Tang C, et al. Cerebral metabolic activity correlates of subsyndromes in chronic schizophrenia. Schizophr Res. 1996;19(1):41-53.

28. Kerns JG, Cohen JD, MacDonald AW, Cho RY, Stenger VA, Carter CS. Anterior cingulate conflict monitoring and adjustments in control. Science. 2004;303:1023-6.

29. Fincham JM, Anderson JR. Distinct roles of the anterior cingulate and prefrontal cortex in the acquisition and performance of a cognitive skill. Proc Natl Acad Sci U S A. 2006;103:12941-6.

30. Lederbogen F, Kirsch P, Haddad L, Streit F, Tost H, Schuch P, et al. City living and urban upbringing affect neural social stress processing in humans. Nature. 2011;474(7353):498-501.

31. Pearlson GD, Petty RG, Ross CA, Tien AY. Schizophrenia: a disease of heteromodal association cortex? Neuropsychopharmacol. 1996;14:1-17.

32. Schmitt A, Steyskal C, Bernstein HG, Parlapani E, Schaeffer EL, Gattaz WF, et al. Stereologic investigation of the posterior part of the hippocampus in schizophrenia. Acta Neuropathol. 2009;117(4):395-407.

33. Minzenberg MJ, Laird AR, Thelen S, Carter CS, Glahn DC. Meta-analysis of 41 functional neuroimaging studies of executive function in schizophrenia. Arch Gen Psychiatry. 2009;66(8):811-22.

34. Gaser C, Nenadic I, Volz HP, Büchel C, Sauer H. Neuroanatomy of "hearing voices": a frontotemporal brain structural abnormality associated with auditory hallucinations in schizophrenia. Cereb Cortex. 2004;14(1):91-6.

35. Brickman AM, Buchsbaum MS, Shihabuddin L, Byne W, Newmark RE, Brand J, et al. Thalamus size and outcome in schizophrenia. Schizophr Res. 2004;71:473-84.

36. Andreasen NC, Arndt S, Swayze V 2nd, Cizadlo T, Flaum M, O'Leary D, et al. Thalamic abnormalities in schizophrenia visualized through magnetic resonance image averaging. Science. 1994;266:294-8.

37. Andreasen NC, Nopoulos P, O'Leary DS, Miller DD, Wassink T, Flaum M. Defining the phenotype of schizophrenia: cognitive dysmetria and its neural mechanisms. Biol Psychiatry. 1999;46:908-20.

38. Schneider F, Habel U, Reske M, Kellermann T, Stöcker T, Shah NJ, et al. Neural correlates of working memory dysfunctions in first-episode schizophrenia patients: An fMRI multicenter study. Schizophr Res. 2007;89:198-210.

39. Gebicke-Härter PJ. Expression profiling in brain disorders. In: Karamanos Y, editor. Expression profiling in neuroscience (neuromethods). New York: Springer; 2011. p. 64. (DOI 10.1007/978-1-61779-448-3_3)

40. Rossner M, Hirrlinger J, Wichert SP, Boehm C, Newrzella D, Hiemisch H, et al. Global transcriptome analysis of genetically identified neurons in the adult cortex. J Neurosci. 2006;26(39):9956-66.

41. Newrzella D, Pahlavan PS, Krüger C, Boehm C, Sorgenfrei O, Schröck H, et al. The functional genome of CA1 and CA3 neurons under native conditions and in response to ischemia. BMC Genomics. 2007;15(8):370.

42. Schmitt A, Bauer M, Feiden W, Heinsen H, the Consortium of Brainnet Europe II, Falkai P. How a neuropsychiatric brain bank should be run – A consensus paper of Brainnet Europe II. J Neural Transm. 2007;114(5):527-37.

43. Schmitt A, Parlapani E, Bauer M, Heinsen H, Falkai P. Is brain banking of psychiatric cases valuable for neurobiological research? Clinics. 2008;63(2):255-66.

44. Schmitt A, Leonardi-Essmann F, Durrenberger P, Parlapani E, Schneider-Axmann T, Spanagel R, et al. Regulation of immune-modulatory genes in left superior temporal cortex of schizophrenia patients: a genome-wide microarray study. W J Biol Psychiatry. 2011;12(3):201-15.

45. Du Y, Dreyfus CF. Oligodendrocytes as providers of growth factors. J Neurosci Res. 2002;68:647-54.

46. Hof PR, Haroutunian V, Copland C, Davis KL, Buxbaum JD. Molecular and cellular evidence for an oligodendrocyte abnormality in schizophrenia. Neurochem Res. 2002;27:1193-200.

47. Hof PR, Haroutunian V, Friedrich VL Jr., Byne W, Buitron C, Perl DP, et al. Loss and altered spatial distribution of oligodendrocytes in the superior frontal gyrus in schizophrenia. Biol Psychiatry. 2003;53:1075-85.

48. Schmitt A, Steyskal C, Bernstein HG, Parlapani E, Schaeffer EL, Gattaz WF, et al. Stereologic investigation of the posterior part of the hippocampus in schizophrenia. Acta Neuropathol. 2009;117(4):395-407.

49. Davis KL, Stewart DG, Friedman JI, Buchsbaum M, Harvey PD, Hof PR, et al. White matter changes in schizophrenia: evidence for myelin-related dysfunction. Arch Gen Psychiatry. 2003;60:443-56.

50. Kolomeets NS, Orlocskaya DD, Rachmanova VI, Uranova NA. Ultrastructural alterations in hippocampal mossy fiber synapses in schizophrenia: a postmortem morphometric study. Synapse. 2005;57(1):47-55.

51. Kolomeets NS, Uranova NA. [Pathology of oligodendroglia and myelinated fibers of the hippocampus in schizophrenia (an ultrastruc-

tural and morphometric study)]. Zh Nevrol Psikhiatr Im S S Korsakova. 2008;108:52-60.

52. Höistad M, Segal D, Takahashi N, Sakurai T, Buxbaum JD, Hof PR. Linking white and grey matter in schizophrenia: oligodendrozyte and neuron pathology in the prefrontal cortex. Frontiers Neuroanatom. 2009;3:9.

53. Hakak Y, Walker JR, Li C, Wong WH, Davis KL, Buxbaum JD, et al. Genome-wide expression analysis reveals dysregulation of myelination-related genes in chronic schizophrenia. Proc Natl Acad Sci U S A. 2001;98:4746-51.

54. Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet. 2003;362:798-805.

55. Aston C, Jiang L, Sokolov BP. Microarray analysis of postmortem temporal cortex from patients with schizophrenia. J Neurosci Res. 2004;77:858-66.

56. Katsel P, Davis KL, Haroutunian V. Variations in myelin and oligo-dendrocyte-related gene expression across multiple brain regions in schizophrenia: a gene ontology study. Schizophr Res. 2005;79:157-73.

57. Dracheva S, Davis KL, Chin B, Woo DA, Schmeidler J, Haroutunian V. Myelin-associated mRNA and protein expression deficits in the anterior cingulate cortex and hippocampus in elderly schizophrenia patients. Neurobiol Dis. 2006;21:531-40.

58. Aberg K, Saetre P, Jareborg N, Jazin E. Human QKI, a potential regulator of mRNA expression of human oligodendrocyte-related genes involved in schizophrenia. Proc Natl Acad Sci U S A. 2006;103:7482-7.

59. Haroutunian V, Katsel P, Dracheva S, Stewart DG, Davis KL. Variations in oligodendrocyte-related gene expression across multiple cortical regions: implications for the pathophysiology of schizophrenia. Int J Neuropsychopharmacol. 2007;10:565-73.

60. Honer WG, Falkai P, Chen C, Arango V, Mann JJ, Dwork AJ. Synaptic and plasticity-associated proteins in anterior frontal cortex in severe mental illness. Neuroscience. 1999;91:1247-55.

61. Tkachev D, Mimmack ML, Ryan MM, Wayland M, Freeman T, Jones PB, et al. Oligodendrocyte dysfunction in schizophrenia and bipolar disorder. Lancet. 2003;362(9386):798-805.

62. Chambers JS, Perrone-Bizzozero NI. Altered myelination of the hippo-campal formation in subjects with schizophrenia and bipolar disorder. Neurochem Res. 2004;29:2293-302.

63. Parlapani E, Schmitt A, Bergmann A, Bernstein HG, Breunig B, Gruber O, et al. Association between myelin basic protein expression and left entorhinal cortex pre-alpha cell layer disorganization in schizophrenia. Brain Res. 2009;1301:126-34.

64. McInnes LA, Lauriat TL. RNA metabolism and dysmyelination in schizophrenia. Neurosci Biobehav Rev. 2006;30(4):551-61.

65. Martins-de-Souza D, Dias-Neto E, Schmitt A, Falkai P, Gormanns P, Maccarrone G, et al. Proteome analysis of schizophrenia brain tissue. World J Biol Psychiatry. 2010;11(2):110-20.

66. Martins-de-Souza D, Gattaz WF, Schmitt A, Novello JC, Marangoni S, Dias-Neto E. Proteome analysis of schizophrenia patients Wernicke's area reveals an energy metabolism dysregulation. BMC Psychiatry. 2009;9(1):17.

67. Martins-de-Souza D, Gattaz WF, Schmitt A, Rewerts C, Maccarone G, Dias-Neto E, et al. Prefrontal cortex shotgun proteome analysis reveals altered calcium homeostasis and immune system imbalance in schizo-phrenia. Eur Arch Psychiatry Clin Neurosci. 2009;259(3):151-63.

68. Brockschnieder D, Sabanay H, Riethmaxher D, Peles E. Ermin, a myelinating oligodendrocyte-specific protein that regulates cell morphol-ogy. J Neurosci. 2006;26(3):757-62.

69. Glantz LA, Lewis DA. Reduction of synaptophysin immunoreactivity in the prefrontal cortex of subjects with schizophrenia. Arch Gen Psychiatry. 1997;54:943-52.

70. Olney JW, Farber NB. Glutamate receptor dysfunction and schizophrenia. Arch Gen Psychiatry. 1995;52:998-1007.

71. Eastwood SL, Brunet PWJ, Harrison PJ. Expression of complexin I and II mRNAs and their regulation by antipsychotic drugs in the rat forebrain. Synapse. 2000;36:167-77.

72. Eastwood SL, Harrison PJ. Hippocampal synaptic pathology in schizo-phrenia, bipolar disorder and major depression: a study of complexin mRNAs. Mol Psychiatry. 2000;5(4):425-32.

73. Eastwood SL, Harrison PJ. Synaptic pathology in the anterior cingulate cortex in schizophrenia and mood disorders. A review and a Western blot study of synaptophysin, GAP-43 and the complexins. Brain Res Bull. 2001;55(5):569-78.

74. Eastwood SL, Harrison PJ. Decreased expression of vesicular glutamate transporter 1 and complexin II mRNAs in schizophrenia: further evi-dence for a synaptic pathology affecting glutamate neurons. Schizophr Res. 2005;73(2-3):159-72.

75. Knable MB, Barci BM, Webster MJ, Meador-Woodruff J, Torrey EF, Stanley Neuropathology Consortium. Molecular abnormalities of the hippocampus in severe psychiatric illness: postmortem findings from the Stanley Neuropathology Consortium. Mol Psychiatry. 2004;9(6):609-20.

76. Kuromitsu J, Yokoi A, Kawai T, Nagasu T, Aizawa T, Haga S, et al. Reduced neuropeptide Y mRNA levels in the frontal cortex of people with schizophrenia and bipolar disorder. Brain Res Gene Expr Patterns. 2001;1(1):17-21.

77. Sawada K, Young CE, Barr AM, Longworth K, Takahashi S, Arango V, et al. Altered immunoreactivity of complexin protein in prefrontal cortex in severe mental illness. Mol Psychiatry. 2002;7(5):484-92.

78. Sawada K, Barr AM, Nakamura M, Arima K, Young CE, Dwork AJ, et al. Hippocampal complexin proteins and cognitive dysfunction in schizophrenia. Arch Gen Psychiatry. 2005;62(3):263-72.

79. Sokolov BP, Tcherepanov AA, Haroutunian V, Davis KL. Levels of mRNAs encoding synaptic vesicle and synaptic plasma membrane proteins in the temporal cortex of elderly schizophrenic patients. Biol Psychiatry. 2000;48(3):184-96.

80. Maycox PR, Kelly F, Taylor A, Bates S, Reid J, Logendra R, et al. Analysis of gene expression in two large schizophrenia cohorts identifies mul-tiple changes associated with nerve terminal function. Mol Psychiatry. 2009;14(12):1083-94.

81. Fatemi SH, Earle JA, Stary JM, Lee S, Sedgewick J. Altered levels of the synaptosomal associated protein SNAP-25 in hippocampus of subjects with mood disorders and schizophrenia. Neuroreport. 2001;12(15):3257-62.

82. Honer WG, Falkai P, Bayer TA, Xie J, Hu L, Li HY, et al. Abnormalities of SNARE mechanism proteins in anterior frontal cortex in severe mental illness. Cereb Cortex. 2002;12(4):349-56.

83. Thompson PM, Sower AC, Perrone-Bizzozero NI. Altered levels of the synaptosomal associated protein SNAP-25 in schizophrenia. Biol Psy-chiatry. 1998;43(4):239-43.

84. Halim ND, Weickert CS, McClintock BW, Hyde TM, Weinberger DR, Kleinman JE, et al. Presynaptic proteins in the prefrontal cortex of patients with schizophrenia and rats with abnormal prefrontal development. Mol Psychiatry. 2003;8(9):797-810.

85. Young CE, Arima K, Xie J, Hu L, Beach TG, Falkai P, et al. SNAP-25 deficit and hippocampal connectivity in schizophrenia. Cereb Cortex. 1998;8(3):261-8.

86. Rosoklija G, Toomayan G, Ellis SP, Keilp J, Mann JJ, Latov N, et al. Structural abnormalities of subicular dendrites in subjects with schizo-phrenia and mood disorders: preliminary findings. Arch Gen Psychiatry. 2000;57(4):349-56.

87. Arnold SE, Franz BR, Gur RC, Gur RE, Shapiro RM, Moberg PJ, et al. Smaller neuron size in schizophrenia in hippocampal subfields that mediate cortical-hippocampal interactions. Am J Psychiatry. 1995;152:738-48.

88. Law AJ, Lipska BK, Weickert CS, Hyde TM, Straub RE, Hashimoto R, et al. Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5' SNPs associated with the disease. Proc Natl Acad Sci U S A. 2006;103(17):6747-52.

89. Liang P, MacRae TH. Molecular chaperones and the cytoskeleton. J Cell Sci. 1997;110(Pt13):1431-40.

90. Arion D, Unger T, Lewis DA, levitt P, Mirnics K. Molecular evidence for increased expression of genes related to immune and chaperone function in the prefrontal cortex in schizophrenia. Biol Psychiatry. 2007;62(7):711-21.

91. Altar CA, Jurata LW, Charles V, Lemire A, Liu P, Bukhman Y, et al. De-ficient hippocampal neuron expression of proteasome, ubiquitin, and mitochondrial genes in multiple schizophrenia cohorts. Biol Psychiatry. 2005;58(2):85-96.

92. Vawter MP, Barrett T, Cheadle C, Sokolov BP, Wood WH 3rd, Donovan DM, et al. Application of cDNA microarrays to exam-ine gene expression differences in schizophrenia. Brain Res Bull. 2001;55(5):641-50.

93. Ben-Shachar D, Bonne O, Chisin R, Klein E, Lester H, Aharon-Peretz J, et al. Cerebral glucose utilization and platelet mitochondria complex I activity in schizophrenia: A FDG_PET study. Prog Neuropsychopharmacol Biol Psychiatry. 2007;31(4):807-13.

94. Ben-Shachar D, Zuk R, Gazawi H, Reshef A, Sheinkman A, Klein E. Increased mitochondrial complex I activity in platelets of schizophrenic patients. Int J Neuropsychopharmacol. 1999;2(4):245-53.

95. Ben-Shachar D, Karry R. Neuroanatomical pattern of mitochondrial complex I pathology varies between schizophrenia, bipolar disorder and major depression. PLoS ONE. 2008;3(11):e3676.

96. Prabakaran S, Swatton JE, Ryan MM, Huffaker SJ, Huang JT, Griffin JL, et al. Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress. Mol Psychiatry. 2004;9(7):684-97.

97. Ben-Shachar D, Zuk R, Gazawi H, Ljubuncic P. Dopamine toxicity involves mitochondrial complex I inhibition: implications to dopamine-related neuropsychiatric disorders. Biochem Pharmacol. 2004;67(10):1965-74.

98. Smalla KH, Mikhaylova M, Sahin J, Bernstein HG, Bogerts B, Schmitt A, et al. A comparison of the synaptic proteome in human schizophrenia and rat ketamine psychosis suggest that Prohibitin is involved in the synaptic pathology of schizophrenia. Mol Psychiatry. 2008;13(9):878-96.

99. Wang S, Fusaro G, Padmanabhan J, Chellappan SP. Prohibitin co-localizes with Rb in the nucleus and recruits N-CoR and HDAC1 for transcriptional repression. Oncogene. 2002;21(55):8388-96.