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NOVEL CANDIDATE GENES FOR TREATMENT RESPONSE TO ANTIPSYCHOTICS IN SCHIZOPHRENIA: EVIDENCE FROM PHARMACOGENETICS IN THE LIGHT OF PERSONALIZED MEDICINE

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Novel candidate genes for treatment response to antipsychotics in schizophrenia: evidence from pharmacogenetics in the light of personalized medicine.

Abstract

The treatment of schizophrenia is based primarily in the use of antipsychotics, however the biological mechanisms of action of these drugs need to be fully elucidated. The measurement of gene expression before and after treatment with an antipsychotic drug can help us understand the genetic mechanisms behind the clinical improvement of the patients, as well as to discern between the genes implicated in the metabolic side effects of antipsychotic drugs. In this review we have selected a series of studies that analyze gene expression from January, 2000 to March, 2015 and we have summarized and compared their results, differentiating between studies carried out on rodents, human blood, post-mortem and in-vitro. The results reflect an increase in the gene expression in a series of pathways including synaptic plasticity, apoptosis, neurotransmitter and lipid metabolism. The data obtained from these studies suggests the implication of an important amount of genes within the mentioned pathways, however the lack of similarities between the characteristics of these investigations make their comparison very difficult and therefore, more researches need to be carried out in order to corroborate these findings.

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Introduction

Schizophrenia is a complex and severe brain disorder which affects approximately 1% of the population and that contributes substantially to the global burden of disease (Whiteford et al. 2013), as it is a very debilitating mental illness that can have a profound, negative impact on the person's life course.

Typically, its clinical onset occurs in late adolescence and early childhood and it encompasses a number of areas of psychopathology: Positive symptoms, which are those that include delusions, hallucinations and thought and movement disorders;

negative symptoms such as lack of motivation, affective flattening, alogia or avolition; and finally cognitive symptoms such as alterations in the working memory and attention (Tandon et al. 2013). Additionally, nearly half of the people suffering from schizophrenia present a lifetime history of substance use disorders (Volkow 2009), and up to 40% of the excess premature mortality can be attributed to suicide and unnatural deaths (Hor and Taylor 2010)

The etiology of schizophrenia is a question which remains yet unresolved and although decades of research have not led to major discoveries, some progress has been made in clarifying the nature of the process. The conception that schizophrenia is a single disease with a specific cause can no longer be assumed and the contemporary view postulates that there is not a clear boundary, with multiple susceptibility genes interacting with environmental insults.

Efforts to understand schizophrenia have focused on the influence of the “dopamine hypothesis”, which is based on the efficacy of the first antipsychotics and has been one of the most enduring ideas in psychiatry. It was based on the observation that drugs that increased levels of dopamine could induce psychosis while those that decreased levels of dopamine could treat psychosis. Neuroimaging techniques later revealed a dopaminergic overactivity in the mesolimbic dopamine as a source of positive symptoms such as delusions and an underactivity in the mesocortical dopamine pathway as a mediator of the negative and cognitive symptoms of schizophrenia. A major current hypothesis suggests that NMDA glutamate receptors may be also involved in the illness, as a descending cortico-brainstem glutamate pathway acts as a brake on the mesolimbic dopamine pathway (via GABAergic neurons) and as accelerator to the mesocortical dopamine neurons. This hypothesis postulates these receptors could be hypofunctional, producing mesolimbic hyperactivity and thus inducing positive symptoms, along with excitation of the mesocortical neurons which would generate the cognitive and negative symptoms of schizophrenia (Siever and Davis 2004; Stahl 2007; Howes and Kapur 2009).

There are approximately 30 antipsychotic drugs approved in North America under two subgroups: typical and atypical antipsychotics, which essentially act on the D2 family of dopamine postsynaptic receptors, with atypical antipsychotics acting on a wider range of receptors. There is an important amount of evidence that supports neurotransmitter receptor abnormalities in psychiatric disorders and therefore, the treatment of schizophrenia is based primarily on the “dopamine hypothesis” although it has become clear that additional systems are required to explain the nature of the disease.

This narrow overview allows for many problems to arise due to the necessity of having to treat an illness with high incidence and prevalence, with a limited spectrum of pharmaceutical options and without a curative prospect on the horizon. Consequently, the use of antipsychotics represents an important expense for the public health system, as the natural trend of schizophrenia is to become chronic, and due to the fact that approximately 1/5 to 1/3 patients are treatment resistant. Additionally, out of those that benefit from the treatment, 54% experience adverse effects (Cascade et al. 2010). Typical antipsychotics exert an anti-dopaminergic effect on the nigrostriatal and tuberoinfundibular pathway producing extrapyramidal symptoms and increasing

prolactin levels. On the other hand, atypical antipsychotics have a multireceptor affinity which reduces these symptoms, however they present a different set of adverse effects concerning weight gain, diabetes mellitus or hyperlipemia leading to an increased risk of cardiovascular disease (Uçok and Gaebel 2008).

The indisputable need for an alternative strategy that allows us to provide a personalized treatment for every patient, thus reducing the rate of treatment failure and important side effects, prompts us to turn to gene expression profiles. Considerable evidence obtained by imaging and genetic studies suggests that the clinical effects of these drugs are due to changes in gene expression and, consequently, an increasing amount of studies have investigated the changes in expression of the genes which are thought to play a major role in the appearance of the mental disease before and after antipsychotic treatment.

These drugs may act at different levels: directly on the genes that are implicated in the illness; they can act on other genes in the same or similar pathways; and finally on the mechanisms that concentrate on the same output systems (neurotransmitters) (Thomas 2006). The latter may be the most important as it is the one with more influential effects on the clinical outcome, however, expression changes may be also responsible for the side effects that usually appear when using antipsychotic treatment.

Although it is not a prevalent topic and the investigation data is still very limited, this allows us to investigate furthermore the genes which are implicated in schizophrenia, but it can also help us understand the correlation between gene expression and the clinical manifestations of a patient, including side effects. It will hopefully throw some light on the matter, as the results can be used as future prediction models of drug response, which will take us a step closer to personalized medicine.

Method:

In preparation for this revision, a literature search was conducted. This review focuses on the period January 2000 to March 2015, because it has been during this period of time that mRNA expression has become an alternative in psychiatrics and this meant that older studies were excluded. Pubmed/MEDLINE, ResearchGate, ScienceDirect and EMBASE were searched using *schizophrenia*, *gene expression*, *antipsychotics* as keywords combined with *microarray and sequencing*.

These studies were screened for inclusion through a review of the abstract and the title. Studies that investigated specifically epigenetic changes such as methylation, chromatin remodeling, phosphorylation, etc. were excluded, as were studies that focused on neuroimaging, specific genetic diseases or other psychiatric diseases different to schizophrenia and those that used antipsychotic drugs that were still in development. Articles that had the following characteristics were included: 1) at least one group receiving typical or atypical antipsychotics with no combinations 2) quantitative measurements of mRNA expression results, 3) human studies were performed on schizophrenic patients with no other mental diseases

Results

Our literature search produced 43 articles which seemed to be potential candidates for inclusion in the analysis.

In this review we classify the studies that analyze gene expression before and after treatment under different headings depending on the nature of the samples they use: human blood, post-mortem human cerebral samples, rats (blood and post-mortem cerebral samples) and in- vitro.

Firstly we have to point out that there are remarkable differences between the studies, as these have different selection and exclusion criteria. When we add the differences in doses, modes and periods of applications of antipsychotic drugs or diversity between the species of laboratory animals, this allows for many important discrepancies to occur. Additionally, when studying gene expression in brain (postmortem and in rats) we have to bare in mind these genetic alterations may not be comparable from one region to another. In the same way, we cannot assume an antipsychotic drug will reproduce the same effect in two regions.

Finally, an important cause of variation between the studies is the methodology that has been used in each investigation. Most of them have been carried out using array based methods (cDNA and oligonucleotide microarrays and cDNA filter arrays) and polymerase chain reaction like TOGA and ATAC-PCR. Although this methods are very precise, the output genes varies greatly between methodologies.

1. Studies on Rodents

In recent years, the analysis of the transcriptome has allowed the study of the effects of antipsychotic drugs in rodents. However, there are a number of difficulties that arise from the use of rodents in these studies.

In first place, we have to keep in mind the differences between the brain of a rodent and the human brain. The complexity of a human brain compared to that of the rodent brain is indisputable, and therefore the arrangement of neuronal connections and dopaminergic pathways may be considerably different. To extend this point, many rodent studies analyze gene expression in the striatum, due to its implications in schizophrenia and neuroleptic therapy. This area is considerably different from the one in humans and this could potentially alter what we can infer from rat studies.

Since the introduction of genetics in the psychiatric field we have obtained an important amount of evidence that associates certain areas or functions with the pathology of schizophrenia. Recently, with the study of antipsychotics, we are starting to observe that these drugs exert their effects by acting on the same systems counteracting the pathologic effects. Therefore, we can divide the results into various categories depending on the biological mechanisms implicated: synaptic function, myelination, ubiquitination, lipid metabolism and insulin resistance, neurotransmitter function, apoptosis and extrapyramidal side effects (as a general concept that englobes various pathways).

To facilitate the understanding of this review, rodent studies have been summarized in Table 1.

Table 1. mRNA expression studies after antipsychotic effect on rodents from 2000-2015

Author	Year	Relevant genes (Gene ID)	Drugs	Brain area	Duration	Technique
Thomas et al.	2001	ApoD	Clozapine, Haloperidol	Striatum	45min, 7h, 24h	TOGA analysis, in situ hybridization
Chong et al.	2002	Syn2, Adora2a,	Haloperidol	Striatum	28 days	cDNA array, immunoblot
Kontkanen et al.	2002	Rab3a, Stx1a, 3,4, 5, Chga, Syt5, Syp	Clozapine	Prefrontal Cortex	1, 6, 24h	cDNA array, in situ hybridization
Kontkanen et al.	2002	Fos, Fosb, Jun, Junb, Jund	Clozapine, haloperidol	Prefrontal cortex	2h, 24h, 6 days, 17 days	In situ hybridization
Polese et al.	2002	Homer1, Fos	Clozapine, Haloperidol, D-cycloserine	Striatum	90min	In situ hybridization
Bartolomeis et al.	2002	Homer1	Haloperidol, Olanzapine	Striatum	3h	In situ hybridization
Thomas et al.	2003	Osbp18, Chn1, Lpcat, H2-Ke6, ApoD	Clozapine, Haloperidol	Striatum, Frontal cortex	24h, 5 days, 12 days, 2 weeks	TOGA, real time PCR, in situ hybridization
Lipska et al.	2003	Drd2, Drd3, Nts, Penk, Pdyn, Gad67	Clozapine, Haloperidol	Prefrontal cortex, Striatum.	28 days	In situ hybridization
Zink. et al.	2004	Gabra, Gad67	Clozapine, Haloperidol	Striatum, prefrontal cortex, Anterior cingulate cortex, Parietal, Temporal cortex.	6 months	In situ hybridization
Chen et al.	2004	Maob, Prkc, Polyubiquitin, Calpain, Cathepsin	Risperidone	Frontal cortex	4 weeks	Microarray, RT-PCR
MacDonald et al.	2005	Stx1a, 1b2, 4, Syn2, Vamp1, 2, Syp, Rab3a, Snap-25	clozapine, Haloperidol	Frontal cortex	26 days	Array, real time RT-PCR
Iwata et al.	2005	Ube2e1, Uchl5, Uchx4, Ube2b	Haloperidol, Biperiden	Frontal cortex	14 days	ATAC-PCR
Jennings et al.	2005	Fos	Ziprasidone	Forebrain	2, 4, 6 hours	Immunohistochemistry
Fehér et al.	2005	Ubc, Syt7, Glur2	Haloperidol, Risperidone	Rat cortex	96h, 4 weeks	Microarray, hybridation, QRT-PCR
Sondhi et al.	2005	GIP, Rab3d	Clozapine	Striatum	28 days	cDNA array, RT-PCR
Fatemi et al.	2006	Calb3, Homer1, Irs2, Pklr, Reln	Olanzapine	Frontal Cortex	21 days	DNA-microarray, Real time quantitative RT-PCR
Minet-Ringuet et al.	2007	Glut1, 4, Lep, Mmp9, Hsl, Fas	Haloperidol, Olanzapine, Ziprasidone	White adipose tissue	5 weeks	RT-PCR

Table 1. mRNA expression studies after antipsychotic effect on rodents from 2000-2015 (continuation)

Narayan et al.	2007	Mbp, Plp, Cnp, Ugt8, Cldn11, Trf, Mog, Mag	Haloperidol, Clozapine	Striatum, frontal cortex, hippocampus	30 days	In situ hybridation
Segnitz et al.	2009	Vglut1, Eaat1, 2, 3, 4	Aripiprazol	Prefrontal cortex	4 weeks	In situ hybridation
Iasevoli et al.	2009	Homer1a	Terguride, Haloperidol	Striatum, Cortex, Hippocampus	90 min	In situ hybridation
Iasevoli et al.	2010	Homer1a	Haloperidol, Risperidone, Olanzapine, Sulpiride	Striatum	90 min	In situ hybridation
Iasevoli et al.	2010	Homer1a, Psd95, m-GluR5, Nr1, Arc.	Haloperidol, Sertindole	Cortex, striatum	90min	In situ hybridation
Segnitz et al.	2011	Nr1, Nr2a, Nr2c, Nr2d	Aripiprazole	Cortex, hippocampus, striatum, thalamus	4 weeks, 4 months	In situ hybridation,
Peselmann et al.	2012	Gad67	Aripiprazole	Frontal cortex, Hippocampus	4 weeks, 4 months	In situ hybridation
Fatemi et al.	2012	Bcl2l1, Comt, Arrb2, Mog, Mbp, Mobp, Gabrβ1, Gad65	Clozapine, Haloperidol	Frontal cortex	21 days	Microarray, QT-PCR
Rizig et al.	2012	Syt1 Sytl4, Synj2, Stx5a, Synpo, Snap25	Haloperidol, Clozapine	Global	12 weeks	cDNA array hybridisation
Kursungoz et al.	2015	Gapdh, Pomc, Npy, Agrp, Cart	Risperidone,	Hypothalamus	4 weeks	QRT-PCR, ELISA

1a. Synaptic function

The evidence we have up to date suggests schizophrenia is a neurodevelopmental disorder, that usually presents in adolescence as a consequence of events that occur in early development, and that synaptic alterations may play a critical role in the development of the disease (Faludi and Mirnics 2011). Feinberg was the first to suggest in 1982 that schizophrenia could be a result of an altered synaptic machinery, and although there is still much to investigate, alterations of gene expression leading to synaptic dysfunction could be an underlying mechanism of action of antipsychotic drugs.

A group of genes that have been found to have an altered differential expression before and after treatment in rat cortex are those that form the SNARE complex (N-ethylmaleimide-sensitive factor attachment protein factor).

This complex is composed by syntaxin (a protein enriched in presynaptic terminals), the synaptosome-associated protein 25kDa (SNAP-25) and the vesicle-associated membrane protein (VAMP). The formation of the SNARE complex brings vesicles and presynaptic membranes together so that fusion with the neurotransmitter can occur. The SNARE proteins also interact with other proteins such as calcium sensors, dysbindin (which modulates the expression of SNAP-25) or synapsin (Johnson et al. 2008). The synapsin family is a group of phosphoproteins (synapsin I, II and III) involved in the

regulation of synaptic vesicle release. Additionally, synapsin II also contributes to nerve growth and formation of synapses, as synapsin IIa is involved in axon elongation and IIb mediates the formation of presynaptic nerve terminals. Phosphorylation of these phosphoproteins releases a pool of synaptic vesicles making them available for neurotransmission.

There is an important amount of human studies that reveal changes in the expression of presynaptic proteins in schizophrenia patients (Mirnics et al. 2000). The direction of the expression changes varies between brain regions, but there certainly are alterations in the SNARE-mediated functions, which has led to the design of specific synaptic function gene expression studies in rats.

In 2002 one of the first studies that assessed gene expression in cerebral rat cortex before and after treatment was carried out by Chong et al. using haloperidol. This study measured cDNA for more than 100 genes involved in schizophrenia after chronic haloperidol administration, and found 14 genes altered, among which was synapsin II (normally down-regulated in schizophrenic patients). Haloperidol caused an increase of this gene (synapsin IIA and IIB), and while atypical antipsychotic activity could not be assessed, Kontkanen et al. carried out a similar study in 2002 in which no change in synapsin II expression was observed. The enhancement of synapsin expression by haloperidol could be result of dopaminergic regulation, as dopamine receptors regulate synapsin phosphorylation in the striatum and it has been proven that haloperidol alters dopaminergic function as it is a D2 antagonist. Haloperidol also regulates the expression of other genes like tyrosine hydroxylase, which can indirectly affect the levels of synapsin. In other words, haloperidol may target a core deficit of synapsin II in schizophrenia but due to the variations of gene expression throughout the brain further studies must be performed to verify this hypothesis.

Many other alterations in the SNARE complex can be found. In a study carried out by MacDonald et al in 2005, VAMP1, VAMP2, syntaxin 1A and SNAP 25 were up-regulated in the frontal cortex by clozapine and haloperidol as opposed to the reduced mRNA expression levels found in rats before the treatment. The up-regulation of these presynaptic genes, when translated in terms of protein function, could increase the probability of vesicle fusion and therefore facilitate synaptic activity.

On the other hand, there are certain down-regulated genes in schizophrenia, that are not changed by typical antipsychotics, but that can be modified by atypical drugs such as clozapine. In the same study we mention previously by Kontkanen et al. in 2002, we find clozapine reduces the expression of genes that participate in the regulation of the synaptic machinery in the prefrontal cortex of rats, such as synaptophysin, synaptotagmin V and Rab3 a, which are known to have a reduced mRNA expression in schizophrenic patients. These genes encode proteins that regulate synaptic vesicle fusion and mobilization. Synaptophysin is involved in the formation of pores between secretory vesicles and plasma membranes, while synaptotagmin acts as a calcium sensor that responds to the influx of calcium and Rab3A is involved in vesicle recycling. E. Thomas, 2006 postulates that the regulation of the genes by clozapine in the same direction as schizophrenia represents a compensatory effect in response to a deficit, due to schizophrenia down regulation, of other synaptic genes such as synapsin II or VAMP.

Nevertheless, a recent study carried out by Rizig et al. in 2012 where synaptotagmin, synaptotagmin-like, synaptotagmin 2, syntaxin 5A and synaptopodin were also assessed, found an up-regulation of these presynaptic genes by clozapine but not by haloperidol. Therefore, although it is visible that clozapine has an influence on this particular presynaptic machinery, but due to controversial results on different studies no conclusions can be drawn. However, genes are being increasingly studied due to the accumulated evidence of their implication in mediating the effects of atypical antipsychotics.

Kontkanen's study was the first to investigate the expression patterns of other Ca^{2+} mediators other than synaptotagmin. Ca^{2+} mediated intracellular signaling affects many elementary processes in neurons including neurotransmission and the adequate release of synaptic vesicles. This includes genes such as chromogranin A, a calcium binding protein enriched in large dense core vesicles that controls vesicle formation and secretion (Kim et al. 2001). In this study, chromogranin A increased after acute administration of clozapine in the prefrontal cortex of the rat, and was down-regulated in the parietal cortex after long-term treatment. Other genes include VSNL-1,2,3, a family of neuronal calcium sensor proteins which have an altered distribution in schizophrenic patients and are down-regulated in response to clozapine treatment in all cortical areas examined in this study; or Calcineurin A (calcium dependent serine-threonine phosphatase), which besides from being implicated in synaptic function, neurotransmission, is also involved in the regulation of gene expression, protein kinase and function of DA receptors. This study suggests that Calcineurin might be induced by clozapine in the prefrontal cortex, corroborating the findings of (Rushlow et al. 2005) and (Gong et al. 1996).

As we will later explain, another gene that has an active role in synaptic activity is Reln, which produces Reelin as a protein product. This gene has been previously linked with other brain disorders apart from schizophrenia such as mood disorders and autism. Levels of this gene are down-regulated in rat models of schizophrenia (Fatemi et al, 2000), however, a study by Fatemi et al. in 2006 demonstrated an up-regulation of this gene in rat frontal cortex after chronic olanzapine treatment. This increase suggests that olanzapine could be modulating synaptic plasticity through reelin, as reelin is a glycoprotein that helps regulate processes of neuronal migration and cell to cell interactions. An interesting link exists between this gene and the glutamate pathway, as recent studies have shown that Reelin receptor (ApoER2) interacts with PSD95, a scaffolding protein in the synapse. This interaction is essential for the coupling of the reelin signaling complex to the NMDA receptors which also play an important role in synaptic plasticity, as opening of NMDA channels leads to a rise in post-synaptic calcium concentration which has been linked to long-term potentiation.

In conclusion, both classes of antipsychotic drugs (typical and atypical) have been found to reverse markers of pathology in schizophrenia. Drugs increased the brain volume and induced neuroplasticity through changes in the synaptic machinery by altering its gene expression in certain brain regions. As we can see, from the findings of the studies cited above not every type antipsychotic drug affects every presynaptic gene, and this can be explained because antipsychotic drugs do not induce changes in synaptic mechanisms directly, but rather modulate neurotransmitters which in ultimate terms will modulate

synaptic activity. Most antipsychotics modulate D2 receptor activity which can lead to glutamatergic signaling, and evidence points towards glutamate receptors playing an important role in synapse formation and stabilization. This hypothesis could indeed explain the differences between atypical and typical antipsychotic effects on synaptic machinery, as their affinity for dopamine receptors is not equal.

1b. Myelination

Another hypothesis, that arises from neuroimaging and postmortem studies, supports that altered neuronal connectivity could be due to an altered myelination. Many studies have provided evidence of oligodendrocyte abnormalities and imaging studies show a reduction in white matter volume as well as a reduction in the mRNA of certain genes involved in myelination (Pongrac et al. 2002) (Sugai et al. 2004).

Myelin is an electrical insulator that also facilitates conduction in axons. Oligodendrocytes supply the myelin for the central nervous system and its composition is approximately 40% water, with 70-85% of lipids and consequently a low proportion of proteins (15-30%). CNS myelin is rich in certain lipids, and although these may vary depending on the species, we can underline certain lipids such as cerebroside, sulfatide, cholesterol and phospholipids. On the other hand, the protein composition of myelin in the CNS includes the myelin basic protein (MBP), the myelin associated glycoprotein (MAG) and the proteolipid protein (PLP); as well as TRF which is involved in oligodendrocyte differentiation.

When studying the expression of the genes that code these components, although some studies like (Chong et al. 2002; Fasulo and Hemby 2003) do demonstrate gene expression response to antipsychotic drugs, these studies focus in gray matter regions. However, in 2001 a study by Thomas et al. analyzed the increases of apolipoprotein D expression in rodent brain, and provided evidence that apolipoprotein D was regulated by clozapine, but not by haloperidol. ApoD is an atypical apolipoprotein which participates in the transport of small hydrophobic molecules, and therefore, binds to sterols, steroid hormones and arachidonic acid, which suggests it may function as a transporter of lipids in the brain. ApoD may also act as a chaperone for the extra and intracellular transport of arachidonic acid when released from membrane phospholipids which prevents the toxicity that would result from its peroxidation, a possible cause behind the pathology of schizophrenia (Khan et al. 2002). Its increased expression when the rats are treated with clozapine may suggest that ApoD contributes to clozapine's antipsychotic benefits. Elevated levels of ApoD in oligodendrocytes induced by treatment could lead to stabilization of the membrane and proper myelin function which would reduce the negative symptoms, as white matter deficits have been associated with negative symptoms.

Posteriorly, in 2003, these findings have been corroborated in studies carried out by Khan et al, by measuring the expression of ApoD in response to the same antipsychotics. Khan also postulated that increased apoD with atypical antipsychotics could protect arachidonic acid from degradation, making it available in neuronal membranes contributing to an improved neurotransmission.

To continue, in 2007 a study by Narayan et al, the team found a reduction in the expression levels of certain genes after haloperidol treatment in corpus callosum,

anterior commissure and the internal capsule: MBP (myelin basic protein) and PLP (protelipid protein) which are components of the myelin membrane in compact myelin; UGT8 (UDP-galactose ceramide galactosyltransferase), which synthesizes galactocerebroside, TRF (transferrin) involved in oligodendrocyte differentiation; CLDN11 (claudin 11), a component of tight junctions in oligodendrocytes and MAG (myelin associated glycoprotein). The finding that the expression of 6 essential genes in myelin function is decreased by the use of haloperidol could be indicative that myelin dysfunction is a consequence of haloperidol treatment. There are various hypothesis that could explain this alteration, however scientists seem to agree on the possibility of it being due to the effect of haloperidol on the D2 receptors which are expressed in mature interfascicular oligodendrocytes. This idea gains strength by the fact that clozapine cannot induce these changes in myelin and oligodendrocyte gene expression, as clozapine has a lower affinity for D2 class receptors. This could be seen as the first demonstration that haloperidol has an effect on the expression of myelinating genes, and that it can exacerbate the preexisting deficits.

These findings seem to correlate with posterior studies carried out on human brain (Konopaske et al. 2008; Farkas et al. 2010). However, a recent study undertaken by Fatemi et al. 2011 found an upregulation of Mbp (myelin basic protein), mog (myelinating oligodendrocyte protein) and Mobp (myelinating oligodendrocyte basic protein) by chronic treatment with haloperidol. Mbp was also significantly increased by chronic olanzapine treatment.

The differences between the studies can be due to the different areas of the brain examined, as Fatemi et al. analyze the expression of the genes in the frontal cortex of the rats, while Narayan et al. limit to corpus callosum, anterior commissure and internal capsule. However, the general idea we can extract from this studies is that atypical antipsychotics seem to increase myelin related genes in rat brain, while the effect of typical antipsychotics seem contradictory. Authors postulate this could be due to a high-affinity D2 antagonism by haloperidol, however, another hypothesis we have studied in depth in the Lipid Metabolism section defends that increase in myelin related gene expression by clozapine could be due to its action on lipid metabolism, as myelin is composed primarily of lipids and clozapine seems to increase central (SNC) and peripheral (blood) lipid metabolism, leading to adverse effects.

1c. Ubiquitination

The ubiquitin proteasome system is a protein degradation system that has been associated with neuropsychiatric disorders such as Alzheimer's disease, bipolar disorder and schizophrenia (Lam et al. 2000, Rubio et al. 2013). Ubiquitin is a small regulatory protein encoded by a family of genes whose products are fusion proteins UBB, UBC, UBA52 and RPS27A. Its function is to carry out ubiquitination, a process where ubiquitin is attached to a substrate protein leading to its degradation via proteasome; or to alter its cellular location or promote/prevent protein interaction. In addition to ubiquitin, many related proteins have been described. They receive the name UBL modifiers and they have a similar 3D structure but have a divergent sequence. Among them we can find NEDD8 which regulates ubiquitin E3 ligases and leads to proteasome degradation; UFG1 and SUMO.

Microarray studies on humans have provided evidence of decreased protein ubiquitination, along with decreased ubiquitin and UBL activases and ligases which

suggests that an alteration of these processes may be an underlying cause of the pathology of schizophrenia.

When analyzing the effects of antipsychotics on the gene expression of the proteins involved in ubiquitination, some studies have proven key. The first study to analyze this effect was carried out by Thomas et al. in 2003 using rats, and it provided evidence of an increase in the gene of the ubiquitin-conjugating enzyme E2R (UBE2r2) and the ubiquitin gene (UBA52) in the striatum after treatment with clozapine or haloperidol. UBA52 maintained a high expression compared to controls in the cortex when treated with clozapine, while the ubiquitin conjugated enzyme E2R reduced its expression.

More evidence of Ubiquitination pathway alteration was obtained when Chen and Chen undertook a study in 2004 that exposed rats to 4 weeks of risperidone treatment and polyubiquitin was among the 17 of 1536 genes studied which were found to be up-regulated in the rat frontal cortex when compared to control animals.

Iwata et al. in 2005 used co-administration of haloperidol and biperiden (an antiparkinsonian agent of the anticholinergic type) to investigate the alteration of additional ubiquitin-related genes. These increased their mRNA levels (UBE2E1, UCHL5) while UCHX4 decreased in comparison with the saline-treated control. However, no significant differences could be appreciated in this group of genes after the administration of haloperidol alone. On the other hand, other studies like (Fehér et al. 2005) demonstrated a decrease of Ubiquitin C in rat cortex after risperidone treatment and in 2013, Rubio et al. found no differences in the expression in rats of any of the ubiquitin genes that were found altered in human experiments.

In global terms, these studies reflect that antipsychotic drugs may help normalize protein metabolism in the schizophrenic brain. Although the specific mechanisms by which these drugs may affect the ubiquitin system remain unknown, dendritic spine structure is dependent on the coordinated balance between protein synthesis and degradation which is one of the functions the ubiquitin system carries out. As this system has been found to be altered in schizophrenia, antipsychotics could be restoring the synaptic dysfunction present in schizophrenia. This idea is more reasonable when we learn that recent findings have linked the ubiquitin proteasome system with vesicle trafficking and trafficking of vacuoles in the endoplasmic reticulum and Golgi systems (Haas and Broadie 2008).

1d. Lipid metabolism and insulin resistance

Schizophrenia has been associated with cardiovascular disorders, making it the main cause of death due to the increased risk factors these patients have: obesity, arterial hypertension, metabolic syndrome and type 2 diabetes. Epidemiological data suggests that patients with this disease are more likely to suffer from sedentarism, high fat diet or smoking due to the inherent neglect of personal care. Not only does schizophrenia increase the risk of cardiovascular disease, but antipsychotic medication has also been associated with important weight gain, which is also considered an indirect pathway to diabetes through insulin resistance.

Although the risk of metabolic side effects differs from typical and atypical antipsychotics and within the same group, we still cannot completely understand the pathways involved in these processes. Studies that assess the effects of antipsychotics

in gene expression are very diverse, however some conclusions can be drawn when their data is compared.

As commented above, in 2003 Thomas et al. carried out a study which assessed the changes of 17325 mRNAs in rat striatum and frontal cortex after clozapine and haloperidol treatment. The most notable differences were observed in the lipid metabolism genes, especially those related with phospholipids and fatty acid signaling. OSBPL-8, an oxysterol binding protein, was the first gene to find altered. It binds to oxysterol which is a 27-carbon product of cholesterol, thereby regulating cholesterol and lipid homeostasis. This gene exhibited an increased expression both in the striatum and in the cortex by both clozapine and haloperidol.

Another gene which displayed an increase in the experiment after clozapine administration is ApoD, which we have mentioned when discussing the effects of antipsychotic drugs on myelination. It encodes apolipoprotein D which is structurally similar to the lipocalins, a family of lipid-binding proteins that are responsible for the transport of lipids, and it is increased in atherosclerosis, schizophrenia, Alzheimer's disease as well as in other risk conditions such as hypertension, obesity, dyslipemia, diabetes or smoking (Perdomo and Henry Dong 2009).

Furthermore, oxysterol levels have been shown to elevate apoD levels, which provides a link towards a common function of the two genes.

To end with this study, N-chimerin, LPA acyltransferase and steroid dehydrogenase ke6, also displayed an altered expression. N-chimerin belongs to a family of lipid receptors which act as GTPase accelerating proteins and enhance transduction of signals, similarly to LPA acyltransferase with whom it appears to have a common function.

Khan et al. carried out, in 2003, a study that also provides evidence of increased expression of ApoD after atypical antipsychotic administration and postulates that increased cholesterol synthesis that accompanies clozapine and olanzapine treatment may be a complication of peripheral cholesterol metabolism which develops as a consequence of increased CNS cholesterol synthesis and that may be vital to correct defective myelination, as cholesterol constitutes the most important myelin lipid.

Studies continue in 2005 with an investigation by Sondhi et al. where GIP (glucose dependent insulinotropic polypeptide) was analyzed. GIP is one of the most important incretins in the body which binds to its receptor and produces glucose-dependent insulin secretion, induction of β -cell proliferation and resistance to apoptosis. Additionally, GIP promotes energy storage by adipose tissue and enhances bone synthesis (Baggio et al, 2007). When the presence of glucose in blood is elevated, GIP stimulates the excretion of insulin from β cells and therefore its alteration has been linked to type II diabetes.

mRNA of this gene had not been detected before in brain tissue, however it had been shown to be expressed in pancreatic β cells, stomach or small intestine due to its function as an incretin (Usdin et al. 1993). In first place, this study provided evidence of GIP mRNA expression in rat striatum and describes the distribution of GIP and its receptor throughout the brain. Interestingly, GIP is co-localized with tyrosine hydroxylase which suggests a modulatory role in the brain.

In second place, in this study, the presence of clozapine increases levels of GIP mRNA in plasma and in the striatum, as well as the levels of glucose and insulin. This suggests that

antipsychotic drugs could lead to an increase of glucose resistance. The hypothesis is that as clozapine antagonizes α_2 adrenergic receptors, the physiological inhibition by which α_2 inhibits GIP response to glucose ingestion is lost. However, clozapine has an affinity for multiple receptors, and therefore the increase of GIP could be due to the activation of another receptor.

Following the chronological order, a study by Fatemi et al. in 2006 used microarray technology to investigate brain response to chronic olanzapine treatment in rats. This provided evidence of up-regulation of several genes including insulin 2, pyruvate kinase, CART, muscle glycogen phosphorylase and calpain 8. On the other hand, several metabolic genes were down-regulated like sucrose-isomaltase, UDP glycosyltransferase 2 and carbohydrate sulfotransferase 3. This leads to the hypothesis that chronic olanzapine treatment increases energy production in the brain with the objective of increasing glucose for local use as pyruvate kinase produces pyruvate and ATP, and glycogen phosphorylase is involved in glycogen degradation to glucose. This increase in glucose serves to increase insulin 2, which in ultimate terms could contribute to insulin resistance. Additionally, an interesting link has been discovered between pyruvate kinase polymorphism and type III diabetes, which could be related to the fact that olanzapine also increases the risk for diabetes in patients with schizophrenia.

In 2007 a study by Minet-Ringuet et al. that measured gene expression of several genes involved in trophic or metabolic adipocyte response to investigate if they were related to the obesity that antipsychotic drugs have as a side effect. Among the antipsychotics studied, olanzapine was the only drug that significantly altered gene expression of FAS (fatty acid synthetase) and HSL (hormone-sensitive lipase), up-regulating FAS and decreasing the latter. These two genes could be related to the adipocyte hypertrophy which was only seen after the treatment with olanzapine, as opposed to haloperidol and ziprasidone. In other words, olanzapine administration could decrease lipolysis in adipocytes and induce fatty acid synthase, which could result in fat accumulation in rats.

Finally, in 2015 Kursungoz et al investigated the effect the administration of risperidone on rats on gene expression. This study measured the food intake, weight gain, leptin levels and finally gene expression of hypothalamic peptides such as NPY, POMC or CART. These peptides are thought to have several functions including increasing food intake and storage of fat. Gene expression of POMC (proopiomelanocortin) and NPY (neuropeptide Y) were significantly decreased in the risperidone group, while CART (cocaine and amphetamine regulated transcript) expression was up-regulated, corroborating the findings of Fatemi et al. in 2006. The author postulates that serotonergic antagonism could explain the reductions of POMC and NPY as these genes are regulated by the serotonergic system. Dopaminergic and histaminergic neurons effects were also considered, leading to another possible hypothesis where atypical antipsychotics (which have an important effect on weight gain) could increase appetite stimulation by activation hypothalamic factor AMP kinase which is normally blocked by histamine-1 receptor. This suggests that risperidone, using different pathways could affect body weight not only by altering mRNA expression of genes involved in lipid metabolism but also by affecting genes that encode neuropeptides in charge of appetite regulation.

These findings support the idea that lipid metabolism is indeed altered by the administration of antipsychotic drugs. When comparing the effects of both of types of antipsychotics, most of the studies investigate the effect of atypical drugs in lipid metabolism and only Thomas et al. in 2003 reviews the effects of haloperidol. This does not permit comparison between the two groups, however we can conclude that atypical antipsychotics can alter lipid metabolism and can produce insulin resistance. Many genes and pathways could be involved in this dysregulation and therefore we cannot point to a specific mechanism but to a combination of alterations in gene expression.

1e. Dopamine/GABA/Glutamate function

Dopamine

It is a widely assumed as a result of years of investigations that there are alterations in the dopamine and GABA systems, and that several components of these neurotransmitter systems can be modulated by antipsychotic treatment. The latter has been studied by following in time the clinical improvement of the patients, as well as anatomical and electrophysiological changes in animal models of the disease. However, it is only recently that studies have started to analyze functional changes in these systems by measuring mRNA before and after treatment.

The first study that starts using this method is carried out in 2003 by Lipska et al. where they observe the changes in the gene expression of dopamine receptors that occur after the use chronic treatment with antipsychotic drugs in neonatally lesioned rats, a popular model of schizophrenia in these animals. However, this study concludes that the expression of dopamine receptors was not changed in response to antipsychotic treatment, but neither was the profile of the expression modified by the neonatal lesion.

However, in 2005, a study conducted by Chen and Chen, assessed gene expression changes in rats after 4 weeks of risperidone administration. One of the genes which presented an altered expression after the treatment was Monoamine oxidase B. The protein encoded by this gene catalyzes the oxidative deamination of biogenic amines, including dopamine. Various studies dating from 1970 have reported a reduction of MAOB activity in platelets after haloperidol treatment, which contradicts the findings in this study where monamine oxidase expression was increased after risperidone treatment. The hypothesis is that MAOB gene expression could be increased to compensate the inhibitory effect of risperidone on MAOB activity. This could suggest that risperidone may also affect the genes involved in the degradation of the biogenic amine neurotransmitters.

Another interesting finding comes with the alteration of several genes in a study conducted by Fatemi et al. in 2011 when expression of COMT, a gene that encodes an enzyme with the same name (Catechol O-methyltransferase), was found altered after treatment with clozapine and haloperidol. COMT is one of the enzymes that degrade catecholamines, including dopamine and previous studies have also identified this enzyme as being altered in schizophrenia (Goghari and Sponheim 2008). This study shows a down-regulation of this gene in prefrontal cortex, which can be understood if we bear in mind our knowledge of the dopamine hypothesis where dopamine has a lower

activity in schizophrenia, and therefore these drugs could be increasing dopamine and reducing positive symptoms.

Both of these studies suggest that antipsychotic drugs could be affecting the expression of enzymes in charge of regulating the concentrations of dopamine in schizophrenia, as well as directly antagonizing D2 receptors.

GABA

Alterations in the metabolism of GABA in postmortem brain samples in human and rats has also been repeatedly reported since 1987. In 1995, a study by Akbarian et al., provided evidence of a reduced expression in the prefrontal cortex of schizophrenic patients of GAD67, a GABA synthesizing enzyme, which could indicate the loss of GABAergic neurons. Dopaminergic and glutamatergic neurons converge onto GABAergic neurons in the prefrontal cortex, therefore a dysregulation at this level could be key in the pathophysiology of schizophrenia. In 1995, mRNA expression of GAD67 was analyzed by Delfs et al. displaying an increased expression in striatum after haloperidol treatment (but not with olanzapine), as well as a reduction in the globus pallidus, which was later contradicted by a second study the same group carried out in the same year.

In 2003, (Lipska et al. also analyzed the expression of GAD67, in the prefrontal cortex and the striatum of adult rats before and after treatment. The findings demonstrate an increase GAD67 mRNA in the prefrontal cortex and striatum after haloperidol treatment, but not by clozapine administration, whereas in the nucleus accumbens both drugs increase the expression. Zink et al. in 2004, carry out a similar study where an increase in the expression of GAD67 could be observed after clozapine or haloperidol administration in the infralimbic cortex. Additionally, the gene for the GABA_A receptor was also analyzed in this study, and the results presented an increased expression in the striatum, accumbens, infralimbic nucleus and in the anterior cingulate cortex with haloperidol and clozapine, as well as a decrease in expression in the parietal and temporal cortex with haloperidol.

These findings suggest that increase in GAD67 may have therapeutic value, as the reduction of GAD67 expression in schizophrenia may be explained by reduced dopamine in the prefrontal cortex as cortical GABA neurons are under the stimulatory control of the dopaminergic neurons of the ventral tegmental area. This suggests that antipsychotic drugs increase GABA expression by increasing dopamine in the prefrontal cortex.

A recent study from 2013 by Peselmann et al. shows GAD67 increased after 4 months of treatment with aripiprazol (a partial dopaminergic and serotonergic receptor agonist) in hippocampus, amygdala and cerebral cortex, as opposed to post-mortem studies that present a reduced expression of GAD67 in several areas of the brain. On the other hand, the same study shows a suppression of GAD67 that was observed in subcortical and frontocortical regions. This could be the result of the partial agonism of aripiprazol, which acts as a functional antagonist in the mesolimbic dopamine pathway and shows an agonist activity in the mesocortical pathway.

As we can see from the results above the data available from the different studies is quite ambiguous due to the differences in periods of applications, laboratory animals and doses. It is not clear whether or not these genes increase or decrease GABA activity

as a whole. However, a constant finding is that GAD67 is induced by first generation antipsychotics (haloperidol, sulpiride) in basal ganglia while second generation antipsychotics like clozapine and olanzapine decrease or do not alter the expression of GAD67. This leads us to think GAD67 is regulated by the dopaminergic system, as typical drugs have a high affinity for dopamine receptors. It appears that aripiprazol exerts different effects in the mesolimbic and mesocortical system which can be considered beneficial as region specific effects could indeed be an answer to the treatment of schizophrenia if we apply the dopamine hypothesis.

Glutamate

The glutamate pathway, which has also been implicated in schizophrenia, also has altered genes after exposure to antipsychotic treatment. Glutamate is one of the major excitatory neurotransmitters in the brain. Many post-mortem studies have evaluated the changes of the genes related to glutamate in schizophrenic patients (Ohnuma et al. 1998, Smith et al 2001, Matute et al 2005, Eastwood et al, 2005). However, when analyzing mRNA expression after antipsychotic treatment animal studies remain necessary.

In 2003 a study Zink et al. , analyzed mRNA expression of EAAT2 in rats after 6 months of haloperidol or clozapine treatment. EAAT are excitatory aminoacid transporters that control glutamate concentrations in the synaptic cleft by regulating reuptake into the neurons (Danbolt et al, 2001). In this study we observed suppressed expression of EAAT2 and EAAT 3 in hippocampus and cortical subregions. These findings were confirmed in 2006, in a study by Bragina et al where EAAT2 mRNA was down-regulated in the frontal cortex of rats after 9 weeks of clozapine treatment. This suggests one possible molecular mechanism by which clozapine and haloperidol increase cortical glutamate levels. In this study, vGluT expression (vesicular glutamate transporters that participate in the presynaptic assembly of glutamate into synaptic vesicles) was not altered, however other studies like those carried out by Moutsimilli et al, in 2005 and 2008 reported clozapine increased the expression of vGluT1. Furthermore, in 2009, a study was carried out by Segnitz et al. which studied these genes and their response to an atypical antipsychotic: aripiprazol. They reported a transient induction of vGluT1, suppression of the glial transporters EAAT1, EAAT2, EAAT3 and EAAT4.

As we can see, all these studies provide evidence that the suppression of the glial transporters is present in studies that use haloperidol, clozapine and aripiprazol, and a possible hypothesis for these changes suggests that antipsychotics might reduce glutamate clearance from the synaptic cleft and therefore increase the glutamate concentrations potentiating glutamatergic neurotransmission.

In the study by MacDonald et al. in 2005, transcripts of the glutamate system also showed dysregulation, however the genes seemed to be altered in different directions and no conclusions can be made from this data.

In 2011, Segnitz et al. investigated the effects of aripiprazole on the expression of NMDA subunits, which demonstrated that aripiprazol affects the expression of these genes. It induced NR1, NR2A, NR2C and NR2D and suppressed NR2B. It is important to bear in mind aripiprazol interacts with serotonergic and dopaminergic receptors, however it has a low affinity for glutamate receptors. The author proposes a regulation of the NMDA receptors by dopaminergic D1 and D2 receptors as well as serotonergic 5HT1A receptors

to explain these alterations, based on the findings of Wirkner et al in 2004 where the stimulation of D1 receptors increased NMDA currents in rat prefrontal cortex and this improved cognitive deficits induced by phencyclidine.

Homer

Increasing evidence has been provided of the modulation of the glutamatergic systems by antipsychotics. However, not many studies have emphasized the importance of Homer, a postsynaptic density protein located at the excitatory synapse which interacts with the C-terminal intracellular tail of group 1 metabotropic glutamate receptors-mGluRs (Polese et al. 2002). Due to its implication in the modulation of glutamate receptors we have decided to include a brief analysis of this gene under this section.

Previous studies have demonstrated that Homer expression is linked to D2 antagonism, and therefore a link could exist between Homer expression and the degree of D2 antagonism an antipsychotic drug has. An important molecule that mediates the function of Homer is PSD-95 scaffolding protein that couples mGluRs + Homer to NMDA receptors. Previous studies have also linked PSD-95 function to schizophrenia.

The effects of antipsychotics in the expression of Homer were only recently studied by de Bartolomeis et al. in 2002 where the acute effects of haloperidol and olanzapine on rat brain were assessed. This study provided evidence of Homer gene expression being significantly increased in caudate-putamen subregions as well as accumbens in haloperidol treated rats, while only in the core of accumbens for olanzapine treated rats. In the same year, Polese et al. corroborated this findings by analyzing chronic exposition to the same drugs.

The results of these studies indicate a modulation of Homer gene expression by antipsychotic drugs, although variations between atypical and typical drugs should not be undermined. Haloperidol presents a more pronounced action, while olanzapine has a discrete effect, limiting its actions to the core of the accumbens which could be explained by the limbic selectivity of this compound correlating with its low incidence of extrapyramidal effects (de Bartolomeis et al. 2002). Studies have also shown that Homer proteins interact with PSD95, a protein that has been demonstrated to cause NMDA glutamate receptor clustering (Kornau et al. 1995; Tu et al. 1999), therefore Homer may indirectly modulate NMDA function.

In 2006, a study by Fatemi et al. showed an increase in the levels of Homer1 after chronic treatment with olanzapine. Thus, it appears that haloperidol, clozapine and olanzapine could be indeed increasing levels of Homer, with the objective of modulating the glutamate system.

Finally, in 2010, a study by Iasevoli et al. , demonstrated an increase in the transcripts of Homer after chronic haloperidol treatment in the striatum, an increase in mGluR in the striatal and cortical subregions and an increased expression in the PSD-95 gene. Homer expression again appears to be higher for compounds such as haloperidol that have high affinity for D2 receptors, compared to other atypical antipsychotics such as clozapine.

The same group of investigators carried out a similar study to compare haloperidol with other antipsychotic drugs and in total, the results provided evidence that doses of these

drugs that potentially impair motor behavior, induced Homer in the lateral striatum, which receives connections from the corticostriatal and the mesostriatal pathway and has the function of controlling the motor performance. Additionally, the difference between Homer expression when using haloperidol compared to other atypical antipsychotics was similar to the findings obtained by Bartolomeis et al and Polese et al.

This supports the hypothesis that Homer is induced by blockade of D2 receptors and that induction of Homer could have a role in motor side effects by these antipsychotic drugs (Iasevoli et al. 2010). This could also explain the differences between the expression of homer when using haloperidol compared to other atypical drugs. This could be highlighting a hidden relationship between dopamine receptor antagonism and the modulation of the glutamatergic function.

1f. Apoptosis

The apoptosis pathway is another mechanism that has been proposed to be involved in the pathogenesis of schizophrenia. While schizophrenia is considered a neurodevelopmental disorder the clinical deterioration and neurostructural changes have led to the hypothesis that an alteration of the apoptotic mechanisms could be behind them.

As with other pathways we have reviewed, the apoptotic implication in schizophrenia has been repeatedly studied (Jarskog et al., 2005; Jarksog et al., 2006; Boyajan et al., 2013), however there are not many studies that assess the effect of antipsychotics nor the effect on DNA expression.

In 2005, Chen and Chen 2005 analyse the effect of risperidone on a series of genes and find an alteration in the expression of certain genes involved in apoptosis. One example of this genes is Amida, which encodes an associated protein of Arc, a non-transcriptional immediate early gene specific to brain (Irie et al., 2000). It is not until recently that there has been evidence of an association between overexpression of Amida and apoptosis. However when contranfectated with Arc, Arc can interfere with the apoptosis triggered by Amida. This study found that chronic risperidone treatment induced Amida in rat cerebral cortex, and although this needs further study and clarification, it could suggest that risperidone may change synaptic plasticity by regulation of apoptosis.

Additionally, other 2 protease- related genes were found to be up-regulated by risperidone in this study: Calpain 2, cathepsin D. Calpains belong to the family of calcium-dependent non lysosomal cysteine proteases and although their physiological role is poorly understood, they are thought to participate in cell mobility and cell cycle progression by remodeling of cytoskeletal and membrane attachments. In 2003 Ray et al., linked calpain activity with neuronal apoptosis. On the other hand, Cathepsin D encodes a lysosomal aspartyl protease that also plays a role in mediating apoptosis.

The up-regulation in this study of 3 genes related to proteases suggests that long term use of risperidone could affect proteolysis in the brain, however this association needs to be investigated in more studies.

In 2006, He et al, studied the expression of another apoptotic gene Bclx. This gene has to splice variants Bclxl which has an anti-apoptotic effect, and Bclxs with a pro-apoptotic effect. This study found that in a rat model of schizophrenia using phencyclidine the

ratio of Bclxl to Bclxs was reduced, increasing apoptosis with rats displaying an impaired spatial memory. However, the administration of quetiapine reversed the effect and improved the symptomatology, which could suggest a neuroprotective effect. A recent study carried out in 2012, by Fatemi et al, corroborates these alterations.

1g. Extrapyramidal side effects

Long term treatment with typical antipsychotic drugs has been associated with the development of extrapyramidal side effects such as tardive dyskinesia as a result from prolonged blockage of dopamine receptors in the striatum. These side effects affect approximately 60% of the patients that take these type of drugs. Based on this idea, authors tend to hypothesize that genes with predominant expression in the striatum are more likely to be the ones associated with the motor alterations than those that are expressed ubiquitously throughout other brain regions.

In a study by Thomas et al. in 2003 clozapine and haloperidol were administered before the measurement of gene expression in rat striatum. The results revealed a predominant expression of PDE1B (phosphodiesterase 1B), striatin and oxysterol. While striatin was only found to be elevated by haloperidol treatment, PDE1B and OSBPL-9 were elevated by both drugs, although greater changes were observed with haloperidol. Increases and decreases of these genes in the striatum could lead to extrapyramidal side effects and if that was the case, they could be targeted by other drugs to inhibit this tardive dyskinesia, however this is only a hypothesis and evidence of the link between this genes and the side effects remains unavailable.

On the other hand, high-throughput gene studies have revealed a consistent elevation in the mRNA of Fos in response to antipsychotics, and it is a widely accepted idea that Fos could be related to the extrapyramidal side effects.

The Fos family consists of 4 members: Fos, FosB, FosL1 and FosL2, and they encode leucine zipper proteins that can dimerize with the JUN family (Jun, JunB and Jun D) to form a transcription factor also known as AP-1. Therefore, Fos proteins are regulators of cell proliferation, differentiation and transformation, as well as apoptotic cell death (RefSeq, 2008). Fos is also known as an immediate gene which rapidly increases in areas of neuronal activation, which has led to its use in studies as a marker and proposed as a predictor of therapeutic response and motor side effects.

Many studies have analyzed the effect of acute haloperidol and clozapine treatment during the 1990's and it is widely accepted that both typical and atypical antipsychotics increase Fos in the nucleus accumbens. Additionally, some atypical drugs may also increase its expression in the prefrontal cortex, and similarly some typical drugs like haloperidol up-regulate the gene in the striatum which could produce extrapyramidal side effects by blockade of D2 receptors, however this is not made completely clear by studies that investigate acute administration of these drugs because clinical antipsychotic effect develop slowly over weeks of treatment (Merchant and Dorsa 1993; Robertson et al. 1994), and there are only a few studies that investigated the effect of chronic treatment in Fos expression.

In 2002, Kontkanen et al. studied by in situ hybridation the expression of fos and jun family genes following chronic treatment with haloperidol and clozapine. When administering Clozapine a marked induction of the fos and jun family gene was detected

in various parts of the rat forebrain (caudate, accumbens and cortical areas), while haloperidol administration led to increases of fos and jun family in cortical regions, putamen, and frontal cortex, and contrary to other previous published studies there was a discrete increase of c-fos expression in the PFC.

A study by Jennings et al. in 2006, extends the study of c-Fos expression. In this case, it is measured after acute administration of Ziprasidone, a recent atypical antipsychotic. C-Fos expression was induced in nucleus accumbens, corroborating the findings of previous studies with typical and atypical antipsychotics. Posteriorly, increases were also observed in rostral and caudal nucleus, lateral septum and cingulate gyrus. C-fos induction in the accumbens nucleus has been linked previously to clinical efficacy while response in the putamen has also been linked to the propensity to cause motor side effects (Deutch et al. 1992)

2. Human postmortem studies

The first postmortem brain studies using microarray techniques started in 1999, years after the first successful microarray experiments which took place in 1994. This was due to the fact that while other diseases could use samples from living patients, most of the psychiatric diseases required brain tissue, which had to be extracted post-mortem and therefore quality of the sample was an issue. Additionally, in most brain diseases, only a fraction of the cells is affected by a disorder, which led to misleading results.

Many of the studies we have obtained through the bibliographic ([Table 2](#)) research investigate altered genes in schizophrenia and not many analyze the repercussion of antipsychotic treatment, these made the amount of studies obtained comparatively much less in number compared to the ones carried out in rats.

Table 2. Human post-mortem studies evaluating mRNA expression after antipsychotic treatment from 2000-2015

Author	Year	Patients	Antipsychotic drug	Duration of the treatment	Relevant Genes	Tissue	Technique
Urigüen et al.	2009	31 schizophrenia, 46 control	Quetiapine, olanzapine, clozapine, risperidone, levopromazine, haloperidol	Not specified, long-term treatment	A2A, D2, CB ₁	Frontal cortex	Quantitative real-time PCR
Choi et al.	2009	50 schizophrenia, 34 controls	Phenothiazines, haloperidol, olanzapine, risperidone	Long term	CYP7A1, CEBPA, AR1, ABCG5, CYP51A1, FOSL2, SOD2, IL1RN	Liver	Microarray and quantitative PCR

Table 2. Human post-mortem studies evaluating mRNA expression after antipsychotic treatment from 2000-2015 (continuation)

Schmitt et al.	2010	10 Schizophrenia, 9 control	Clozapine, zotepine, olanzapine, flupenthixole, haloperidol, perphenazine, pipamperone, promethazine, tiapride, zuclopenthixol	Long term (mean: 35 years)	NR1, NR2A, NR2B, NR2C, NR2D, NR1	Cerebellum	In-situ hybridation
Tan et al.	2014	35 schizophrenia, 35 controls, 35 bipolar	Dopamine, olanzapine	Long- term	Synapsin II	Dorsolateral prefrontal cortex	RT-PCR

The first studies we obtained investigated dopamine and other neurotransmitters in the frontal cortex of patients that had undertaken antipsychotic treatment. This is the case of the study carried out by Urigüen et al. in 2009 where they studied the expression of A2A, CB₁ and D₂ receptor in the prefrontal cortex.

This study indicated there wasn't a significant alteration of the mRNA expression in none of the genes investigated, and although previous evidence had demonstrated a reduced expression of CB₁ receptor mRNA in the prefrontal cortex of patients under antipsychotic treatment, studies in rats have corroborated the findings of Urigüen et al.

Neurotransmitter studies continue with Schmitt et al. in 2010 with analysis of the glutamate receptors. Although the study of glutamate receptors is a popular choice, this study concentrates on gene expression in the cerebellum which has also shown involvement in different cognitive tasks and in the pathophysiology of schizophrenia. This study revealed an over-expression of the NR2D subunit of the NMDA receptor in the molecular and granular layer of the right cerebellar hemisphere and vermis of schizophrenic patients, while no significant alterations in the left side were observed. An increased expression of the NR2D subunit could be interpreted as a regulation to glutamatergic hypoactivity. Another regulation would be to lower the threshold of the glutamate receptor making it hyperexcitable to ensure an effective postsynaptic depolarization when presynaptic activity is reduced. As these patients had been receiving antipsychotic treatment, medication effects could be the reason for these changes. In this study, clozapine treated patients showed a higher expression of NR2D compared to haloperidol.

The last post-mortem brain study we obtained was carried out by Tan et al. in 2014 to investigate the effects of antipsychotic treatment on the presynaptic machinery, specifically in Synapsin II. As we mentioned before in rat studies, synapsin II has been found to be decreased in schizophrenic patients, a finding that is also corroborated by Tan et al.. Additionally in this study, multiple regression analysis showed that antipsychotic use was associated with synapsin IIa expression in schizophrenia, a repeated finding throughout the different studies, which can lead us to think that presynaptic machinery must be altered by antipsychotic administration. Dorsolateral

prefrontal cortical synaptic alterations have also been associated with attention deficits and cognitive alterations, therefore this results could lead us to think antipsychotics could be modulating this presynaptic pathway to achieve clinical improvement.

Finally, post-mortem evidence of lipid metabolism alterations has also been obtained by Choi et al. in 2009. Interestingly, this study compares gene expression between typical and atypical antipsychotics in liver instead of comparing antipsychotic treatment versus controls in brain regions. Typical antipsychotics affected genes related with nuclear proteins, stress and phosphorylation in the liver, while atypical antipsychotics modulated the Golgi apparatus and endoplasmic reticulum. This could provide important information on the metabolic side effects of these drugs at an hepatic level.

3. Human blood studies

One of the main difficulties when investigating psychiatric disorders arises from the unavailability of brain tissue to investigate gene expression. While in other disorders a brain biopsy would seem timely, biopsies from psychiatric patients are not feasible for diagnostic purposes. However, the advances in the fields of biomarkers have allowed the use of peripheral blood as a substitute. Studies that have compared gene expression in circulating blood and brain have found high correlation of the expression of the total transcriptome (Sullivan et al. 2006; Rollins et al. 2010). Additionally, many candidate genes for psychiatric illnesses have been studied individually such as dopamine receptors D3, D4 or DISC1 (Maeda et al. 2006; Padín et al. 2006). Sullivan et al. in 2006 suggest that expression of the biologically relevant genes are statistically similar between blood and brain and have calculated that the median non-parametric correlation between transcripts present in whole blood and CNS is around 0.5.

The studies we have selected for this analysis are reflected in [Table 3](#).

Table 3. Human blood studies analyzing gene expression before and after antipsychotic treatment from 2000-2015

Author	Year	Patients	Antipsychotic drugs	Duration of the treatment	Relevant genes	Technique
Suzuki et al.	2008	40 schizophrenia (20 drug naïve, 20 medicated); 40 healthy	Variety of typical and atypical antipsychotic drugs.	Long-term	VLDLR, ApoeER2	Quantitative real time RT-PCR
Zhang et al.	2008	31 treatment naïve	Chlorpromazine, Risperidone, Quetiapine	1 month	NRG-1	Semi-quantitative RT-PCR
Van Beveren et al.	2012	41 schizophrenic (naïve/free) patients, 29 controls.	Variety of typical and atypical antipsychotic drugs.	Long term	AKT-1	Microarray
Kumarasinghe, et al	2013	10 schizophrenic treatment naïve, 11 controls	Risperidone, Haloperidol	6-8 weeks	AKT-1, DISC1, DGCR6, RXRA, MMP9, MAL, ABCF1, BTBD11, BCL11B	Microarray and real time RT-PCR

Table 3. Human blood studies analyzing gene expression before and after antipsychotic treatment from 2000-2015 (continuation)

Chervenkov et al.	2013	21 schizophrenic patients, 10 controls	Haloperidol	3 weeks	CCDC86	RT-PCR
Crespo-Facorro et al.	2014	22 schizophrenic antipsychotic naïve patients	Aripiprazol, Risperidone, Olanzapine, Quetiapine	3 months	ADAMTS2, CD177, CNTNAP3, ENTPD2, RFX2	Quantitative RT-PCR
Ota et al.	2014	44 schizophrenic antipsychotic naïve patients	Risperidone	10 weeks	ABAT, TSPO, CHRN1, CHRNE, COMT, GABRR2, TACR2, NRG1	Quantitative RT-PCR

3a. Glutamate and GABA pathways

Two genes were studied in 2008 by Suzuki et al. , VLDLR and ApoER2, as they encode two Reelin receptors. Reelin is involved in neurotransmission, memory formation and synaptic plasticity and there is increasing evidence that suggests that Reelin is implicated in schizophrenia, as it's expression is significantly lower in untreated schizophrenic patients. This study measured the expression of the two receptors in drug-naïve and medicated schizophrenic patients and discovered that mRNA levels were increased after 6 months of antipsychotic medication and the presence of a negative correlation between the expression of VLDLR and the clinical symptoms. ApoER2, on the other hand, was found to decrease after 6 months of treatment with antipsychotics, with a negative correlation between ApoER2 mRNA levels and antipsychotic dose and a positive correlation between the clinical symptoms and ApoER2 levels.

These findings suggest that antipsychotic treatment affects the transcriptional expression of these genes in lymphocytes and could be used as a biological marker in people with schizophrenia.

In the same year, Zhang et al. study the expression of Nrg-1, which has been considered a susceptibility gene for schizophrenia, before and after 4 weeks with atypical antipsychotic treatment. Nrg-1 (neuregulin) is one of the 4 proteins in the neuregulin family which act as substrates for the EGF receptor, and therefore is related to neurodevelopment, neuronal migration, synaptogenesis, gliogenesis, myelination and neuronal neurotransmission. Additionally, recent studies have provided evidence of Nrg-1 regulating the expression of NMDA and therefore modulating synaptic plasticity. They found that the expression of Nrg-1 mRNA increased with the administration of antipsychotics and the improvement of the symptoms, however after 4 weeks Nrg-1 mRNA was still significantly lower than in the controls, which suggests that it could take a long time for the patient to reach the levels of expression of a healthy control.

In 2014, another study was carried out to analyze the changes in gene expression and methylation in the blood of patients with schizophrenia. This study conducted by Ota et al. assessed the expression of 10 genes: ABAT, TSPO, CHRN1, CHRNE, COMT, GABRR2,

GCH1, GCHFR, TACR2 and NRG1. Out of these genes COMT (catechol- O-methyltransferase) and GCHFR (GTP cyclohydrolase I feedback regulator) showed an increase in the expression levels after treatment with risperidone, while GABRR2 (gamma aminobutyric acid receptor) showed a decrease in the expression. An interesting fact is that COMT up-regulation contradicts previous findings in rats by Fatemi et al. in 2011 where COMT was down-regulated by clozapine treatment. Additionally, GABRR2 was down-regulated after 10 weeks of risperidone treatment and interestingly, the higher the GABRR2 levels, the more severe the negative symptoms that the patients displayed. This can be explained by the understanding that GABRR2 encodes a GABA receptor subunit, and the GABA system is involved in the pathophysiology of schizophrenia through GABAergic interneurons that can be modulated by other neurotransmitter pathways, as risperidone does not show affinity for GABA receptors. One of the hypothesis is that GABRR2 expression levels could be reduced via D2 blockade by risperidone, however no differences were observed in the expression of the dopamine receptors.

Taken together, this study suggests that risperidone could induce GABRR2 down-regulation, which could in ultimate terms reflect changes in GABA concentrations and be one of the reasons behind the clinical improvement achieved by antipsychotic drugs.

3b. Apoptosis

The AKT-1 gene has also been studied in more than one occasion. Evidence from association studies has highlighted the implication in schizophrenia of AKT-1, a member of the serine-threonine protein kinase AKT gene family that possesses redundant functions that contribute to cell growth, survival and metabolism, and therefore, gain or loss of AKT has been linked with several human diseases. As a general idea, one of the main function of AKT1 is to promote cell growth mediated survival and to block apoptosis.

This gene was studied by Van Beveren et al. in 2012, using the evidence provided by previous studies (Emamian et al. 2004), but expression levels of AKT-1 in antipsychotic free patients was not significantly different from the levels obtained from medicated patients. Posteriorly, another study carried out by Kumarasinghe et al. in 2013 found expression of AKT-1 to be decreased after antipsychotic treatment and returned to control levels. The correction of AKT1 by antipsychotics may be due to a link between dopamine D2 receptors (antagonized by antipsychotics) and AKT1. In any case, this study identified by IPA 3 differentially expressed pathways than contain AKT1: EIF2 signalling, regulation of EIF4 and mTOR signaling which have roles in regulating cell growth and survival and that respond to glutamate via NMDA receptors.

3c. Inflammation

The previous study also provides evidence of the alteration of the immune and inflammation after antipsychotic treatment. Three genes: BTBD11 (BTB/POZ domain containing protein 11), BCL11B (B-cell CLL/lymphoma 11B zinc finger protein) and ABCF1 (ATP-binding cassette subfamily F) were found to be down-regulated after treatment with antipsychotic drugs. BCL11B is a transcription factor involved in the commitment of the T cell lineage during haematopoiesis and ABCF-1 is located in the major histocompatibility complex locus reported to be associated to schizophrenia. These findings along with the fact the study shows an enrichment of the biological functions

related to immune function suggests that biological functions related to immunity, inflammation and infectious disease are altered in schizophrenia and can be partially compensated by antipsychotic drugs through the regulation of gene expression.

Additionally, a study was conducted in 2013 by (Chervenkov et al. 2013) to analyze the differential expression of the gene Cyclon or CCDC86 which has appeared in a number of investigations. Cyclon is a cytokine-induced protein with coiled-coil domain that is strongly induced by IL-3 in hematopoietic cell lines. Therefore modulation of Cyclon levels has been known to affect the activation of leukocytes, specifically T cells, but also their apoptosis by regulating the expression of a death receptor (Fas).

In this study we can see how Cyclon mRNA levels are higher in schizophrenic patients compared to controls, and that these correlate with the clinical appearance of the disease: high levels appear in patients with positive symptoms while low levels can be found in patients with negative symptoms. Levels of Cyclon are reduced after administration of antipsychotic treatment. Additionally, before applying the treatment, T-cells were reduced while B cells were increased, and after the treatment the opposite effect was observed, with T cells increasing and B cells declining. Therefore, we can conclude from this study that a modulation of Cyclon levels might affect the immune system and contribute to it's the dysfunction of the disease.

We can also include under this heading the data obtained by Crespo-Facorro et al. in 2014, where 6 genes out of the 17 that showed a modified expression in the study (ALPL, GPER, LTF, MMP8, OLR1, CRISP3) have been found to be related to inflammation (Sainz et al. 2012). Additional findings of this study related to lipid metabolism can be found below.

3d. Lipid Metabolism

In 2014, Crespo-Facorro et al. carried out a high throughput study where 22 treatment naïve patients were followed during 3 months of antipsychotic treatment to assess differential gene expression. In first place, an interesting fact is that out of the 17 differentially-expressed genes that were found, 8 were associated to 24 diseases including schizophrenia (ADAMTS2, CD177, CNTNAP3, ENTPD2, RFX2 and UNC45B), bipolar disorder or obesity. These 6 genes implicated in schizophrenia reverted to normal levels after medication with atypical antipsychotics which suggests that they could be related to the positive symptoms of schizophrenia as the positive symptoms in these patients also improved (Sainz et al. 2012; Crespo-Facorro et al. 2014).

On the other hand, 5 genes (GPER, LTF, MMP8, OLR1 and OLFM4) have been found to be implicated with obesity while 4 genes (ALPL, LTF, MMP8 and OLR1) presented an association with diabetes. When analyzing the functions of these genes it is not surprising to find some of them have lipid metabolism related functions such as OLR1, a gene that encodes low density lipoprotein receptor and is considered to be a marker of atherosclerosis, while others have functions related to cell growth and differentiation (OLFM4).

Although many pathways are involved in this genetic activity, the GPER pathway is especially interesting as it encodes the G-coupled estrogen receptor 1, which along with LTF (lactotransferrin) and OLFM4 (Olfactomedin-4) confirms the existence of an

association between estrogen and schizophrenia. Animal studies provide evidence that estrogen modulates the dopamine pathways by increasing the dissociation constant of the binding of dopamine to its receptor, thereby blockading them like neuroleptics (McEwen and Alves 1999; Chavez et al. 2010).

4. In vitro studies.

The molecular study of mental illnesses remains a difficult task due to the difficulties when accessing the tissue where the pathology underlies, in this case, the brain, but to the risks it implies for the patients which make this kind of studies ethically impossible. The study of rat brain has facilitated a lot of information on the physiopathology of schizophrenia, however, differences between animal and human genes, molecules and pathways, as well as the lack of a clear animal model of schizophrenia make the results less applicable. Blood studies seem to be the way to go, however this technique has only been discovered recently and its reliability is still being studied.

This situations leaves us with one more alternative, in vitro studies. Limitations of this studies are evident: they do not capture the interacting genetic variables that contribute to the development of schizophrenia, cell types may not be the same, environmental variables and the lack of a clinical correlation. However, the advances in genetics and stem cell biology are providing in-vitro approaches allowing molecular, developmental and pathophysiological alterations to be studied with precision.

Most of the in-vitro studies we have found are related to lipid metabolism and they are carried out using cell cultures of different kinds. They investigate the effects of antipsychotic drugs on gene expression with the objective of extrapolating these findings to in-vivo studies. These studies are listed in the table below ([Table 4](#)).

Table 4. In-vitro studies assessing gene expression changes before and after antipsychotic treatment from 2000 to 2015

Author	Year	Cell-culture	Antipsychotic drugs	Harvesting time	Relevant genes	Technique
Ferno et al.	2005	Human glioma cell line	Clozapine, haloperidol	24h	HMGCR, HMGC1, SCD, FADS1, FADS2	Microarray and RT PCR
Polymeropoulos et al.	2009	Retinal pigment epithelial cell line	Aripiprazole, clozapine, iloperidone, olanzapine, quetiapine, risperidone, ziprasidone, chlorpromazine, fluphenazine, haloperidol, lozapine, mesoridazine, molindone, perfenazine, promazine, thioridazine, trifluoperazine and triflupromazine.	24h	INSIG1, SCD, FADS1, FADS2, ACAT2, LDLR	Microarray

Table 4. In-vitro studies assessing gene expression changes before and after antipsychotic treatment from 2000 to 2015 (continuation)

Kristiana et al.	2010	Chinese Hamster ovary cells	Haloperidol, pimozide, aripiprazole, clozapine, quetiapine, olanzapine, risperidone, ziprasidone.	6h	HMGCoA reductase, LDLR, ACC, FAS	Quantitative RT-PCR
Sárvari et al.	2014	Human adipose-derived stem cells	Olanzapine, ziprasidone, clozapine, quetiapine, aripiprazole, risperidone, haloperidol	34 days	LEP, NF-KB1, TNF- α , IL-1B, IL-8, MCP-1	PCR array
Steiner et al.	2014	Oligodendroglial cultures	Clozapine, haloperidol.	24h	GLUT1, GLUT3, MCT1, ACC1.	RT-PCR

4a. Lipid metabolism

The first study was carried out in 2005 Fernø et al. to evaluate the effects of antipsychotic drugs lipid biosynthesis genes in human glioma cells. The study provides evidence of clozapine and haloperidol-induced up-regulation of a cluster of cholesterol and fatty acid biosynthetic genes 24 hours after adding the antipsychotic drugs. Among the up-regulated genes we can find HMGCR (3-hydroxy-3methylglutaryl-coenzyme A reductase) and HMGCS1 (3-hydroxy-3methylglutaryl-coenzyme A synthase-1) which control cholesterol biosynthesis and SCD (Stearoyl-CoA desaturase), FADS1 and FADS2 (fatty acid desaturase 1 and 2) which control fatty acid biosynthesis. These findings were supported by enhanced HMGCR enzyme activity and elevated levels of cholesterol and triglycerides. All of the up-regulated genes appear to have in common a regulatory gene which controls the levels of the mentioned genes which suggests that antipsychotics could induce SREBP, thereby increasing the metabolic side effects of antipsychotics.

Another study was carried out 4 years later by Polymeropoulos et al. where 18 antipsychotic drugs were used in retinal pigment epithelial cell lines to analyse the expression of genes involved in fatty acid and cholesterol biosynthesis. Some of the genes that showed an up-regulation were common to the previous study (SCD, FADS1, FADS2), and this increase was maximum for fluphenazine, perphenazine or iloperidone with molindone having the lowest effect. The author proposes that activation of the antipsychotic could be the central mechanism of their actions instead of a side effect, in other words, these drugs could be achieving their results by altering lipid homeostasis, as fatty acids and cholesterol alter the fluidity of cell membranes and neurons. On the other hand, this study also finds an interesting relationship between the effect of antipsychotics and SERMS (selective estrogen receptor modulators), which could be due to the fact that dopamine can activate estrogen receptors. Additionally, modulation of the estrogen receptor can result in the activation of SREBP responsive genes, as we mentioned in reference to Fernø et al.'s study. This idea, which is also present in (Crespo-Facorro et al. 2014) study suggests that estrogen could be involved in the therapeutic effect of antipsychotics.

The findings of both of these studies were corroborated by Kristiana et al. in 2010 where Chinese Hamster ovary cells were used to investigate if a variety of antipsychotic drugs upregulated the SREBP target genes. Some of the genes were common to the previous studies (HMG-CoA reductase, LDLR, ACC, FAS) and in the same way as previous studies, antipsychotic drugs were found to up-regulate these genes.

A study by Oh et al. in 2012 examined the effect of antipsychotic drugs (clozapine, quetiapine and ziprasidone) on brown adipocytes differentiation in rats. Brown adipose tissue has a specialized role in thermogenesis as it burns metabolic substrate to produce energy. Only two studies had suggested before the possibility of brown adipose tissue being related to antipsychotic use: first with lithium (Rodríguez de la Concepción et al. 2005) and posteriorly, clozapine (Blessing et al. 2006). When analyzing the effects of the drugs on the expression of brown adipocyte the results revealed that clozapine inhibited the expression of all the genes examined: PRDM16, PPAR γ 2, UCP-1, PGC1 α and Cidea. Quetiapine also down-regulated the expression of these genes but not with the same intensity, while ziprasidone only showed activity in the early stages of the differentiation. These results support the hypothesis that the inhibition of brown adipogenesis may be one of the mechanisms by which atypical antipsychotics induce weight gain. Additionally, the expression of adipokines like resistin, leptin and adiponectin was also increased by these drugs. This occurs because clozapine impairs insulin signaling, and insulin is a regulator of leptin synthesis. In contrast to these findings, in a study by Hauner et al. in 2003 clozapine did not alter the levels of leptin.

Finally, in 2014 Steiner et al. conducted a study to compare the effects of clozapine and haloperidol on the metabolism of oligodendrocytes analyzing availability of glucose, mitochondrial respiration and myelin and lipid synthesis. When analyzing the data on glucose availability we can see that haloperidol and clozapine had no influence on the expression of GLUT1, GLUT3, or MCT1, although haloperidol decreased glycolysis while clozapine increased it. Secondly, the data for both drugs suggests an increased expression of ACC1, an enzyme related to free fatty acid synthesis, however, clozapine but not haloperidol increased the expression of galactocerebroside. In conclusion, the study suggests that clozapine and haloperidol modulate differently glucose and myelin synthesis, leading to the idea that clozapine, due to its increased lipid metabolism activity compared to haloperidol, might improve energy supply and maturation of the oligodendrocytes and therefore could maintain the integrity of myelinated fibers. However, this could also have an important repercussion on weight gain and other side effects.

4b. Inflammation

In 2014, Sárvári et al. conducted another study investigating pro-inflammatory gene expression and apoptosis in human adipocytes. In this study, antipsychotics produced an increase in the mRNA levels of the transcription factor NF-KB1 and target genes, pro-inflammatory cytokines like TNF- α , IL1B, IL-8 and MCP-1 which suggests chronic treatment with antipsychotics produces an inflammation in adipocytes. When the expression was analyzed 34 days later, NF-KB1 increased along with TNF- α , IL-1B and IL8 and MCP (which in vivo could produce infiltration of monocytes or macrophages in adipose tissue increasing inflammation). An interesting finding is that TNF- α increase was accompanied by a reduction of IRS1 and glucose transport 4, while maintaining a high expression of IL-8. These findings could inhibit insulin activity, leading to an insulin resistance and metabolic disorders.

These findings suggest that antipsychotic treatment can alter gene expression in adipocytes creating an initial inflammation that could be behind the metabolic adverse effects we find in these patients.

Conclusion

Schizophrenia is a complex and devastating disorder characterized by progressive clinical deterioration. Until the date, the main treatment of the disease is based on the chronic administration of antipsychotic drugs, however this solution appears to be merely symptomatic with no alternative curative treatments. On the other hand, antipsychotic drugs are characterized by important side effects that appear as a consequence of their prolonged use and that can be very invalidating for the patients, making them one of the main causes of treatment failure.

Although antipsychotic drugs appear to have a clinical effect on the diseases, we cannot be certain of the underlying mechanisms of action. However, with the new introduction of genetic studies in the psychiatric field this is starting to change, as certain pathways appear to be consistently affected by antipsychotic drugs, pointing towards different mechanisms of action.

One of the most important findings, which has been consistently studied since 2000 is the implication of genes involved in the synaptic machinery. An essential part of this process is carried out by the SNARE complex, along with its regulating proteins like synapsin or synaptotagmin. Although, this complex was initially investigated in rodent studies, the idea has been extrapolated to human blood. These molecules are repeatedly up-regulated in response to both typical and atypical antipsychotics in rodent and post-mortem, which could suggest the increase or decrease of neurotransmitter concentrations in certain parts of the pathways implicated in the “dopamine hypothesis” by increasing the probability of vesicle fusion and facilitating synaptic activity.

Another constant finding is the alteration of the myelination pathway as a result of the administration of drugs. Our review highlights an important relationship between myelination and lipid metabolism. An interesting finding is that repeated studies inform of the alteration in the expression of molecules such as ApoD, MBP or PLP, proteins (normally found in the myelin membrane) in different directions. Although more studies need to be carried out in order to explain this, it is important to note that these effects were carried mainly by atypical antipsychotics, which also have important adverse effects when referring to lipid metabolism. It is postulated that atypical antipsychotics could stabilize the lipid membrane, leading to a reduction of the negative symptoms that appear as a consequence of the alteration of the white matter. This effect could be also responsible for the negative adverse effects of this drug at a peripheral level, increasing the risk of cardiovascular disease and obesity. Haloperidol on the other hand, with reduced lipidic effects and an increase of the D2 affinity, could be increasing extrapyramidal side effects exacerbating preexisting deficits of the myelin membrane.

When analyzing the effects on gene expression as a whole, the main affected pathway is lipid metabolism, which appears to be altered in all the different subsections. In this pathway, there is an evident difference between the effects of atypical and typical antipsychotics, with atypical drugs leading to more noticeable results. The different studies show that not only do these drugs affect lipidic molecules such as cholesterol or

oxysterol, but they also affect the gene expression of hormones (incretins) that can increase appetite and decrease saturation, which could lead to an indirect weight gain. Not only have atypical drugs been implicated with obesity and cardiovascular risk, but diabetes and insulin resistance is also induced as a result of the alteration of the genes that control glucose concentration in the blood such as muscle glycogen phosphorylase or pyruvate kinase. Another interesting finding in this section, appeared when studies started assessing the gene expression in adipocytes to find that atypical drugs increased FAS, a gene in charge of the synthesis of fatty acids. This could be related to posterior findings where antipsychotic drugs also produce an elevation of the genes that encode interleukins and other cytokines, producing an increase in the inflammatory pathway in the adipocytes creating an initial subclinical inflammation that could be behind the metabolic adverse effects. Another recent hypothesis that has been postulated thanks to in-vitro studies, is that brown adipocyte differentiation may decrease as a result of atypical antipsychotics, which could also lead to weight gain, as this causes a reduction of the burning of the metabolic substrates.

The most contradictory information comes from the analyses of the genes implicated in the dopamine, glutamate and GABA pathway. The main idea we can extract from these results is the need for more studies to investigate these specific pathways. Many of the experiments we have obtained in this section contradict each other and some are highly inconclusive. This is mainly due to variations in the methodology of the study: different laboratory rats, administration period, genetic analysis, antipsychotic drugs, brain regions, etc. However, other conclusions can also be made from these results. In first place, we can assume that antipsychotics could be affecting dopamine, GABA and glutamate concentrations through an indirect mechanism that involves the regulation of enzymes in charge of regulating neurotransmitter concentrations such as COMT or MAOB for dopamine, and EAAT 1, 2 or 3 in the case of glutamate, which have shown an altered expression, as supposed to the sole D2 receptor antagonism that the dopamine hypothesis defends. In second place, we can assume from our findings that there is a neuronal network that communicates these three neurotransmitter systems. In this way, our findings show evidence of the dopamine pathway regulating the receptors of the GABA pathway as a consequence of antipsychotic treatment, as well as the glutamate pathway through the regulation of NR1, NR2A, NR2C and NR2D. It is interesting to point out the increasing evidence of the implication of Homer in the effects of antipsychotics. The studies we have analyzed point towards a link between Homer and the motor side effects produced by long-term treatment with medication.

Finally, another pathway we can highlight from our research is the inflammation pathway. Although inflammation is not a popular pathway when it comes to mRNA expression studies, it is one of the most important theories of schizophrenia. Many authors postulate that the etiology of these diseases could lie in a prenatal inflammation, or an infection. Gene expression has been repeatedly studied in patients with schizophrenia compared to control patients, and alterations of the inflammatory genes are evident, however there is not much evidence of these alterations after an exposition to antipsychotic treatment. Only 4 of the studies we have analyzed (1 in vitro, 3 in human blood) investigated the effects of drugs in inflammatory gene expression and all of them show a reduction of the inflammation (decreased interleukin and Cyclon

gene expression) with treatment compared to non-treated patients. Not surprisingly, there seems to be a link between the reduction of the inflammation and the reduction of the apoptosis both in rats and human studies. On the other side, ubiquitination, responsible for the degradation of proteins, is up-regulated by both clozapine and haloperidol, and authors postulate this could be a result of a normalization of the protein metabolism, which was previously altered in schizophrenia, reducing the equilibrium between protein synthesis and degradation.

When comparing the studies carried out with atypical and typical antipsychotics we can observe a difference between their effect on different pathways. In the same way that lipid metabolism and myelination are much more affected by atypical antipsychotics, typical drugs are much more effective when increasing the metabolism of synaptic machinery and when altering gene expression related to extrapyramidal effects. This doesn't come as a surprise, as it is directly linked with the adverse effects of these drugs. In other pathways such as glutamate neurotransmission where there is an important difference between the effects of these drugs, authors postulate it is due to the variations in the specificity for dopamine receptors, with haloperidol having a higher affinity for this drug and clozapine or olanzapine, acting on a wider range of receptors.

In summary, although further studies need to be carried out in order to obtain a general view of the results, as the studies have very few similarities between them, both in the methodology and in the pathways studied, the data we have reviewed in the present article suggests that antipsychotics produce an alteration in the genetic expression of certain pathways involved in the symptomatology of schizophrenia. While most of the alterations contribute to an improvement in the clinical spectrum of the disease, some of the pathways demonstrate their contribution to the adverse effects that often appear with long term treatment. With these studies we can characterize the genes behind these changes and hopefully, this view will eventually lead to the development of markers that are able to predict and measure the adverse effects the drugs will have on certain patients, taking a step closer to personalized medicine. On the other hand, these findings contribute to the development of novel drugs and to a further understanding of the ones we have at our disposition, in order to change the way we prescribe these antipsychotics in time or combinations and to translate these results to the clinical settings.

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References

- De Bartolomeis A, Aloj L, Ambesi-Impiombato A, Bravi D, Caracò C, Muscettola G, et al. Acute administration of antipsychotics modulates Homer striatal gene expression differentially. *Molecular Brain Research*. sciencedirect; 2002;98(1-2):124–9.
- Van Beveren NJ, Buitendijk G, Swagemakers S, Krab L, Röder C, Haan L, et al. Marked Reduction of AKT1 Expression and Deregulation of AKT1-Associated Pathways in Peripheral Blood Mononuclear Cells of Schizophrenia Patients. *PLoS ONE*. PLoS ONE; 2012;7(2).
- Blessing WW, Zilm A, Ootsuka Y. Clozapine reverses increased brown adipose tissue thermogenesis induced by 3,4-methylenedioxymethamphetamine and by cold exposure in conscious rats. *Neuroscience*. 2006 Sep 5;141(4):2067–73.
- Cascade E, Kalali A, Mehra S, Meyer J. Real-world Data on Atypical Antipsychotic Medication Side Effects. *Psychiatry (Edgmont (Pa : Township))*. 2010;7(7):9–12.
- Chavez C, Hollaus M, Scarr E, Pavey G, Gogos A, van den Buuse M. The effect of estrogen on dopamine and serotonin receptor and transporter levels in the brain: an autoradiography study. *Brain research*. 2010;1321:51–9.
- Chen M-L, Chen C-H. Microarray Analysis of Differentially Expressed Genes in Rat Frontal Cortex Under Chronic Risperidone Treatment. *Neuropsychopharmacology*. nature; 2004;30(2):268–77.
- Chervakov T, Shishkov R, Tonchev AB. Expression and differential response to haloperidol treatment of Cyclon/CCDC86 mRNA in schizophrenia patients. *Neurochem Int* [Internet]. Elsevier Ltd; 2013;62(6):870–2. Available from: <http://linkinghub.elsevier.com/retrieve/pii/S0197018613000624>
- Choi K, Higgs B, Weis S, Song J, Llenos I, Dulay J, et al. Effects of typical and atypical antipsychotic drugs on gene expression profiles in the liver of schizophrenia subjects. *BMC Psychiatry*. 2009;9(1):57.
- Chong V, Young T, Mishra R. cDNA array reveals differential gene expression following chronic neuroleptic administration: implications of synapsin II in haloperidol treatment. *Journal of Neurochemistry*. wiley; 2002;82(6):1533–9.
- Crespo-Facorro B, Prieto C, Sainz J. Schizophrenia Gene Expression Profile Reverted to Normal Levels by Antipsychotics. *International Journal of Neuropsychopharmacology*. 2014;pyu066.
- Delfs JM, Ellison GD, Mercugliano M, Chesselet MF. Expression of glutamic acid decarboxylase mRNA in striatum and pallidum in an animal model of tardive dyskinesia. *Exp Neurol*. 1995 Jun 4;133(2):175–88.
- Emamian ES, Hall D, Birnbaum MJ, Karayiorgou M, Gogos JA. Convergent evidence for impaired AKT1-GSK3 β signaling in schizophrenia. *Nat Genet*. 2004 Feb;36(2):131–7.
- Faludi G, Mirnics K. Synaptic changes in the brain of subjects with schizophrenia. *Int J Dev Neurosci*. 2011 May;29(3):305–9.
- Farkas N, Lendeckel U, Dobrowolny H, Funke S, Steiner J, Keilhoff G, et al. Reduced density of ADAM 12-immunoreactive oligodendrocytes in the anterior cingulate white matter of patients with schizophrenia. *World J Biol Psychiatry*. 2010 Apr 4;11(3):556–66.

- Fasulo W, Hemby S. Time-dependent changes in gene expression profiles of midbrain dopamine neurons following haloperidol administration. *Journal of Neurochemistry*. 2003;87(1):205–19.
- Fatemi H, Folsom T, Reutiman T, Novak J, Engel R. Comparative gene expression study of the chronic exposure to clozapine and haloperidol in rat frontal cortex. *Schizophr Res. sciencedirect*; 2011;134(2-3):211-218.
- Fatemi H, Reutiman T, Folsom T, Bell C, Nos L, Fried P, et al. Chronic Olanzapine Treatment Causes Differential Expression of Genes in Frontal Cortex of Rats as Revealed by DNA Microarray Technique. *Neuropsychopharmacology. nature*; 2006;31(9):1888–99.
- Fehér L, Kálmán J, Puskás L, Gyülvézi G, Kitajka K, Penke B, et al. Impact of haloperidol and risperidone on gene expression profile in the rat cortex. *Neurochemistry International. sciencedirect*; 2005;47(4):271-280.
- Fernø J, Raeder M, Vik-Mo A, Skrede S, Glambek M, Tronstad K-J, et al. Antipsychotic drugs activate SREBP-regulated expression of lipid biosynthetic genes in cultured human glioma cells: a novel mechanism of action? *Pharmacogenomics J*. 2005;5(5):298–304.
- Goghari VM, Sponheim SR. Differential association of the COMT Val158Met polymorphism with clinical phenotypes in schizophrenia and bipolar disorder. *Schizophr Res*. 2008 Aug 5;103(1-3):186–91.
- Gong CX, Shaikh S, Grundke-Iqbal I, Iqbal K. Inhibition of protein phosphatase-2B (calcineurin) activity towards Alzheimer abnormally phosphorylated tau by neuroleptics. *Brain Res*. 1996 Nov 1;741(1-2):95–102.
- Haas KF, Broadie K. Roles of ubiquitination at the synapse. *Biochim Biophys Acta*. 2008 Aug 5;1779(8):495–506.
- Hauner H, Röhrig K, Hebebrand J, Skurk T. No evidence for a direct effect of clozapine on fat-cell formation and production of leptin and other fat-cell-derived factors. *Mol Psychiatry*. 2003 Mar 6;8(3):258–9.
- Hor K, Taylor M. Review: Suicide and schizophrenia: a systematic review of rates and risk factors. *Journal of Psychopharmacology*. 2010;24(4):81–90.
- Howes O, Kapur S. The Dopamine Hypothesis of Schizophrenia: Version III—The Final Common Pathway. *Schizophrenia Bulletin*. 2009;35(3):549–62.
- Iasevoli F, Fiore G, Cicale M, Muscettola G, de Bartolomeis A. Haloperidol induces higher Homer1a expression than risperidone, olanzapine and sulpiride in striatal sub-regions. *Psychiatry Res [Internet]. Elsevier Ltd*; 2010;177(1-2):255–60. Available from: <http://dx.doi.org/10.1016/j.psychres.2010.02.009>
- Iwata S-I, Morioka H, Iwabuchi M, Shinohara K, Maeda M, Shimizu T, et al. Administration of haloperidol with biperiden reduces mRNAs related to the ubiquitin-proteasome system in mice. *Synapse*. 2005 Jun 3;56(4):175–84.
- Jennings C a., Cluderay JE, Gartlon J, Cilia J, Lloyd a., Jones DNC, et al. The effects of ziprasidone on regional c-Fos expression in the rat forebrain. *Psychopharmacology (Berl)*. 2006;184(1):13–20.
- Johnson RD, Oliver PL, Davies KE. SNARE proteins and schizophrenia: linking synaptic and neurodevelopmental hypotheses. *Acta biochimica Polonica*. 2008 Feb 5;55(4):619–28.
- Khan MM, Evans DR, Gunna V, Scheffer RE, Parikh VV, Mahadik SP. Reduced erythrocyte membrane essential fatty acids and increased lipid peroxides in schizophrenia at the never-medicated first-episode of psychosis and after years of treatment with antipsychotics. *Schizophr Res*. 2002 Nov 5;58(1):1–10.
- Khan, Parikh, Mahadik. Antipsychotic drugs differentially modulate apolipoprotein D in rat brain. *Journal of Neurochemistry. wiley*; 2003;86(5):1089–100.
- Kim T, Tao-Cheng JH, Eiden LE, Loh YP. Chromogranin A, an “on/off” switch controlling dense-core secretory granule biogenesis. *Cell*. 2001 Aug 5;106(4):499–509.
- Konopaske GT, Dorph-Petersen K-AA, Sweet RA, Pierri JN, Zhang W, Sampson AR, et al.

Effect of chronic antipsychotic exposure on astrocyte and oligodendrocyte numbers in macaque monkeys. *Biol Psychiatry*. 2008 Apr 2;63(8):759–65.

Kontkanen O, Lakso M, Wong G, Castrén E. Chronic Antipsychotic Drug Treatment Induces Long-lasting Expression of fos and jun Family Genes and Activator Protein 1 Complex in the Rat Prefrontal Cortex. *Neuropsychopharmacology*. nature; 2002 a;27(2):152–62.

Kontkanen O, Törönen P, Lakso M, Wong G, Castrén E. Antipsychotic drug treatment induces differential gene expression in the rat cortex. *J Neurochem*. wiley; 2002 b;83(5):1043–53.

Kornau HC, Schenker LT, Kennedy MB, Seeburg PH. Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science*. 1995 Sep 5;269(5231):1737–40.

Kristiana I, Sharpe LJ, Catts VS, Lutze-Mann LH, Brown a J. Antipsychotic drugs upregulate lipogenic gene expression by disrupting intracellular trafficking of lipoprotein-derived cholesterol. *Pharmacogenomics J*. 2010;10(5):396–407.

Kumarasinghe N, Beveridge N, Gardiner E, Scott R, Yasawardene S, Perera A, et al. Gene expression profiling in treatment-naïve schizophrenia patients identifies abnormalities in biological pathways involving AKT1 that are corrected by antipsychotic medication. *The international journal of neuropsychopharmacology / official scientific journal of the Collegium Internationale Neuropsychopharmacologicum (CINP)*. 2013;16(7):1483–503.

Kursungoz C, Ak M, Yanik T. Effects of risperidone treatment on the expression of hypothalamic neuropeptide in appetite regulation in Wistar rats. *Brain Research*. sciencedirect; 2015;1596:146155.

Lam YA, Pickart CM, Alban A, Landon M, Jamieson C, Ramage R, et al. Inhibition of the ubiquitin-proteasome system in Alzheimer's disease. *Proc Natl Acad Sci USA*. 2000 Aug 2;97(18):9902–6.

Lipska B, Lerman D, Khaing Z, Weickert C, Weinberger D. Gene expression in dopamine and GABA systems in an animal model of schizophrenia: effects of antipsychotic drugs. *Eur J Neurosci*. wiley; 2003;18(2):391–402.

Maeda K, Nwulia E, Chang J, Balkissoon R, Ishizuka K, Chen H, et al. Differential expression of disrupted-in-schizophrenia (DISC1) in bipolar disorder. *Biological psychiatry*. 2006;60(9):929–35.

MacDonald ML, Eaton ME, Dudman JT, Konradi C. Antipsychotic drugs elevate mRNA levels of presynaptic proteins in the frontal cortex of the rat. *Biol Psychiatry*. 2005;57(9):1041–51.

McEwen, Alves. Estrogen actions in the central nervous system. *Endocrine reviews*. 1999;20(3):279–307.

Merchant KM, Dorsa DM. Differential induction of neurotensin and c-fos gene expression by typical versus atypical antipsychotics. *Proc Natl Acad Sci USA*. 1993 Apr 4;90(8):3447–51.

Minet-Ringuet, Even, Valet, Carpéné, Visentin, Prévot, et al. Alterations of lipid metabolism and gene expression in rat adipocytes during chronic olanzapine treatment. *Mol Psychiatry*. nature; 2007;12(6):562–71.

Mirnic K, Middleton FA, Marquez A, Lewis DA, Levitt P. Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron*. 2000 Oct;28(1):53–67.

Narayan S, Kass K, Thomas E. Chronic haloperidol treatment results in a decrease in the expression of myelin/oligodendrocyte-related genes in the mouse brain. *J Neurosci Res*. wiley; 2007;85(4):757–65.

Oh J-EE, Cho YM, Kwak S-NN, Kim J-HH, Lee KW, Jung H, et al. Inhibition of mouse brown adipocyte differentiation by second-generation antipsychotics. *Exp Mol Med*. 2012 Sep;44(9):545–53.

Ohnuma T, Augood SJ, Arai H, McKenna PJ, Emson PC. Expression of the human excitatory amino acid transporter 2 and metabotropic glutamate receptors 3 and 5 in the prefrontal cortex from normal individuals and patients with schizophrenia. *Brain Res Mol Brain Res*. 1998 May 5;56(1-2):207–17.

Ota V, Noto C, Gadelha A, Santoro M, Spindola L, Gouvea E, et al. Changes in gene expression and methylation in the blood of patients with first-episode psychosis. *Schizophrenia research. sciencedirect*; 2014;159(2-3):358364.

Padín, Rodríguez M, Domínguez E, Dopeso-Reyes I, Buceta M, Cano E, et al. Parallel regulation by olanzapine of the patterns of expression of 5-HT_{2A} and D₃ receptors in rat central nervous system and blood cells. *Neuropharmacology*. 2006;51(4):923–32.

Perdomo G, Henry Dong H. Apolipoprotein D in lipid metabolism and its functional implication in atherosclerosis and aging. *Aging (Albany NY)*. 2009 Jan 4;1(1):17–27.

Pesemann N, Schmitt A, Gebicke-Haerter P, Zink M. Aripiprazole differentially regulates the expression of Gad67 and γ -aminobutyric acid transporters in rat brain. *Eur Arch Psychiatry Clin Neurosci*. springer; 2012;263(4):285–97.

Polesse D, de Serpis A, Ambesi-Impimbato A, Muscettola G, de Bartolomeis A. Homer 1a Gene Expression Modulation by Antipsychotic Drugs: Involvement of the Glutamate Metabotropic System and Effects of D-Cycloserine. *Neuropsychopharmacology. nature*; 2002;27(6):906–13.

Polymeropoulos MH, Licamele L, Volpi S, Mack K, Mitkus SN, Carstea ED, et al. Common effect of antipsychotics on the biosynthesis and regulation of fatty acids and cholesterol supports a key role of lipid homeostasis in schizophrenia. *Schizophr Res [Internet]*. Elsevier B.V.; 2009;108(1-3):134–42. Available from: <http://dx.doi.org/10.1016/j.schres.2008.11.025>

Pongrac J, Middleton FA, Lewis DA, Levitt P, Mirnics K. Gene expression profiling with DNA microarrays: advancing our understanding of psychiatric disorders. *Neurochem Res*. 2002 Oct 2;27(10):1049–63.

Rizig MA, McQuillin A, Ng A, Robinson M, Harrison A, Zvelebil M, et al. A gene expression and systems pathway analysis of the effects of clozapine compared to haloperidol in the mouse brain implicates susceptibility genes for schizophrenia. *J Psychopharmacol (Oxford)*. 2012 Sep 6;26(9):1218–30.

Robertson GS, Matsumura H, Fibiger HC. Induction patterns of Fos-like immunoreactivity in the forebrain as predictors of atypical antipsychotic activity. *J Pharmacol Exp Ther*. 1994 Nov 2;271(2):1058–66.

Rodríguez de la Concepción ML, Yubero P, Iglesias R, Giralt M, Villarroja F. Lithium inhibits brown adipocyte differentiation. *FEBS Lett*. 2005 Mar 1;579(7):1670–4.

Rollins B, Martin M, Morgan L, Vawter M. Analysis of whole genome biomarker expression in blood and brain. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2010;153B(4):919–36.

Rubio MD, Wood K, Haroutunian V, Meador-Woodruff JH. Dysfunction of the ubiquitin proteasome and ubiquitin-like systems in schizophrenia. *Neuropsychopharmacology*. 2013 Sep;38(10):1910–20.

Rushlow, Seah, Belliveau, Rajakumar. Changes in calcineurin expression induced in the rat brain by the administration of antipsychotics. *Journal of Neurochemistry*. 2005;94(3):587–96.

Sainz, Mata, Barrera, Perez-Iglesias, Varela, Arranz, et al. Inflammatory and immune response genes have significantly altered expression in schizophrenia. *Molecular Psychiatry*. 2012;18(10):1056–7.

Sárvári AK, Veréb Z, Uray IP, Fésüs L, Balajthy Z. Atypical antipsychotics induce both proinflammatory and adipogenic gene expression in human adipocytes in vitro. *Biochem Biophys Res Commun*. 2014;450(4):1383–9.

Segnitz N, Ferbert T, Schmitt A, Gass P, Gebicke-Haerter PJ, Zink M. Effects of chronic oral treatment with aripiprazole on the expression of NMDA receptor subunits and binding sites in rat brain. *Psychopharmacology (Berl)*. 2011;217(1):127–42.

Segnitz N, Schmitt A, Gebicke-Härter PJ, Zink M. Differential expression of glutamate transporter genes after chronic oral treatment with aripiprazole in rats. *Neurochem Int*. 2009;55(7):619–28.

Schmitt A, Koschel J, Zink M, Bauer M, Sommer C, Frank J, et al. Gene expression of NMDA receptor subunits in the cerebellum of elderly patients with schizophrenia. *Eur Arch Psychiatry Clin Neurosci*. 2010;260(2).

Siever L, Davis K. The Pathophysiology of Schizophrenia Disorders: Perspectives From the Spectrum. *American Journal of Psychiatry*. 2004;161(3):398–413.

Sondhi, Castellano, Chong, Rogoza, Skoblenick, Dyck, et al. cDNA array reveals increased expression of glucose-dependent insulinotropic polypeptide following chronic clozapine treatment: role in atypical antipsychotic drug-induced adverse metabolic effects. *Pharmacogenomics J. nature*; 2005;6(2):131–40.

Stahl SM. Beyond the Dopamine Hypothesis to the NMDA Glutamate Receptor Hypofunction Hypothesis of Schizophrenia. *CNS Spectrums*. 2007;12(4):265–8.

Sugai T, Kawamura M, Iritani S, Araki K, Makifuchi T, Imai C, et al. Prefrontal abnormality of schizophrenia revealed by DNA microarray: impact on glial and neurotrophic gene expression. *Ann N Y Acad Sci*. 2004 Oct 5;1025:84–91.

Steiner J, Martins-de-Souza D, Schiltz K, Sarnyai Z, Westphal S, Isermann B, et al. Clozapine promotes glycolysis and myelin lipid synthesis in cultured oligodendrocytes. *Front Cell Neurosci* [Internet]. 2014;8(November):1–11. Available from: <http://journal.frontiersin.org/article/10.3389/fncel.2014.00384/abstract>

Sullivan P, Fan C, Perou C. Evaluating the comparability of gene expression in blood and brain. *American journal of medical genetics Part B, Neuropsychiatric genetics : the official publication of the International Society of Psychiatric Genetics*. 2006;141B(3):261–8.

Tan, Dyck, Gabriele, Daya, Thomas, Sookram, et al. Synapsin II gene expression in the dorsolateral prefrontal cortex of brain specimens from patients with schizophrenia and bipolar disorder: effect of lifetime intake of antipsychotic drugs. *The Pharmacogenomics Journal*. 2014;14(1):63–9.

Tandon R, Gaebel W, Barch D, Bustillo J, Gur R, Heckers S, et al. Definition and description of schizophrenia in the DSM-5. *Schizophrenia Research*. 2013;150(1).

Thomas E. Molecular profiling of antipsychotic drug function: convergent mechanisms in the pathology and treatment of psychiatric disorders. *Molecular neurobiology*. 2006;34(2):109–28.

Thomas E, Danielson P, Nelson A, Pribyl T, Hilbush B, Hasel K, et al. Clozapine increases apolipoprotein D expression in rodent brain: towards a mechanism for neuroleptic pharmacotherapy. *Journal of Neurochemistry*. wiley; 2001;76(3):789–96.

Thomas, George, Danielson, Nelson, Warren, Lo, et al. Antipsychotic drug treatment alters expression of mRNAs encoding lipid metabolism-related proteins. *Mol Psychiatry*. nature; 2003;8(12):983–93.

Tu JC, Xiao B, Naisbitt S, Yuan JP, Petralia RS, Brakeman P, et al. Coupling of mGluR/Homer and PSD-95 complexes by the Shank family of postsynaptic density proteins. *Neuron*. 1999 Jul 4;23(3):583–92.

Uçok A, Gaebel W. Side effects of atypical antipsychotics: a brief overview. *World psychiatry : official journal of the World Psychiatric Association (WPA)*. 2008 Mar 2;7(1):58–62.

Urigüen L, García-Fuster J, Callado L, Morentin B, Harpe R, Casadó V, et al. Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: effect of antipsychotic treatment. *Psychopharmacology*. 2009;206(2):313–24.

Usdin TB, Mezey E, Button DC, Brownstein MJ, Bonner TI. Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. *Endocrinology*. 1993 Dec 3;133(6):2861–70.

Volkow N. Substance Use Disorders in Schizophrenia—Clinical Implications of Comorbidity. *Schizophrenia Bulletin*. 2009;35(3):469–72.

Whiteford H, Degenhardt L, Rehm J, Baxter A, Ferrari A, Erskine H, et al. Global burden of disease attributable to mental and substance use disorders: findings from the Global Burden of Disease Study 2010. *Lancet*. 2013;382(9904):1575–86.

Zhang H-X, Zhao J-P, Lv L-X, Li W-Q, Xu L, Ouyang X, et al. Explorative study on the expression of neuregulin-1 gene in peripheral blood of schizophrenia. *Neuroscience Letters*. 2008;438(1):15.

Zink M, Schmitt A, May B, Müller B, Demirkaya T, Braus D, et al. Differential effects of long-term treatment with clozapine or haloperidol on GABAA receptor binding and GAD67 expression. *Schizophrenia Research*. sciencedirect; 2004;66(2-3):151157.